



Extended Data Figure 1. Activation of animal and plant PARPs by 5'-phosphorylated DNA damage mimetics.

(a) Chemical stability of *Af*-PARP1-derived ADP-ribosylation was assessed post-auto(ADP-ribosylation). Stability under acidic and alkaline condition was assessed with HCl and NaOH, respectively. Stability under high ionic strength conditions and in presences of hydroxylamine was assessed at pH 7 stabilised with MOPS•NH₄OH.

(b) EMSA assay assessing the DNA damage binding specificity of *h*PARP2, 3, and *Lo*PARP2. PARPs (5 μM) were incubated with DNA mimetics (1 μM) for 1 h, separated via native PAGE and DNA visualised with SYBR safe staining.

(c) Assessment of *h*PARP2, 3, and *Lo*PARP2 catalytic activities in presence of different DNA damage mimetics. PARPs (0.5 μM) were incubated with DNA (0.6 μM) and NAD⁺ (500 μM) and formation of PAR detected using immunoblot.