Supplementary Methods

Cell culture, transfection of siRNA and overexpression of PARP1

HEK293T cells were grown in DMEM Glutamax-1 (Invitrogen), supplemented with 10% (v/v) fetal calf serum (Invitrogen) and 50 units/ml penicillin and 50 μg/ml streptomycin (Sigma). Cells were grown in 5% CO_2 and 37°C in a humidified incubator. HEK293T cells were transfected with RNAi-max (Invitrogen) and siRNA directed against PARP1 (Qiagen, Cat.No. S102662989) or control siRNA (Qiagen, Cat.No. S103650318) for 48 hours. Overexpression of PARP1 was performed by transfection of HEK293T cells by standard Calcium-Phosphate precipitation method with a pcDNA3-HA-PARP1 expression vector or an empty vector control, respectively. 8 hours after transfection the medium was replaced and the cells were grown for another 20 hours before they were harvested.

ADP-ribosylation of isolated nuclei

Nuclei were isolated from 5x 10^6 HEK293T cells by the addition of cold hypotonic lysis buffer (5 mM HEPES pH 7.4, 0.5% NP-40, 85 mM KCl, 1 μg/ul Pepstatin, Leupetin, Bestatin). After 2 minutes of incubation, the nuclei were centrifuged for 4 min at 7000g and washed twice with suspension buffer (33.3 mM Tris-HCl pH 7.8, 40 mM $MgCl_2$, 1 μg/ul Pepstatin, Leupetin, Bestatin). The pellet was resuspended in permeabilization buffer (38.3 mM Tris-HCl pH 7.8, 42.1 mM $MgCl_2$, 0.53 mM EDTA, 13.9 mM β-Mercaptoethanol, 1 μg/ul Pepstatin, Leupetin, Bestatin), supplemented with 400 μM etheno-NAD^+ (Sigma Aldrich) or 4 mM PJ34 (Enzo Life Science) and incubated for 20 min at 37°C at 900 rpm in a rotator. After centrifugation the pellet was resuspended in SDS-lysis buffer and run on a 18% SDS-PAGE. Western blotting was performed with anti Ig4 antibody hybridoma serum (kindly provided by Dr. R. Santella, Columbia University, USA) in a vacuum blot apparatus (Millipore SNAP i.d).

Supplementary Figures

Supplementary Figure 1:

PARP1 poly(ADP-ribosyl)ates chromatin associated histones. (A) HEK293T cells were depleted of PARP1 and nuclei were prepared. The nuclei were incubated with 400 μM etheno-NAD^+ at 37°C for 20 min and lysed in SDS-lysis buffer. Western blotting was performed with the Ig4 antibody, which specifically recognizes the etheno-group of NAD^+. (B) HEK293T cells were transiently transfected with HA-PARP1 (ov. PARP1) or with an empty vector (control). Nuclei were prepared and incubated with 400 μM etheno-NAD^+ in presence or absence of the PARP-Inhibitor PJ34.

Supplementary Figure 2:

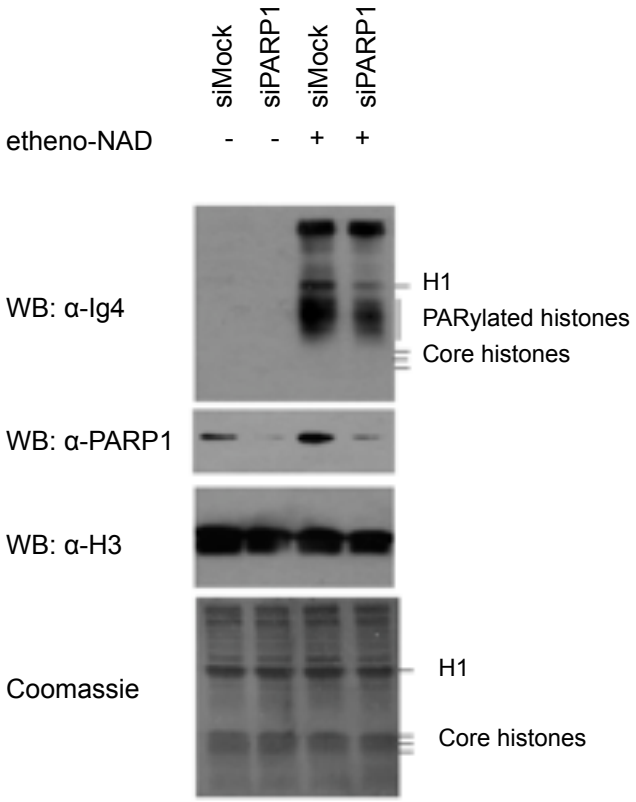
(A) Trans-ADP-ribosylation of histone tails by PARP1. 1.5 μg of full-length and truncated histones were incubated with 10 pmol PARP1 and 100 nM ^{32}P-NAD^+ for 15 min at 30°C. His-tagged H2B (36-122) was generated by PCR and cloned into pET3a with NdeI and BamHI restriction enzymes. The protein was expressed in inclusion bodies, solubilized, purified by a nickel-column and dialyzed against water. The other histones were expressed and purified as in Luger K. et al, 1997, JMB, 272, 301-311. (B) Identical to Fig. 1C of the main manuscript. The automodification of PARP2 was adjusted to the automodification of PARP1 by ImageQuant-Software. (C) Trans-ADP-ribosylation of H2B is not impaired in an H2B E2A mutant, in which the only glutamic acid residue is substituted by an alanine. Shown are autoradiographs and Coomassie stained gels. (D) Poly-L-lysine but not poly-L-glutamate are modified by hPARP1. Poly-L-amino acids were coupled onto cyanogen-bromide activated agarose beads over night as suggested by the provider (Sigma-Aldrich). Excess poly-L-amino acids were washed away and unoccupied reactive sites were blocked over night. The beads were washed and equilibrated in PARP1 reaction buffer. Reactions were performed for 5 minutes at 30°C in the presence of 100 nM radiolabeled NAD^+. The beads were washed 3 times in PARP1 reaction buffer containing 500 mM NaCl before scintillation counts in two different channels were determined. (E) Extracted ion chromatogram of the biotin tagged H3 (23-42) peptide, ADP-ribosylated by PARP1. ETD fragment spectrum of ADP-ribosylated

H3 peptide (590.29 m/z) at K37, indicated by the sequence plot. (F) Automodification of wild-type PARP1 and PARP1 D756K mutant for 10 min at 30°C in presence of activating DNA (EcoRI-linker) and 100 nM ^{32}P-NAD^{+}. Shown is an autoradiography and the coomassie stained gel. (G) Histone H4 (aa 1-22) peptide was ADP-ribosylated with PARP1 or PARP1 D756K mutant for 15 min at 30°C with 100 nM ^{32}P-NAD^{+}. The peptides were purified by microvolume-C18 reversed phase columns, eluted into scintillation liquid and counted for incorporated ^{32}P. Relative increase of counts per minutes was calculated over background (peptides added after termination of the reaction by 3AB) and the counts obtained for wild-type PARP1 were set to 100.

Supplementary Figure 3:

Time evolution of intermolecular salt bridges and side chain dihedral angles of PARP1 Tyrosine residues in the catalytic cleft.

A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siPARP1
siMock
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etheno-NAD
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H1
PARylated histones
Core histones
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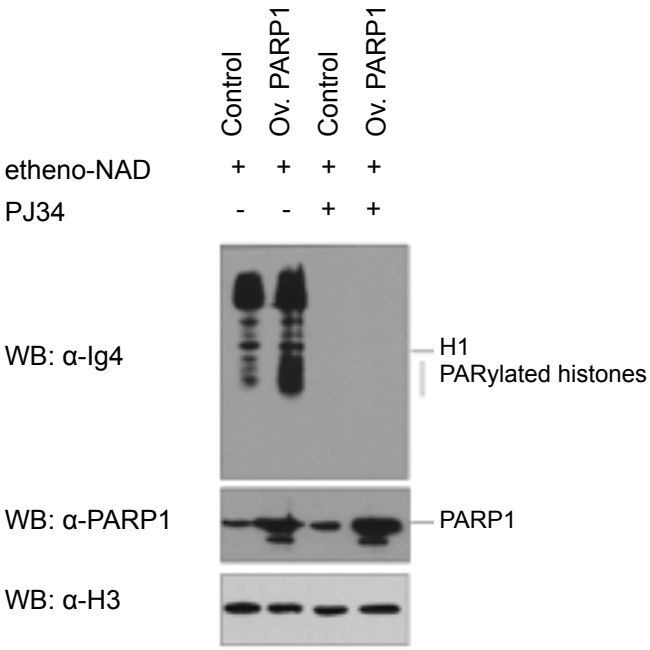
WB: α-H3
Coomassie
H1
Core histones

B


Control
Ov. PARP1
Control
Ov. PARP1
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PJ34
- - + +
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H1
PARylated histones
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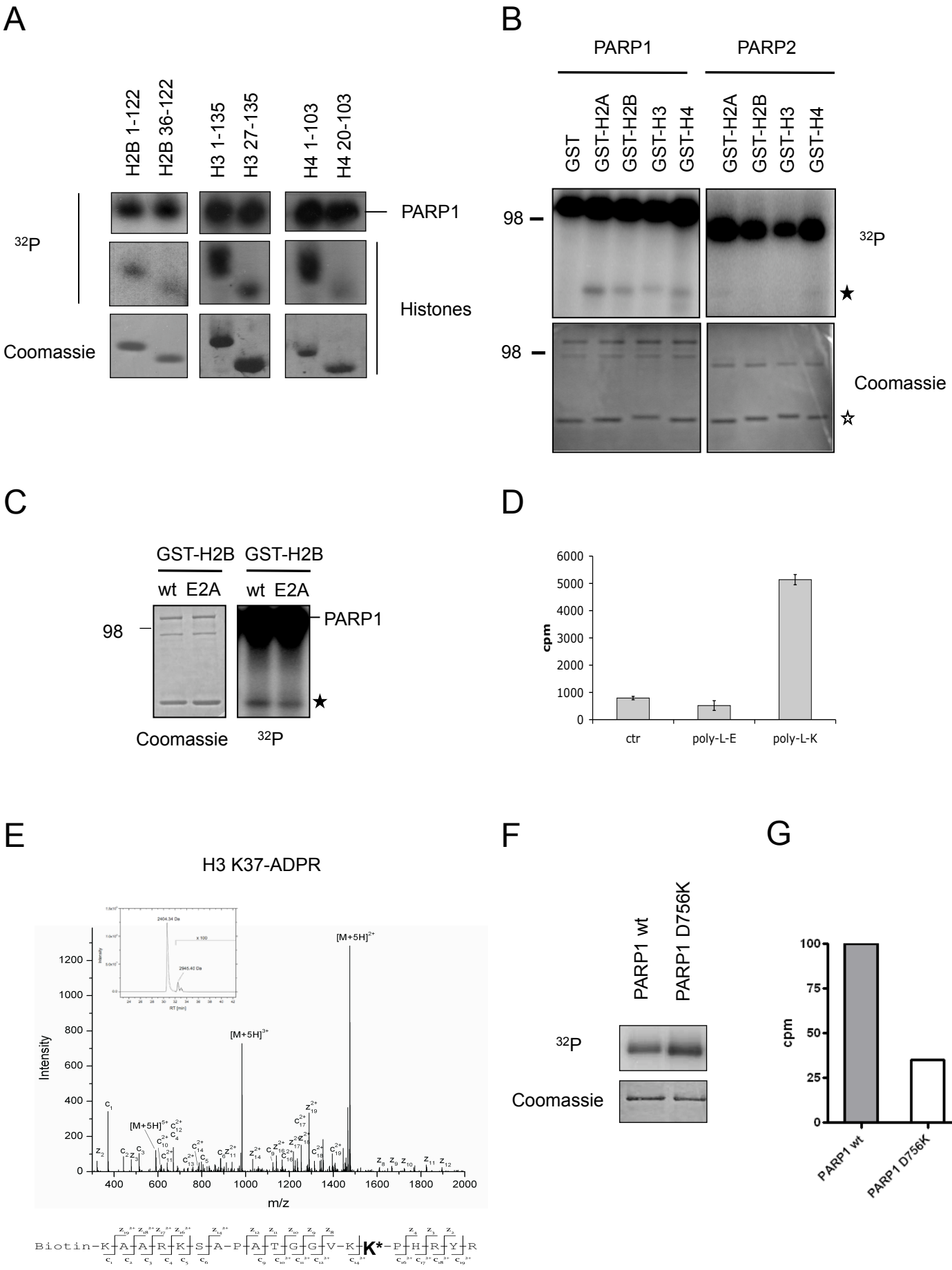
PARP1
WB: α-H3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H3 27-135
H4 1-103
H4 20-103
PARP1
32P
Histones
Coomassie
B
PARP1
PARP2
GST
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GST-H2B
GST-H3
GST-H4
GST-H2A
GST-H2B
GST-H3
GST-H4
98
98
32P
Coomassie
C
GST-H2B
GST-H2B
wt E2A
wt E2A
98
PARP1
Coomassie
32P
D
cpm
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5000
4000
3000
2000
1000
0
ctr
poly-L-E
poly-L-K
E
H3 K37-ADPR
Intensity
m/z
[M+5H]2+
[M+5H]3+
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F
PARP1 wt
PARP1 D756K
32P
Coomassie
G
cpm
100
75
50
25
0
PARP1 wt
PARP1 D756K

Time evolution of intermolecular salt bridges and side chain dihedral angles of PARP1 Tyr residues in the catalytic cleft