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# 🌐 Gene editing of YIPF3, YIPF4, CALCOCO1, FIP200, and ATG7 in HEK293 and HeLa cells V2

Forked from a private protocol

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**We use this protocol and it's working**

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## Abstract

This protocol describes the creation of YIPF3, YIPF4, FIP200, CALCOCO1, and ATG7 knockout cell lines in HEK293 and HeLa cells using CRISPR-Cas9.

## Materials

Puromycin (Gold Biotechnology, P-600-100)

pX459 (Addgene #62988)

HEK293 (ATCC CRL-1573, RRID: CVCL\_0045 )

anti-YIPF4 (Sino Biological 202844-T46)

anti-ATG7 (Cell Signaling Technology, 8558S)

anti-CALCOCO1 (Abclonal A7987)

Dulbecco's MEM (DMEM), high glucose, pyruvate (Gibco / Invitrogen, 11995)

## Troubleshooting

## Cell line maintenance

- 1 Maintain HEK293 and/or HeLa cells in Dulbecco's Modified Eagle's Medium (DMEM) with 10% fetal bovine serum and optional 1% penicillin-streptomycin.

## Targeted knock-out specific genes including YIPF3, YIPF4, CALCOCO1, FIP200, or ATG7: targeting vector creation

- 2 For YIPF4 knock-out, the following sgRNA sequences were designed and ordered (5' ATCTCGCGGCGACTCCCAAC 3' / 5' CGGCCTATGCCCCCACTAAC 3' ), and cloned into a pX459 vector to create pX459-gRNA-YIPF4-KO.

For CALCOCO1 knock-out, the following sgRNA sequences were designed and ordered (5' AAGTTGACTCCACCACGGGA 3' / 5' CTAAGCCGGGCACCATCCCG 3'), and cloned into a pX459 vector to create pX459-gRNA-CALCOCO1-KO.

For ATG7 knock-out, the following sgRNA sequence was designed and ordered (5' ATCCAAGGCACTACTAAAAG 3'), and cloned into a pX459 vector to create pX459-gRNA-ATG7-KO.

For FIP200 knock-out, the following sgRNA sequence was designed and ordered (5' ACTACGATTGACACTAAAGA 3'), and cloned into a pX459 vector to create pX459-gRNA-FIP200-KO.

For YIPF3 knock-out, the following sgRNA sequences were designed and ordered (5' CCATTTCGGGCGCCGCCCGC 3' / 5' GGCGGCGCCCGAAATGGAGC 3'), and cloned into a pX459 vector to create pX459-gRNA-YIPF3-KO.

Sequence validate by Sanger sequencing.

## CRISPR editing and confirmation

- 3 Transfect HEK293 and/or HeLa cells with the pX459-gRNA-YIPF4-KO, pX459-gRNA-CALCOCO1-KO, pX459-gRNA-ATG7-KO, gRNA-FIP200-KO, or 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.
- 3.1 Individual clones were subjected to immunoblotting with anti-YIPF4 (Sino Biological 202844-T46), anti-ATG7 (Cell Signaling Technology, 8558S), anti-FIP200 (Cell Signaling Technology, 12436), anti-YIPF3 (Invitrogen PA566621), or anti-CALCOCO1 Rabbit mAb



antibody (Abclonal A7987) and clones lacking the relevant protein were selected for further analysis by Sanger sequencing of the edited alleles.