Phosphatase 1 Nuclear Targeting Subunit Mediates the Recruitment and Function of Poly (ADP-ribose) Polymerase 1 in DNA Repair

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Figure S1. PNUTS depletion causes endogenous DNA damage. (A, B) HeLa cells were transfected with control or PNUTS siRNA (#1) for 1 day, and then treated with IR, followed by 30 min incubation. Cells were examined by immunofluorescence for γH2AX. The average numbers of γH2AX foci were counted (A), and representative images of cells stained for γH2AX (in red) and DAPI (in blue) are shown (B). At least 100 cells were analyzed for γH2AX foci in panel A. **(C)** SCC38 cells treated with scramble or PNUTS siRNA (#2), were harvested

and analyzed by immunoblotting for γH2AX, and PNUTS.

Figure S2. PNUTS depletion leads to DNA damage hypersensitivity. (A-D) SCC38 cells with control or PNUTS siRNA (#1) were incubated with ionized radiation (IR, 10 Gy, panel A), bleomycin (Bleo, 5 μg/ml, panel B), camptothecin (CPT, 5 μM, panel C), and H_2O_2 (5 μM, panel D). Cells were incubated for 1-4 days. Cell viability was determined and normalized to that of the first day. The mean value and standard deviation were calculated from 3 independent experiments. **(E)** SCC38 cells were treated with scramble or PNUTS siRNA (#2) at day 0, and then with Dox at the indicated concentrations at day 1. The cell viability at day 3 was determined; the cell viability with Dox treatment was normalized to that without Dox treatment. The mean value and standard deviation were calculated from 3 independent experiments.

Figure S3. The recruitment of PNUTS to DNA damage sites is dependent on PARP1. HeLa cells expressing GFP-PNUTS were microirradiated with laser (405 nm). The path of laser microirradiation is marked by white lines (panels on the left). 3 min after laser treatment, the recruitment of GFP-PNUTS to laser induced DNA damage is shown. PARP inhibition with olaparib (10 μM) prevented the recruitment of PNUTS. Quantification of cells exhibiting laser recruitment with or without olaparib is provided in Fig. 5E.

Figure S4. PNUTS is required for the efficient induction of PARylation after DNA damage. HeLa cells transfected with scramble or PNUTS siRNA (#2) were microirradiated with 405nm laser. 5 min post-laser, cells were analyzed by immunofluorescence for PAR and γH2AX. The path of laser microirradiation is marked by white lines.

Figure S5. PNUTS binds the middle segment of PARP1, and promotes its recruitment to laser-induced DNA damage sites. (A) The middle segment of PARP1 (aa 389-669) was

tagged with GFP and expressed in HeLa cells. GFP IP was performed. 20% input, control IP, and GFP IP samples were analyzed by immunoblotting for PNUTS and GFP. **(B)** The middle segment of PARP1 was tagged with GFP, as in panel A, and expressed in HeLa cells with or without PNUTS knockdown. Cells were microirradiated with 405nm laser, and examined for the localization of GFP 3 min post-laser. The path of laser microirradiation is marked by white lines.

Figure S6. PNUTS knockdown impairs the recruitment of GFP-PARP1 to laser-induced DNA damage sites. HeLa cells expressing GFP-PARP1 were treated with control or PNUTS siRNA (#1). Cells were microirradiated with 405nm laser. The localization of GFP-PARP1 3 min post-laser is shown. The white lines mark the regions of laser microirradiation (right panels).

Figure S7. PNUTS knockdown disrupts the recruitment of PARP1 to laser-induced DNA damage sites. HeLa cells were treated with scramble or PNUTS siRNA (#2). Cells were microirradiated with 405nm laser. The localization of PARP1 3 min post-laser is shown by immunofluorescence. The white lines mark the regions of laser microirradiation.

Figure S8. PNUTS targeting sensitizes SCC38 cells to PARP inhibition. SCC38 cells were treated with scramble or PNUTS siRNA (#2) at day 0, and then with PARPi at the indicated concentrations at day 1. The cell viability at day 3 was determined; the cell viability with PARPi treatment was normalized to that without PARPi treatment. The mean value and standard deviation were calculated from 3 independent experiments.

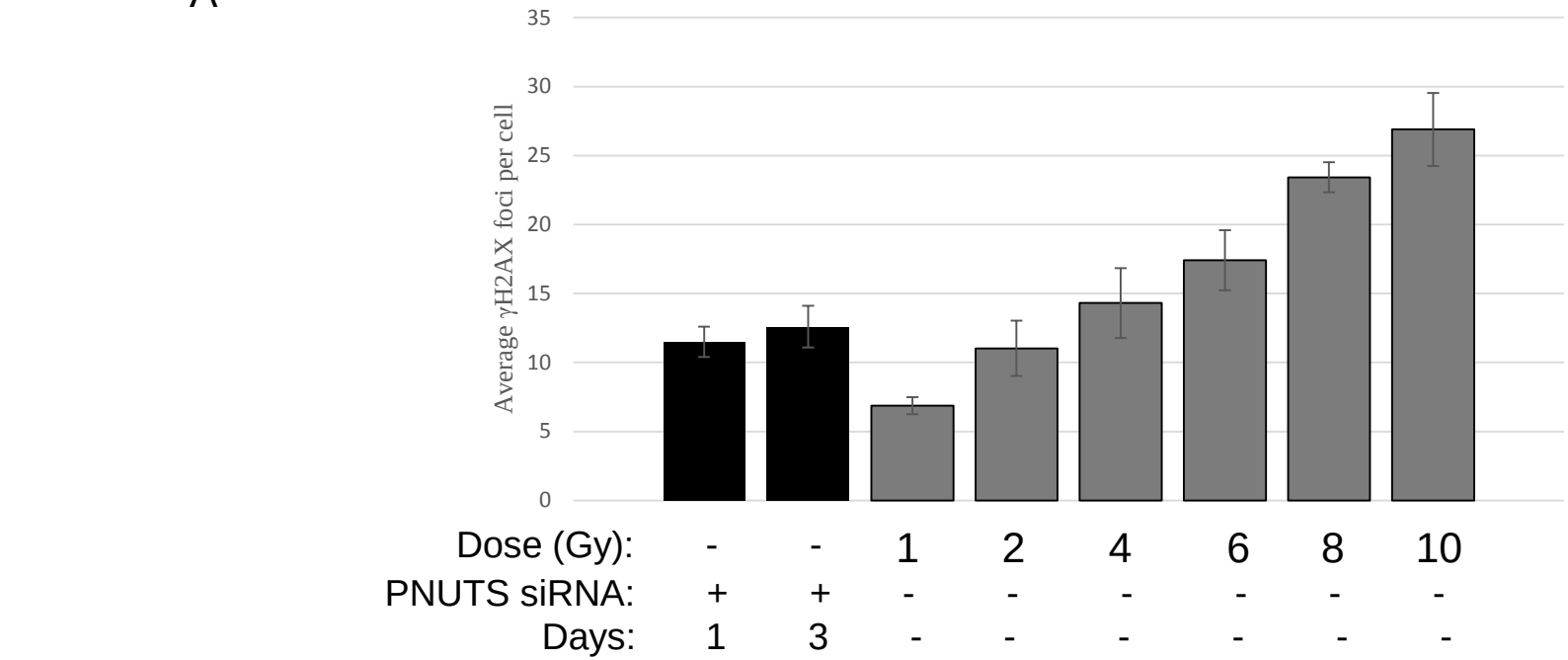
Figure S9. PNUTS targeting sensitizes HeLa cells to PARP inhibition. (A) HeLa cells were treated with PNUTS siRNA (#1) and olaparib (10 μM), as indicated. Cell viability was

determined at days 1-4, and normalized to that of day 1. The mean value and standard deviation were calculated from 3 independent experiments. **(B)** HeLa cells were treated with PNUTS siRNA (#1) and olaparib, as in panel A. The colonogenic assay was performed as described in Materials and Methods. The numbers of colonies were normalized to the untreated control. The mean value and standard deviation were calculated from 3 independent experiments. Statistical significance was analyzed using an unpaired 2-tailed Student's t-test. A p-value<0.01 was considered highly significant (**).

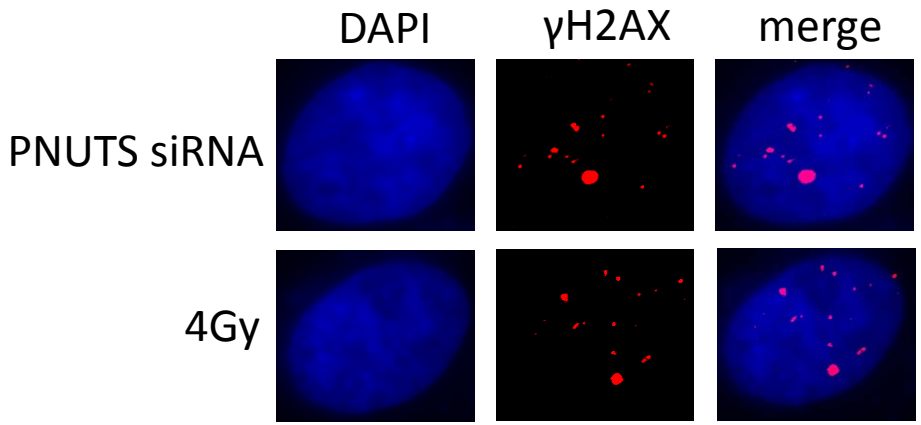
Movie S1. The recruitment of GFP-PNUTS after laser microirradiation is shown in a 10 min period.

Movie S2. The recruitment of GFP-PNUTS after laser microirradiation, in the presence of olaparib is shown in a 10 min period.

A



B



C

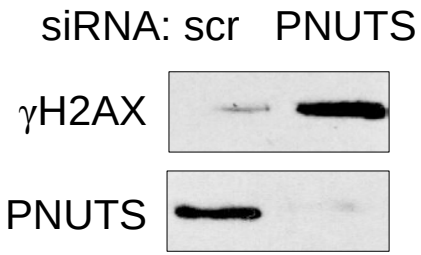


Figure S1

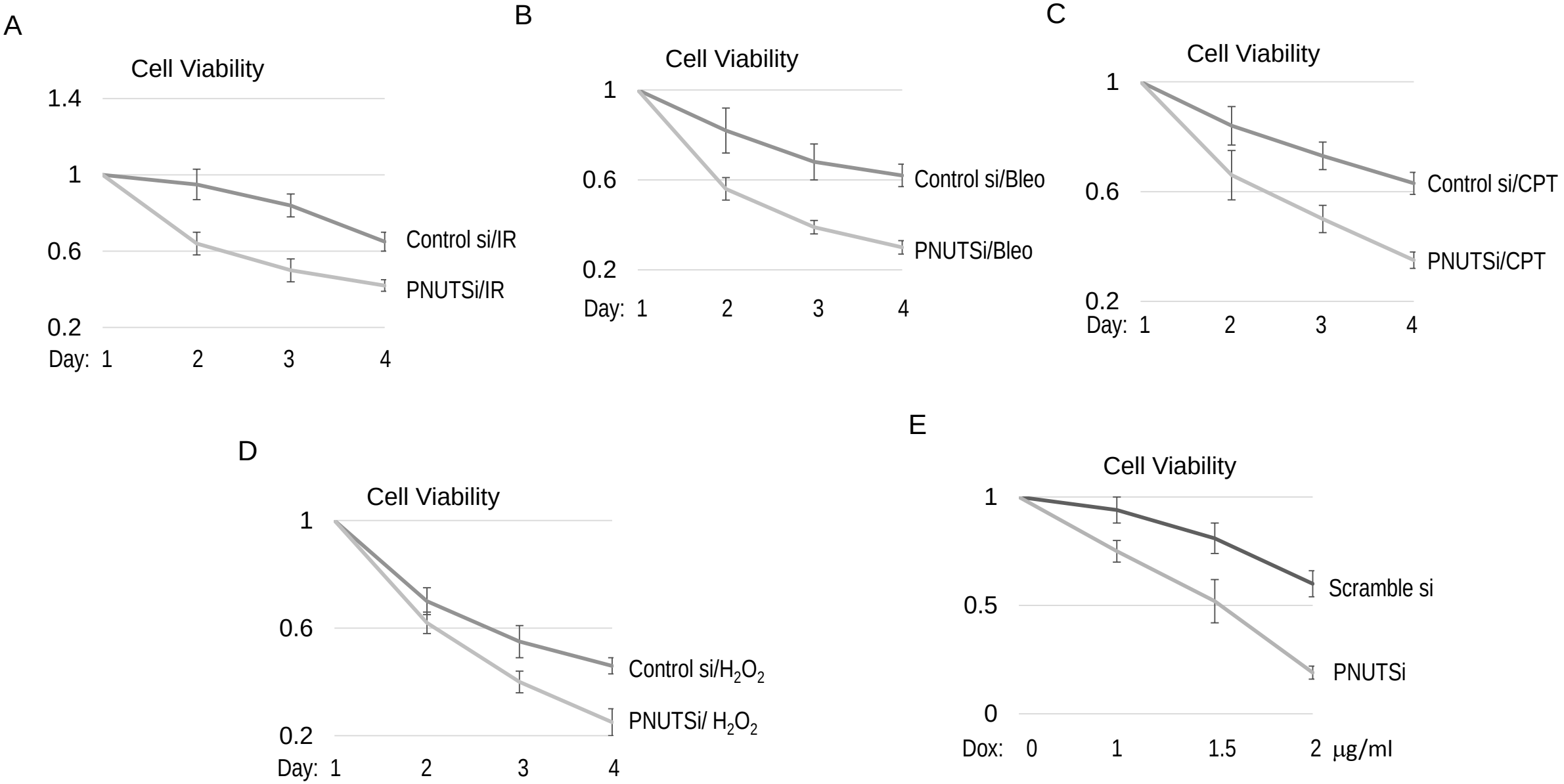

A
Cell Viability
1.4
1
0.6
0.2
Day: 1 2 3 4
Control si/IR
PNUTSi/IR
B
Cell Viability
1
0.6
0.2
Day: 1 2 3 4
Control si/Bleo
PNUTSi/Bleo
C
Cell Viability
1
0.6
0.2
Day: 1 2 3 4
Control si/CPT
PNUTSi/CPT
D
Cell Viability
1
0.6
0.2
Day: 1 2 3 4
Control si/H2O2
PNUTSi/ H2O2
E
Cell Viability
1
0.5
0
Dox: 0 1 1.5 2 μg/ml
Scramble si
PNUTSi

Figure S2

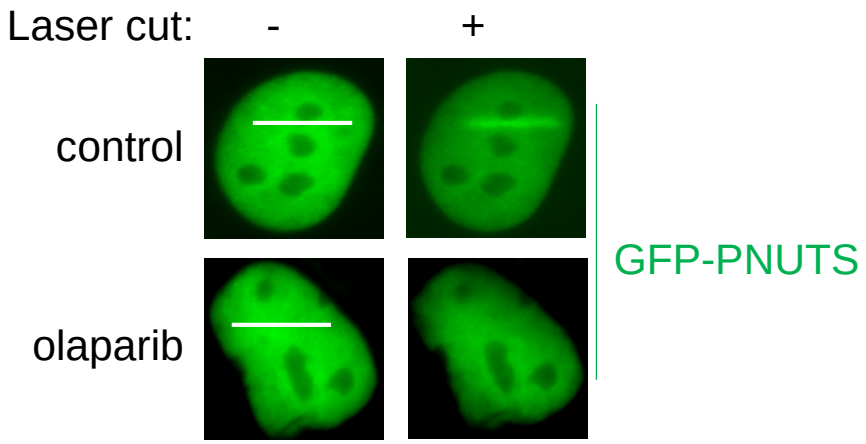


Figure S3

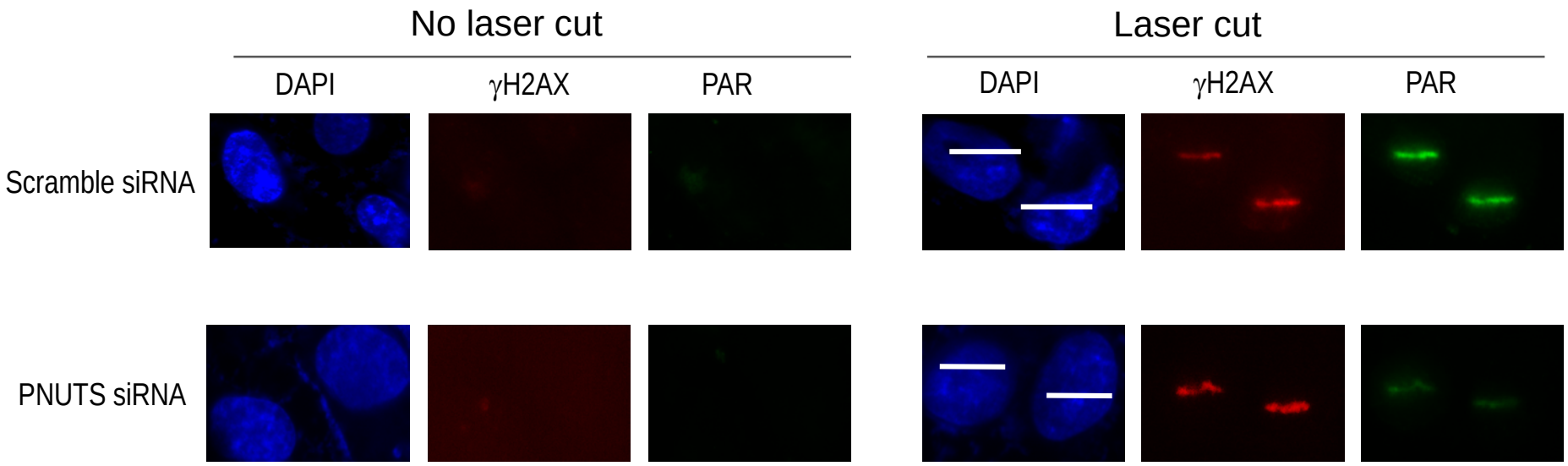

No laser cut
Laser cut
DAPI
γH2AX
PAR
DAPI
γH2AX
PAR
Scramble siRNA
PNUTS siRNA

Figure S4

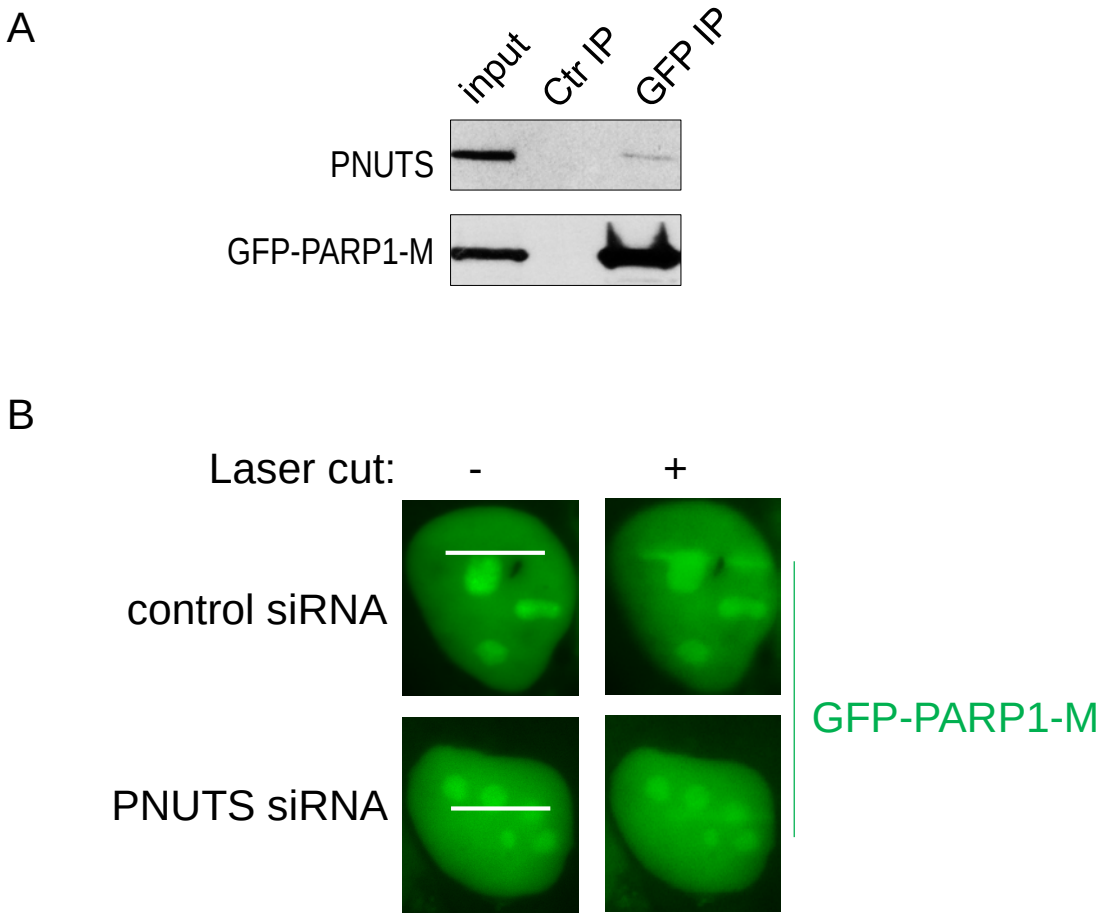

A
input
Ctr IP
GFP IP
PNUTS
GFP-PARP1-M
B
Laser cut:
-
+
control siRNA
PNUTS siRNA
GFP-PARP1-M

Figure S5

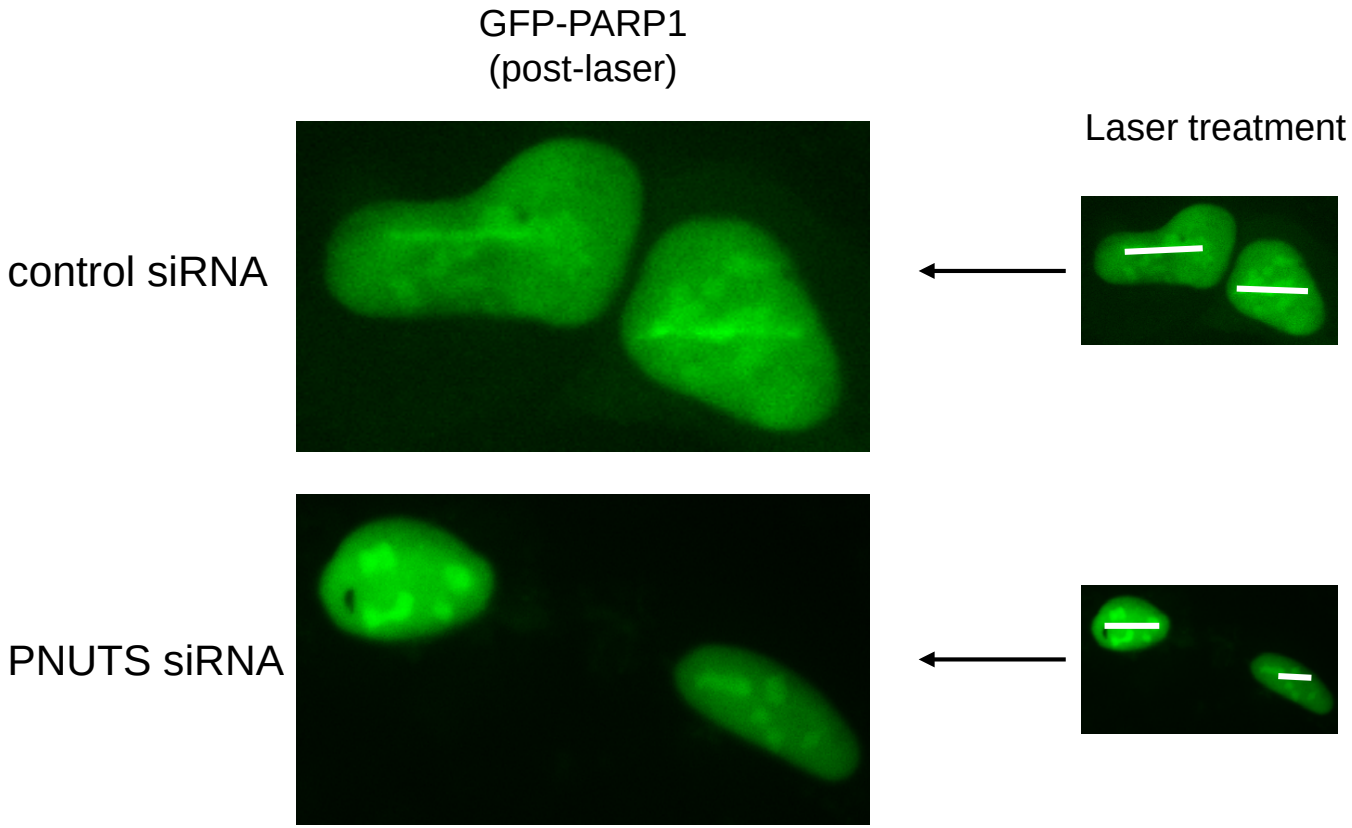

GFP-PARP1
(post-laser)
Laser treatment
control siRNA
PNUTS siRNA

Figure S6

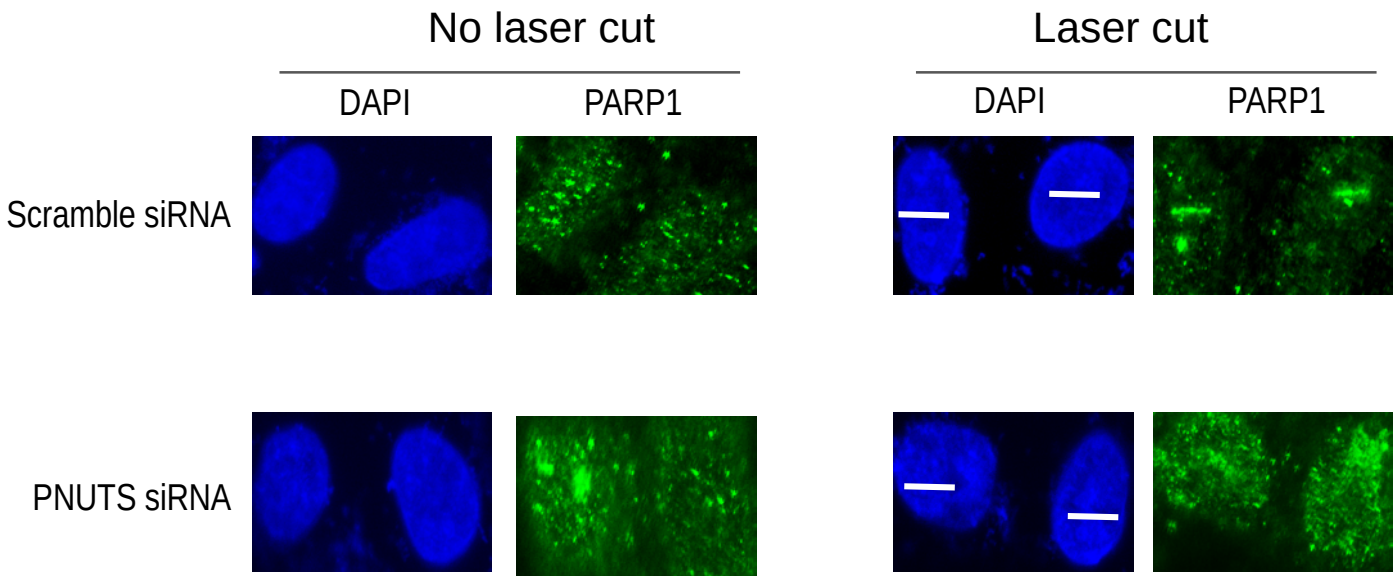

No laser cut
Laser cut
DAPI
PARP1
DAPI
PARP1
Scramble siRNA
PNUTS siRNA

Figure S7

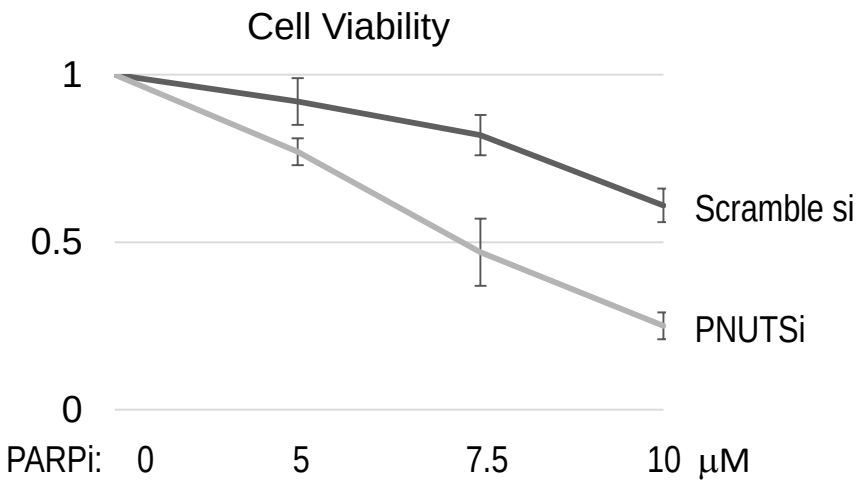
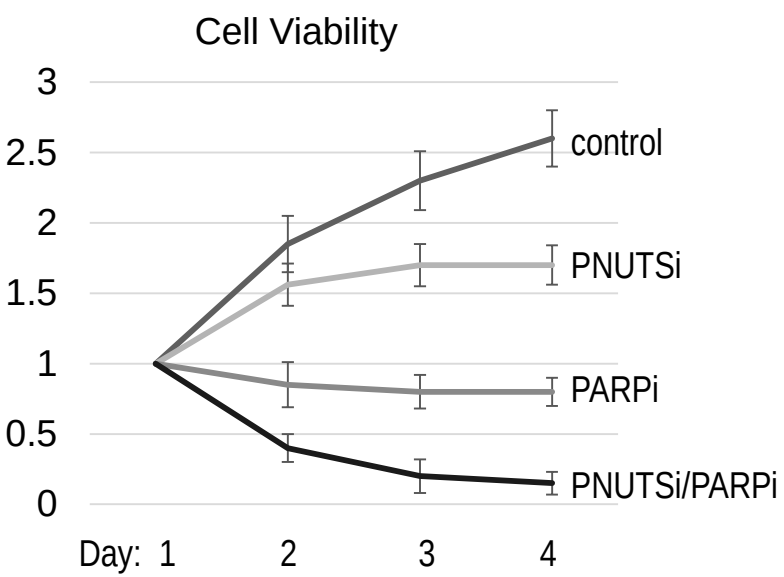


Figure S8

A


Cell Viability
3
2.5
2
1.5
1
0.5
0
Day: 1
2
3
4
control
PNUTSi
PARPi
PNUTSi/PARPi

B

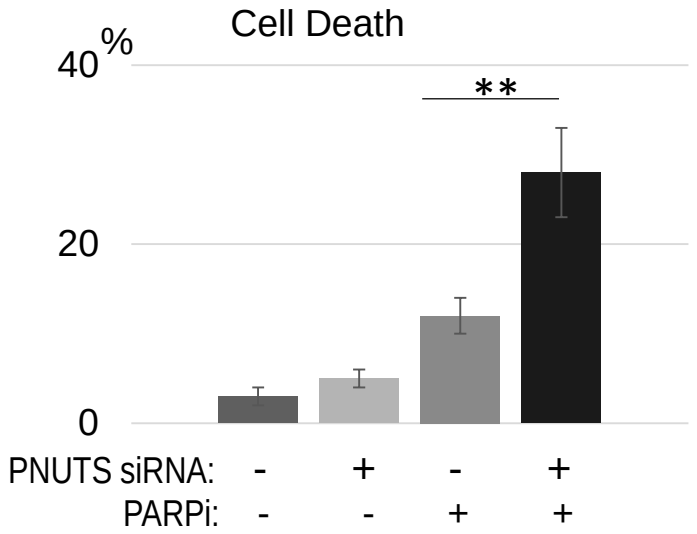

Cell Death
%
40
20
0
**
PNUTS siRNA: - + - +
PARPi: - - + +

Figure S9