Supplementary Material

Suppl. Fig 1. IPIP27 sequence alignment

IPIP27 homologues from the indicated organisms are shown. The N-terminal PH domain is indicated with a black box. The conserved F and H residues required for OCRL1 and Inpp5b binding are highlighted by red boxes. The EI motif required for efficient binding to OCRL1 and Inpp5b is shown in a pink box. The OCRL1/Inpp5b binding site is contained within a predicted helical region, underlined in black.

Suppl. Fig 2. Mapping of the OCRL1/Inpp5b binding region in IPIP27A and B

GFP-tagged truncations of IPIP27A and B were expressed in HeLa cells and tested for interaction with insect cell expressed OCRL1 and Inpp5b. Bound proteins were eluted and Western blotted with anti-GFP antibodies.

Suppl. Fig 3. Controls for GFP-IPIP27 expression

A. Analysis of levels of transfected IPIPs. HeLa cells were untransfected (UT) or transfected with 1-25 ng DNA encoding GFP-IPIP27A or B and analyzed by Western blotting with the indicated antibodies. The bottom row indicates endogenous IPIP27, while the row above shows the GFP-transfected protein detected with anti-IPIP27 antibody. The asterisks indicate non-specific proteins that cross-react with the IPIP27 antibodies. B. hTERT-RPE1cells were transfected with Myc-tagged IPIP27A, labelled with antibodies to EEA1 and analyzed by immunofluorescence microscopy. Arrowheads indicate endosomes positive for Myc-IPIP27A and EEA1. C, HeLa cells transfected with GFP-IPIP27A or B were labelled with antibodies to Golgin-97 and analyzed by immunofluorescence microscopy. Bars, 10 μm.

Suppl. Fig 4. IPIP27 localization

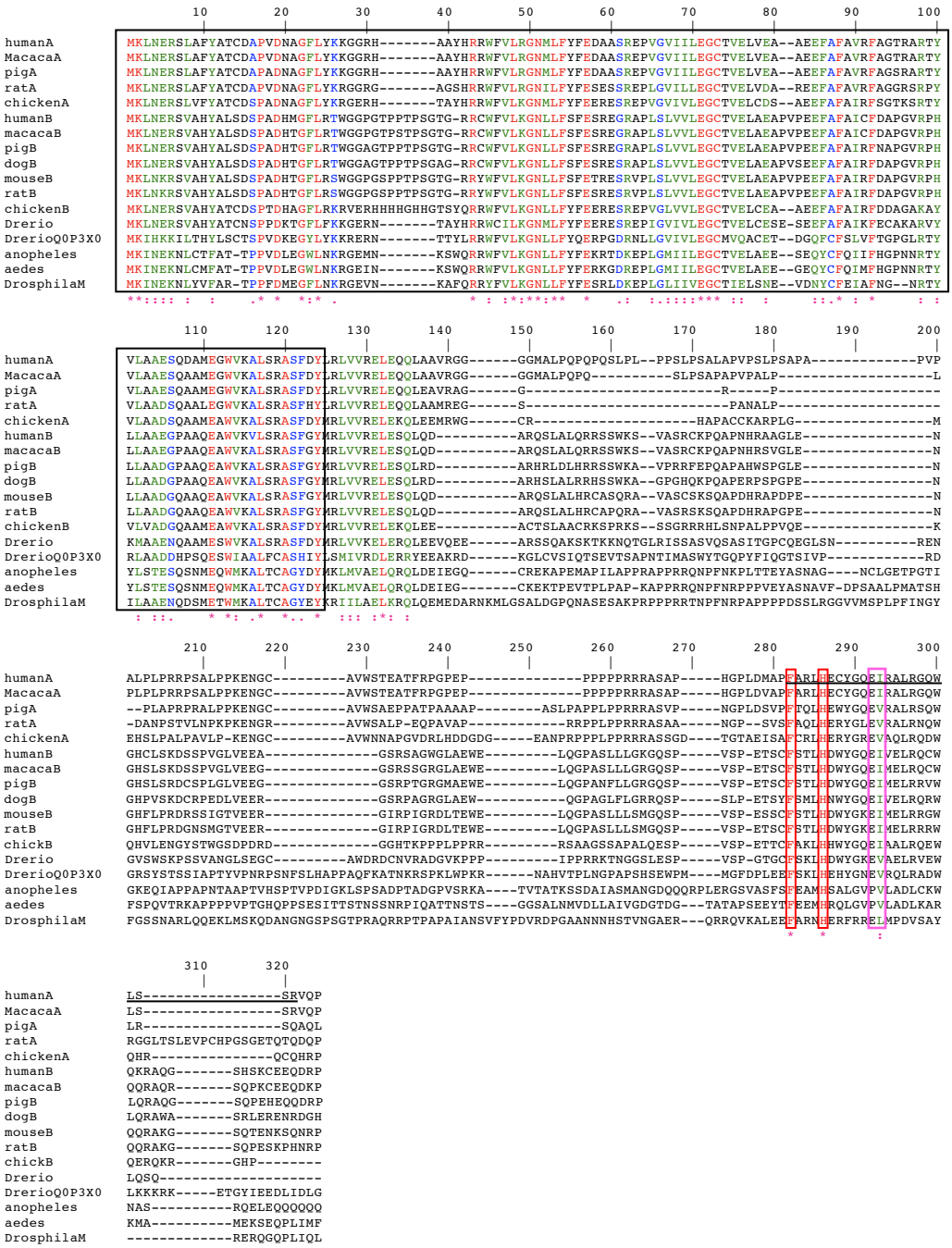
A. Immunofluorescence microscopy of hTERT-RPE1 cells co-expressing low levels of GFP-tagged IPIP27A or B (green) and MApple-Rab11 (red), or transfected with GFP-IPIP27 only and labelled with an antibody to CD63 (red). Bar, 10 µm. B. Nocodazole-treated hTERT-RPE1 cells expressing low levels of GFP-tagged IPIP27A or B (green) were labelled with antibodies to TGN46 or transferrin receptor (TfR) (red), or co-transfected with MApple-Rab11 (red) as indicated. Bar, 5 µm.

Suppl. Fig 5. Targeting of IPIP27 and OCRL1

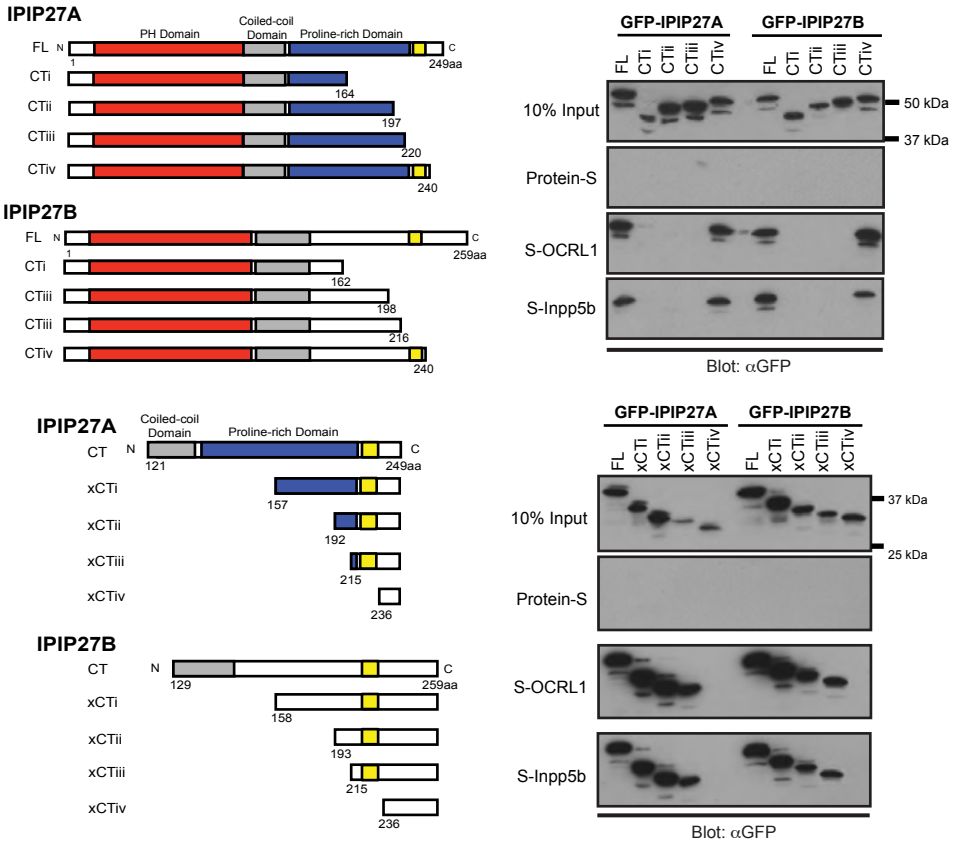
A. Immunofluorescence microscopy of hTERT-RPE1 cells co-expressing low levels of GFP-tagged IPIP27A (green) and mCherry alone or mCherry-OCRL1 (red) and labelled with anti-transferrin receptor antibody (blue). Bar, 10 µm. B. Control, IPIP27A and/or B RNAi-treated HeLa cells were transfected with GFP-OCRL1 (green) and labelled with antibodies to EEA1 (red) and TGN46 (blue). Bar, 10 µm.

Suppl. Fig 6. Co-overexpression of IPIP27 with 5-phosphatase deficient OCRL1 (D499A) alters endosomal morphology and receptor distribution

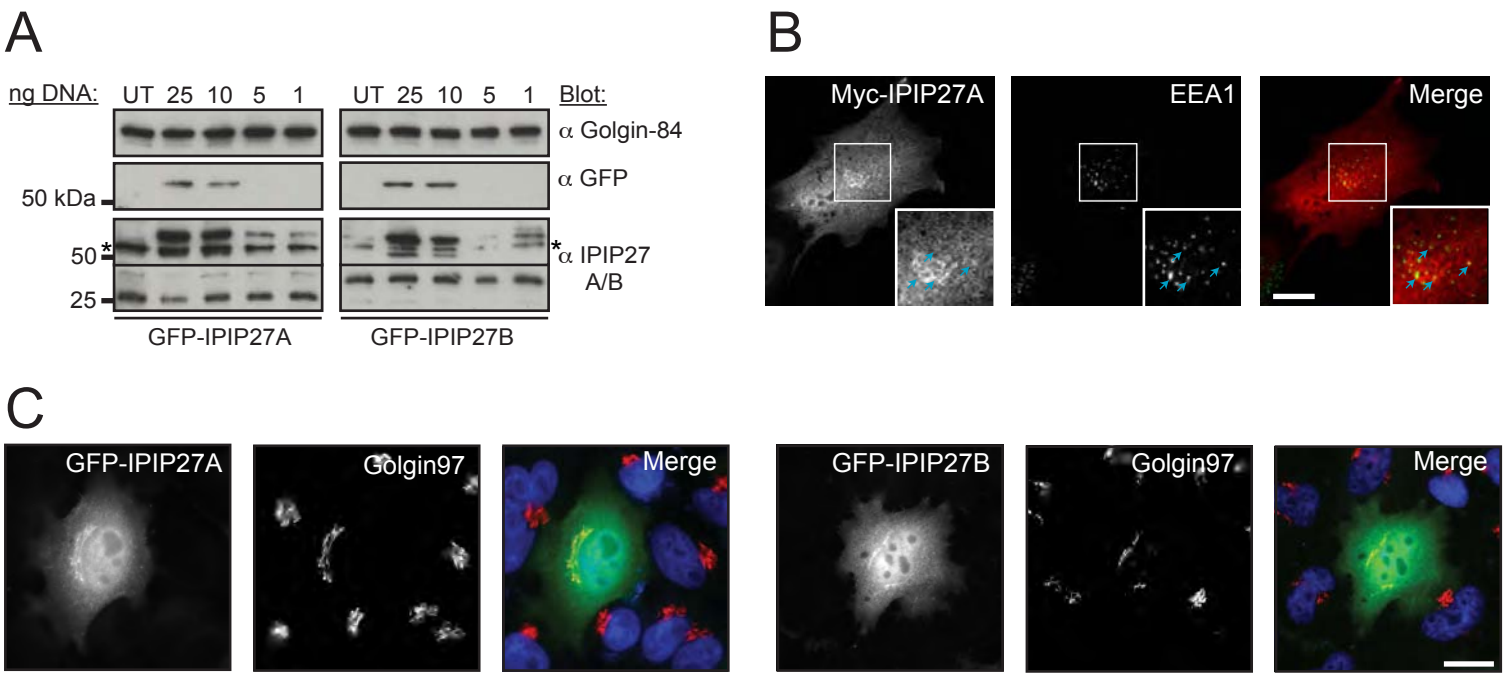
CD8-CIMPR HeLaM cells expressing GFP-OCRL1 D499A (green) without or with co-expression of mCherry wild-type (WT) IPIP27A or the F224A mutant (red) were labelled with antibodies to EEA1 (blue) (A), transferrin receptor (TfR, blue) (B) or CD8 (CD8-CIMPR, blue) (C). Bar, 10 µm.



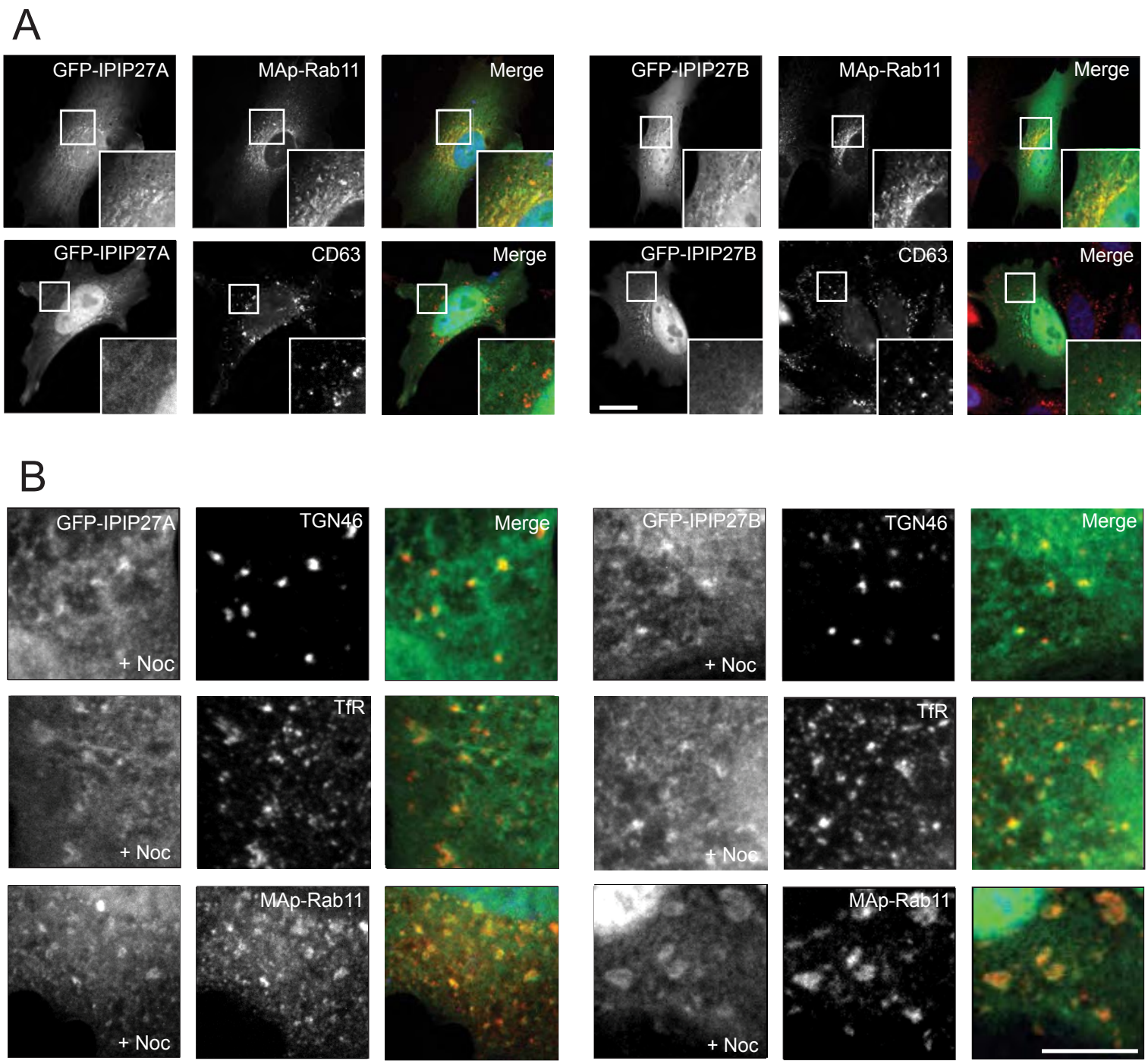
Suppl Fig 1



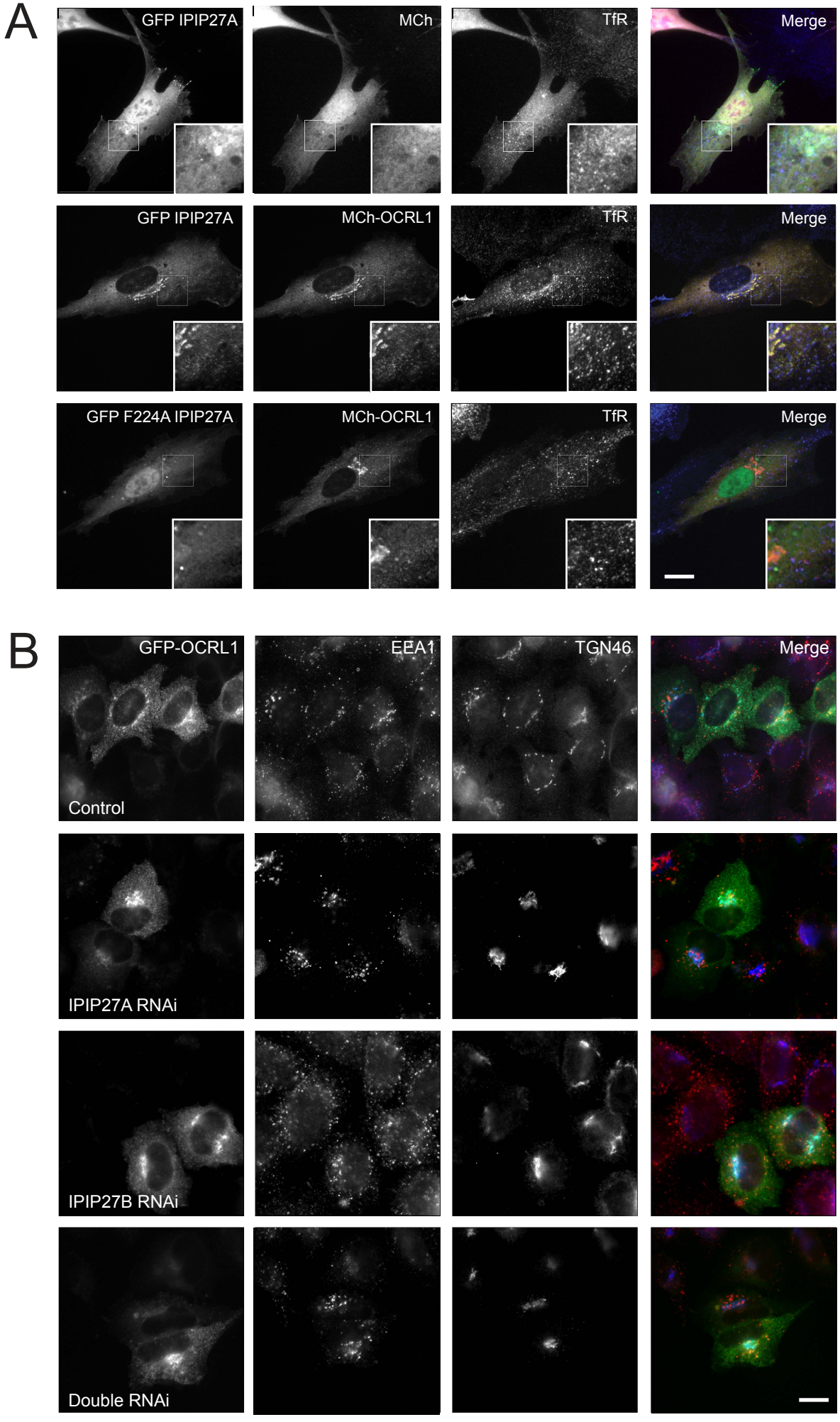
Suppl Figure 2


A
ng DNA:
UT 25 10 5 1
UT 25 10 5 1
Blot:
α Golgin-84
α GFP
50 kDa
50
25
*
*
α IPIP27 A/B
GFP-IPIP27A
GFP-IPIP27B
B
Myc-IPIP27A
EEA1
Merge
C
GFP-IPIP27A
Golgin97
Merge
GFP-IPIP27B
Golgin97
Merge

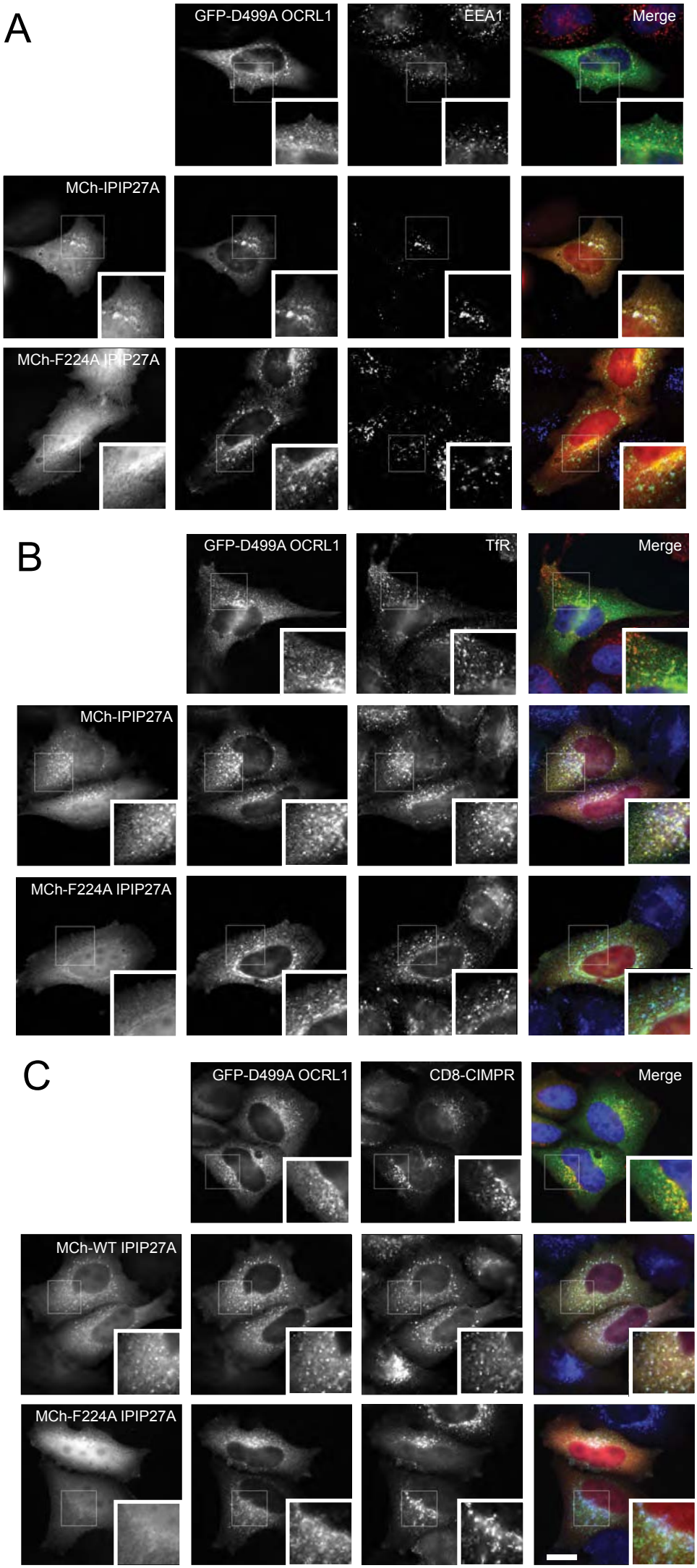
Suppl Fig 3


A
GFP-IPIP27A
MAp-Rab11
Merge
GFP-IPIP27B
MAp-Rab11
Merge
GFP-IPIP27A
CD63
Merge
GFP-IPIP27B
CD63
Merge
B
GFP-IPIP27A
+ Noc
TGN46
Merge
GFP-IPIP27B
+ Noc
TGN46
Merge
+ Noc
TfR
+ Noc
TfR
+ Noc
MAp-Rab11
+ Noc
MAp-Rab11

Suppl Fig 4


A
GFP IPIP27A
MCh
TfR
Merge
GFP IPIP27A
MCh-OCRL1
TfR
Merge
GFP F224A IPIP27A
MCh-OCRL1
TfR
Merge
B
GFP-OCRL1
EEA1
TGN46
Merge
Control
IPIP27A RNAi
IPIP27B RNAi
Double RNAi

Suppl Fig 5


A
GFP-D499A OCRL1
EEA1
Merge
MCh-IPIP27A
MCh-F224A IPIP27A
B
GFP-D499A OCRL1
TfR
Merge
MCh-IPIP27A
MCh-F224A IPIP27A
C
GFP-D499A OCRL1
CD8-CIMPR
Merge
MCh-WT IPIP27A
MCh-F224A IPIP27A

Suppl Fig 6