

To purify MBP-NAP1, human NAP1 cDNA was gene-synthesized (by Genscript) and subcloned into a pGEX-4T1 vector with an N-terminal MBP-tag followed by a TEV cleavage site before wild-type NAP1 (RRID:Addgene_208871), NAP1 delta-NDP52 (S37K/A44E) (RRID:Addgene_208872), or NAP1 delta-TBK1 (L226Q/L233Q) (RRID:Addgene_208873). For expression of MBP-TEV-NAP1 in *E. coli*, the pGEX-4T1 vector encoding MBP-TEV-NAP1 was transformed into *E. coli* Rosetta pLySS cells, cells were grown in 2xTY medium at 37°C until an OD₆₀₀ of 0.4 and then continued at 18°C. Once the cells reached an OD₆₀₀ of 0.8, protein expression was induced with 50 µM IPTG for 16 h at 18°C. Cells were collected by centrifugation and resuspended in lysis buffer (50 mM Tris-HCl pH 7.4, 300 mM NaCl, 1 mM DTT, 2 mM MgCl₂, 5% glycerol, 2 mM β-mercaptoethanol, cOmplete EDTA-free protease inhibitors (Roche), and DNase (Sigma)). Cell lysates were sonicated twice for 30 s and then cleared by centrifugation at 18,000 rpm for 45 min at 4°C in a SORVAL RC6+ centrifuge with an F21S-8x50Y rotor (Thermo Scientific). The supernatant was collected and incubated with pre-equilibrated Amylose beads (Biolabs) for 2 h at 4°C with gentle shaking to bind MBP-TEV-NAP1. Samples were centrifuged to pellet the beads and remove the unbound lysate. Beads were then washed twice with wash buffer (50 mM Tris-HCl pH 7.4, 300 mM NaCl, 5% glycerol, 1 mM DTT), once with high salt wash buffer (50 mM Tris-HCl pH 7.4, 700 mM NaCl, 5% glycerol, 1 mM DTT), and two more times with wash buffer (50 mM Tris-HCl pH 7.4, 300 mM NaCl, 5% glycerol, 1 mM DTT). Beads were incubated overnight at 4°C with 250 mM D-maltose (Santa Cruz) dissolved in wash buffer (50 mM Tris-HCl pH 7.4, 300 mM NaCl, 5% glycerol, 1 mM DTT). After the proteins were released from the beads, the MBP-TEV-NAP1 protein was filtered through a 0.45 µm syringe filter, concentrated using a 30 kDa cut-off Amicon filter (Merck Millipore), and loaded onto a pre-equilibrated Superose 6 Increase 10/300 GL column (Cytiva). Proteins were eluted with SEC buffer (25 mM Tris-HCl pH 7.4, 300 mM NaCl, 1 mM DTT). Fractions were analyzed by SDS-PAGE and Coomassie staining. Fractions containing purified MBP-TEV-NAP1 protein were pooled. After concentrating the purified protein, the protein was aliquoted and snap-frozen in liquid nitrogen. Proteins were stored at -80°C.