Immunoprecipitation

FIP200 knockout HAP1 cells were transiently transfected with pcDNA3.1 NAP1-EGFP (Addgene xxx), or empty pcDNA3.1 vector as a negative control, using Lipofectamine 2000 (Thermo Fisher). This cell line was selected as FIP200 deletion results in TBK1 hyperactivation and thus increased NAP1 phosphorylation. After 48 h, cells were collected by trypsinization and the cell pellet was washed with PBS once before cells were lysed in lysis buffer (100 mM KCl, 2.5 mM MgCl2, 20 mM Tris-HCl pH 7.4, 0.5% NP-40). Samples were lysed for 20 min on ice before cell lysates were cleared by centrifugation at 20,000*g* for 10 min at 4°C. Protein concentrations of the cleared protein lysates were then determined with the Pierce Detergent Compatible Bradford Assay Kit (23246, Thermo Fisher). For both samples, negative control and NAP1-EGFP lysates, 12 mg of cell lysate was incubated overnight with 20 µl or GFP-Trap agarose beads (GTA-20, Chromotek). In the morning, samples were washed three times in lysis buffer before the beads were resuspended in protein loading dye, supplemented with 100 mM DTT, and boiled for 5 min at 95°C. Samples were loaded on 4-12% SDS-PAGE gels (NP0322BOX, Thermo Fisher) with PageRuler Prestained protein marker (Thermo Fisher). After the run, the SDS-PAGE gel was stained with Coomassie and destained overnight. The band corresponding to NAP1-EGFP was cut from the gel with a fresh scalpel and submitted for mass spectrometry analysis.