For OPTN-GST, we cloned human OPTN cDNA in a pETDuet-1 vector with an C-terminal GST-tag. After the transformation of the pETDuet-1 vector encoding OPTN-GST in 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, 2 mM MgCl₂, 5% glycerol, 10 mM Imidazole, 2 mM β-mercaptoethanol, cOmplete EDTA-free protease inhibitors (Roche), CIP protease inhibitor (Sigma), 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 OPTN-GST. Samples were centrifuged to pellet the beads and remove the unbound lysate. Beads were then washed twice with wash buffer (50 mM HEPES pH 7.4, 300 mM NaCl, 1 mM DTT), once with high salt wash buffer (50 mM HEPES pH 7.4, 700 mM NaCl, 1 mM DTT), and two more times with wash buffer (50 mM HEPES pH 7.4, 300 mM NaCl, 1 mM DTT). Proteins were eluted overnight with 20 mM reduced L-glutathione in 50 mM HEPES pH 7.4, 300 mM NaCl, 1 mM DTT buffer. The supernatant was collected, filtered through a 0.45 µm syringe filter, and concentrated using a 50 kDa cut-off Amicon filter (Merck Millipore), and loaded onto a pre-equilibrated Superdex 200 Increase 10/300 GL column (Cytiva). Proteins were eluted with SEC buffer (25 mM Tris-HCl pH 7.4, 150 mM NaCl, 1 mM DTT). Fractions were analyzed by SDS-PAGE and Coomassie staining. Fractions containing purified OPTN-GST were pooled. After concentrating the purified protein, the protein was aliquoted and snap-frozen in liquid nitrogen. Proteins were stored at -80°C.