In vitro phosphatase assay

HAP1 wild-type or FIP200 knockout cells were seeded in 6 well plates and grown until confluency. Cells were collected by trypsinization and pelleted by centrifugation at 300*g* for 5 min at 4°C. After a PBS wash to remove the remaining cell medium, the cell pellets were resuspended in lysis buffer (50 mM HEPES pH 7.4, 150 mM NaCl, 2.5 mM $MgCl_2$, 2 mM DTT, 0.5% NP-40, and protease inhibitor cocktail). 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, wild-type and FIP200 knockout lysates, 100 µg of cell lysate was incubated with 5 µl of 10x NEBuffer for Protein MetalloPhosphatases (P0753, New England Biolabs) and 5 µl of 10 mM of MnCl2 to make a total reaction volume of 50 µl. We added 1 µl of Lambda Protein Phosphatase (NEB) to the reaction and incubated the samples at 30°C for the indicated time. The phosphatase reactions were terminated by the addition of 6x Protein Loading dye and heat inactivation at 95°C for 5 min. Samples were analyzed by western blot analysis as described above.