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GFP-YIPF3 Immunoprecipitation V2

Forked from a private protocol

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Protocol status: Working

We use this protocol and it's working

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Abstract

This is a protocol for the immunoprecipitation of the Golgi protein YIPF3 that is GFP tagged, and probing for ATG8 protein interactions.

Troubleshooting

HEK293 YIPF4 KO Creation

- 1 Maintain HEK293 cells in Dulbecco' Modified Eagles Medium (DMEM) with 10% fetal bovine serum and optional 1x penicillin-streptomycin.
- 1.1 For YIPF4 knock-out, the following sgRNA sequences were designed and ordered within YIPF4 (5' ATCTCGCGGCGACTCCCAAC 3' / 5' CGGCCTATGCCCCCACTAAC 3'), and cloned into a pX459 vector to create pX459-gRNA-YIPF4-KO.
- 1.2 Transfect HEK293 cells with the pX459-gRNA-YIPF4-KO with Lipofectamine 3000, and select with 1.2 µg/mL of puromycin for 24-48 hours. Select monoclonal cells by limiting dilution or by cell sorting (SONY SH800S sorter) in 96 well plates.
- 1.3 Individual clones were subjected to immunoblotting with anti-YIPF4 (Sino Biological 202844-T46), and clones lacking the relevant protein were selected for further analysis by Sanger sequencing of the edited alleles.

HEK293 YIPF3/YIPF4 double KO creation

- 2 For YIPF3/YIPF4 double knock-out, the following sgRNA sequence was designed and ordered within YIPF3 (5' GCGTACAAGCTGAAGGCC 3'), and cloned into a pX459 vector to create pX459-gRNA-YIPF3-KO.
- 2.1 Transfect YIPF4 KO HEK293 cells with the pX459-gRNA-YIPF3-KO with Lipofectamine 3000, and select with 1.2 µg/mL of puromycin for 24-48 hours. Select monoclonal cells by limiting dilution or by cell sorting (SONY SH800S sorter) in 96 well plates.
- 2.2 Individual clones were subjected to immunoblotting with anti-YIPF3 (Invitrogen PA566621), and clones lacking the relevant protein were selected for further analysis by Sanger sequencing of the edited alleles.
- 2.3

IP

- 3 DKO (YIPF3/4) HEK293 cells were reconstituted with mCherry-YIPF4 (WT or LIR mutant) and GFP-YIPF3 (WT or LIR mutant) constructs and sorted for equal expression levels. Immunofluorescence was used to confirm proper localization of both YIPF3 and YIPF4
- 3.1 cells were plated on 10cm plates and grown to 70% confluency. Cells were left untreated or starved using AA withdrawal for 2 hours in the presence of BafA (100nm)



- 3.2 Cells were washed 2x with cold PBS then lysed in 0.8mL of NP-40 lysis buffer (100mM TRIS pH7.4, 150mM KCL, 0.1% NP-40, 0.5mM EDTA, 1x HALT (Roche) protease inhibitors, phos-STOP tabs)
- 3.3 1.5mg of protein from each sample was added to 15ul of washed GFP-TRAP beads and incubated for 2 hours while rotating at 4 degrees. Beads were washed 3 times with lysis buffer and eluted in 1x LDS loading dye at 94 degrees for 5 minutes
- 3.4
- 3.5