

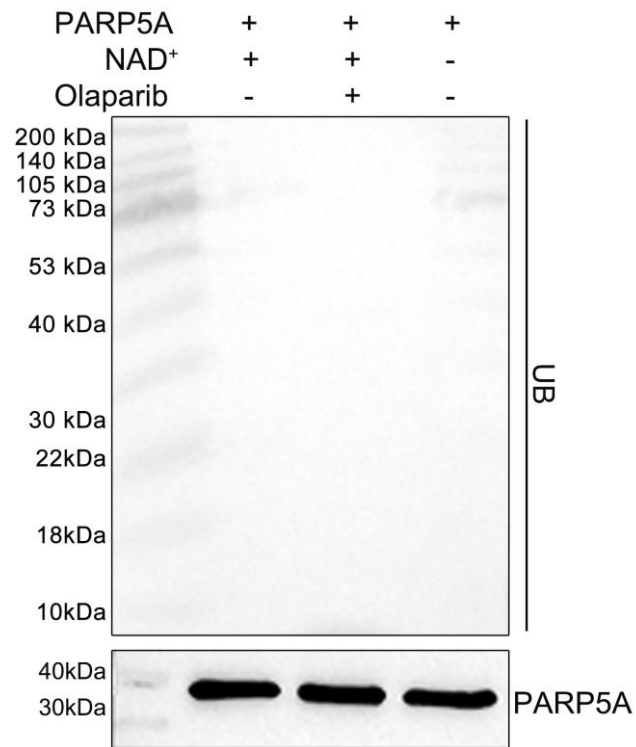


Figure S2. In vitro PAR overlay assay validating the direct PAR-binding capacity of GST-Ub. PAR polymers were synthesized using recombinant PARP5A (10 μ M) with NAD⁺ (1 mM) in the absence (lane 1) or presence (lane 2) of the PARP inhibitor olaparib (100 μ M). PARP5A alone served as a negative control (lane 3). Reaction products were resolved by SDS-PAGE, transferred to membranes, and incubated with purified GST-Ub (20 μ M). Bound GST-Ub was detected by immunoblotting using an anti-GST antibody. PARP5A inputs are shown as loading controls (lower panels).