GFP pull down assay

GFP-tagged TBK1 was mixed with 20 µl of GFP-Trap agarose beads (Chromotek) at a final concentration of 1 µM. To this end, 20 µl of beads were washed twice with dH_2O and equilibrated with bead assay buffer (25 mM Tris-HCl pH 7.4, 150 mM NaCl, 1 mM DTT). Beads were then resuspended in 40 µl bead assay buffer, to which GFP-TBK1 was added at a final concentration of 5 µM. Beads were incubated with GFP-TBK1 for 1 h at 4°C at a horizontal tube roller. Beads were washed three times to remove unbound GFP-tagged bait protein. Protein master mixes with prey protein were prepared in bead assay buffer at the following concentrations: mCherry-OPTN (1 µM), mCherry-NDP52 (1 µM), GST-NAP1 (1-10 µM). The protein master mixes were added to the beads and incubated for 1 h at 4°C at a horizontal tube roller. Beads were washed three times to remove unbound proteins, diluted in 60 µl of 1x Protein Loading dye, and heat-inactivated at 95°C for 5 min. Samples were analyzed by SDS-PAGE and Coomassie staining as described above.