Supplemental data

POLY(ADP-RIBOSE) POLYMERASE-3 (PARP-3) IS A MONO-ADP RIBOSYLASE THAT ACTIVATES PARP-1 IN ABSENCE OF DNA

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Running head: Biochemical characterization of PARP-3
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Table 1

IC_{50} values determined for the PARP inhibitors against PARP-3 and PARP-1

Inhibitor	PARP-3	PARP-1
KU0058948	85 ± 2 nM	0.7 ± 0.03 nM
DR2313	65 ± 0.2 μM	96 ± 11 nM
4-ANI	440 ± 7 μM	23 ± 1 nM
DIQ	370 ± 60 μM	0.19 ± 0.03 μM
NAP	650 ± 160 μM	18 ± 0.3 nM
PJ34	620 ± 60 μM	0.17 ± 0.05 μM
3-ABA	760 ± 10 μM	8.7 ± 0.2 μM

Apparent IC_{50} values of PARP-1 and PARP-3 for the inhibitors were estimated by fitting an inhibition curve to the intensities of the ADP-ribose signal after 5 minutes of reaction in presence of various concentrations of the inhibitors. Quantification of ADP-ribosylation was carried out using Adobe Photoshop CS2.

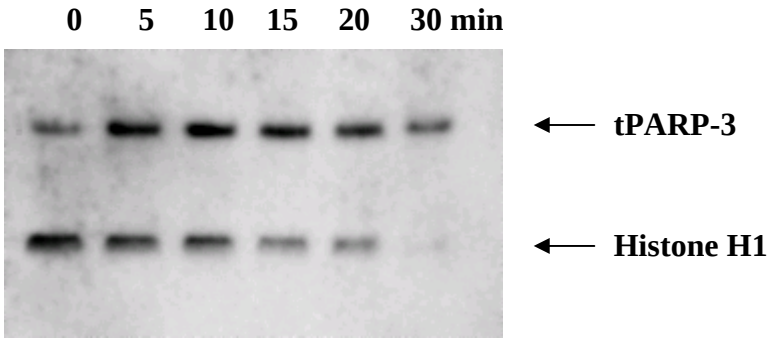
FIGURE LEGEND

Supplemental Fig. 1. tPARP-3 (1 μM) was incubated with 25 μM BioNAD^+, 75 μM NAD^+, Histone H1 (1 μM) and activated DNA (5 μg/ml) in PARP reaction buffer. At times 0, 5, 10, 15, 20 and 30 min samples were removed and the reaction was stopped by addition of LDS sample buffer.

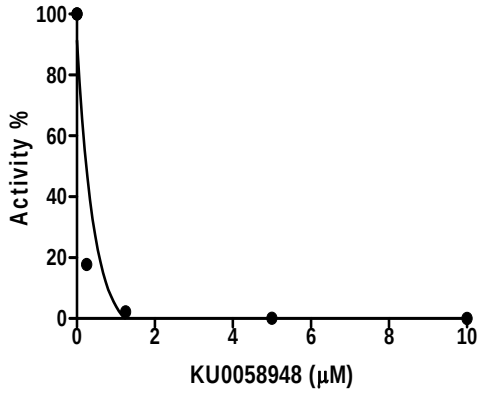
Supplemental Fig. 2. Inhibition curve of PARP-3 using KU0058948.

Supplemental Fig. 3. Activity assay of PARP-3 and PARP-1 in the absence of activated DNA. PARP-3 was incubated with PARP-1, Bio-NAD^+, NAD^+ in presence of Mg^{2+} and Ca^{2+}. At time 0 and after 2.5, 5 and 10 min of incubation at room temperature the reaction was stopped by dilution in LDS sample buffer.

Supplemental Fig. S1



Supplemental Fig. S2



Supplemental Fig. S3