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WIPI2d Coprecipitation Assay

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1 Works for me

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ABSTRACT

Pulldown assay of WIPI2d

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PROTOCOL CITATION

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- 1 Equilibrate 10uL of GST resin to pulldown buffer. To do this, pipette 40uL of slurry (so 20uL of resin) into a 1.7uL eppy. Add >500mL of wash buffer. Slow spin to pellet resin. ~1000rpm for 1 minute should be good. Repeat X3
- 2 Add 10 μ M purified WIPI2d and either with 20 μ M of GST or GST-ATG16L1(207-230). Add buffer (25mM HEPES pH 7.4, 150mM NaCl, 1mM TCEP) to final volume of 150uL.
- 3 Let rock at 4C for 2 hour. Wash x3
- 4 Add 10uL of SDS-Loading dye to beads
- 5 Run beads on SDS-PAGE gel and stain with Coomassie.

