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Protocol status: Working
We use this protocol and it's working

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GFP-YIPF3 Immunoprecipitation V2

Forked from GFP-YIPF3 Immunoprecipitation

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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.

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HEK293 YIPF4 KO Creation

1 Maintain HEK293 cells in Dulbecco' Modifies 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' GCGTACAAGCTGAAGGCCC 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