IMMUNOPRECIPITATIONS

Protein A-Sepharose Bead Processing (for 20 IPs):
Weigh out 50 mg of Protein A-Sepharose beads into 5 1.5 mL eppendorf tubes.
Add 1 mL dH_2O and rotate gently for 15 minutes at RT.
Spin at 10000 rpm for 30 sec and remove supernatant.
Repeat the wash two times.
Incubate with 1 mL of lysis buffer + 1 mg/mL BSA for 30 minutes at RT.
Spin at 10000 rpm for 30 sec and remove supernatant.
Repeat the wash two times.
Resuspend beads with 1 mL of lysis buffer (without BSA).
Spin at 10000 rpm for 30 sec and remove supernatant.
Repeat the wash two times.
After the last wash resuspend beads in 500 uL lysis buffer and store at 4°C.

IP Using 10cm plate of cells:
Harvest plate into 15 mL conical tube.
Ice cells for 20 minutes.
Spin at 1500 x g for 5 minutes at 4°C.
Pour off supernatant.
Resuspend cells in 5 mL cold 1X PBS.
Spin at 1500 x g for 5 minutes at 4°C.
Repeat wash twice.
Lyse pellet with 1 mL of lysis buffer by pipetting.
Transfer lysate to a cold 1.5 mL eppendorf tube.
Rotate in cold room for 30 minutes.
Aliquot 50 uL of this unclarified lysate to run on a gel later if desired.
Spin 10 minutes at 14000 rpm and 4°C to clarify lysate.
Aliquot 50 uL of clarified lysate for a gel.
Transfer supernatant to a cold 1.5 mL eppendorf tube.
Add 125 uL of beads and antibody in proper dilution.
(2.5 uL anti-GFP, 6 uL of Pats1 antibodies)
Rotate for 3 hours in cold room.
Spin for 30 sec at 14000 rpm and 4°C.
Aliquot 50 uL for gel.
Wash with 1 mL of lysis buffer 7 times, aliquoting 50 uL of supernatant from each wash.
After the last wash, add 50 uL of boiled SDS sample buffer.
Flick to mix, and boil for 5 minutes to elute bound protein.
Spin at 14000 rpm for 30 sec at 4°C.
Save supernatant. Store at -20°C and boil for 5 minutes before loading onto a gel.

Lysis Buffer:
50 mM Tris, pH 7.4
150 mM NaCl
1% Triton X-100
100 mM EDTA, pH 8.0

1:100 of 0.1 M PMSF in 100% EtOH
1:1000 of 5 mg/mL leupeptin in dH_2O
1:1000 of 1.4 mg/mL pepstatinA in MeOH