

To purify NAP1 or GST-NAP1, human NAP1 cDNA was synthesized and cloned in a pcDNA3.1 vector (Genscript), from where it was subcloned into a pGEX-4T1 vector with an N-terminal GST tag followed by a TEV cleavage site (RRID:Addgene\_208870). For expression of GST-TEV-NAP1 in *E. coli*, which we used in Figure 5B, Figure 6E, and Figure S3, Figure S5, the pGEX-4T1 vector encoding GST-TEV-NAP1 was transformed into *E. coli* Rosetta pLySS cells, cells were grown in 2xTY medium at 37°C until an OD<sub>600</sub> of 0.4 and then continued at 18°C. Once the cells reached an OD<sub>600</sub> 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 MgCl<sub>2</sub>, 5% glycerol, 2 mM β-mercaptoethanol, cOmplete EDTA-free protease inhibitors (Roche), and DNase (Sigma)). Cell lysates were sonicated twice for 30 s. Lysates were 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 Glutathione Sepharose 4B beads (GE Healthcare) for 2 h at 4°C with gentle shaking to bind GST-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 TEV protease or 4 ml of 50 mM reduced glutathione 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 GST-NAP1 protein was filtered through a 0.45 µm syringe filter, concentrated using a 30 kDa cut-off Amicon filter (Merck Millipore), or 10 kDa cut-off in case of unlabeled NAP1, 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 NAP1 or GST-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.