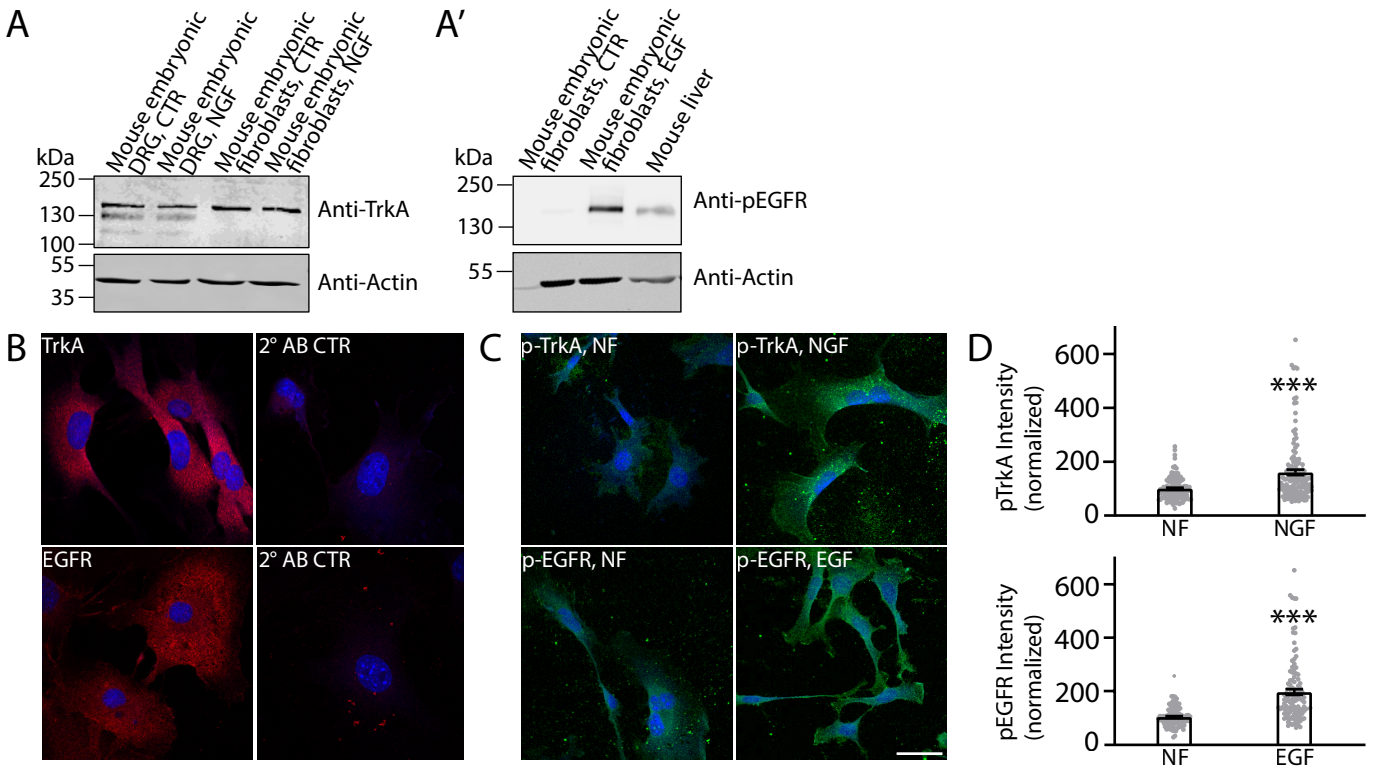


Fig. S1. Trk and EGFR expression and functionality in MEFs. (**A, A'**) Expression of TrkA and EGFR in MEFs using Western Blot. (**B**) Expression of TrkA and EGFR in MEFS using immunochemistry. (**C**) Images of pTrkA, and pEGFR immunostaining in cultured MEFs in "no factor" (NF) untreated conditions and following treatment with respective ligand (100ng/ml NGF or EGF) for 20 minutes; scale bar= 40 µm. (**D**) Quantification of pTrkA and pEGFR signal in MEFS in NF and ligand stimulated conditions, normalized to NF. PTrkA NF vs NGF: df=254, $p<0.0001$; pEGFR NF vs EGF: df=108, $p<0.0001$. Significance was determined by Student's t-test, n=30 images per condition in three independent experiments; error=SEM, ***$p<0.001$.

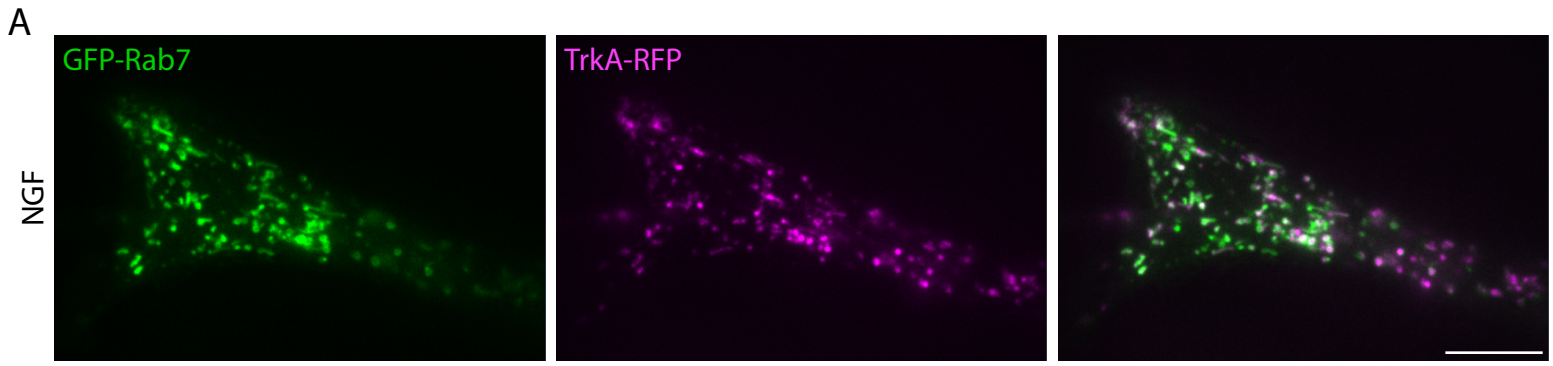
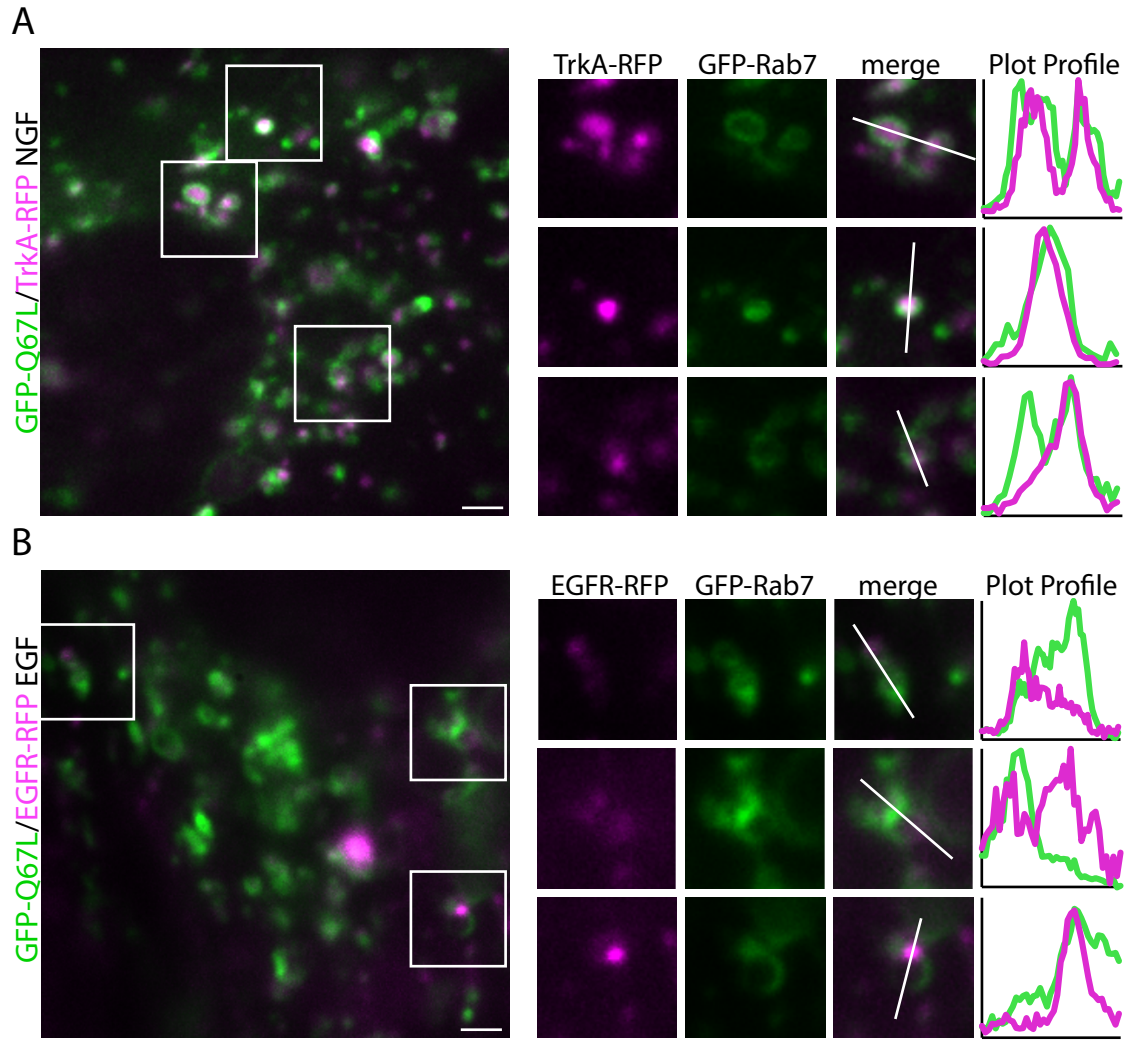
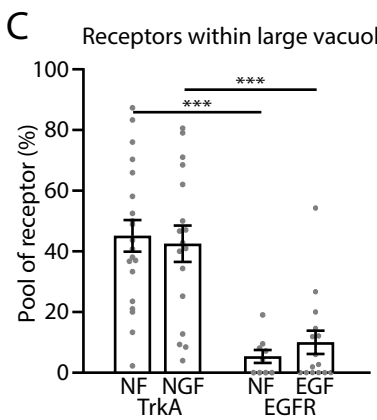
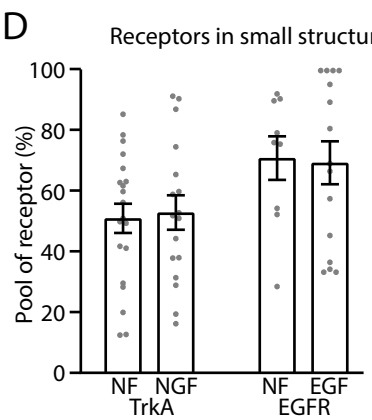


Fig. S2. TrkA-RFP expression in MEFs in live-TIRF. (**A,**) Representative TIRF microscopy images of MEFs co-transfected with GFP-tagged Rab7 and RFP-tagged TrkA in the presence of NGF from the same culture as images in Figure 2G; scale bar= 10µm.


A
GFP-Q67L/TrkA-RFP NGF
TrkA-RFP
GFP-Rab7
merge
Plot Profile
B
GFP-Q67L/EGFR-RFP EGF
EGFR-RFP
GFP-Rab7
merge
Plot Profile


C
Receptors within large vacuoles
Pool of receptor (%)
100
80
60
40
20
0
***
***
NF NGF
TrkA
NF EGF
EGFR


D
Receptors in small structures
Pool of receptor (%)
100
80
60
40
20
0
NF NGF
TrkA
NF EGF
EGFR

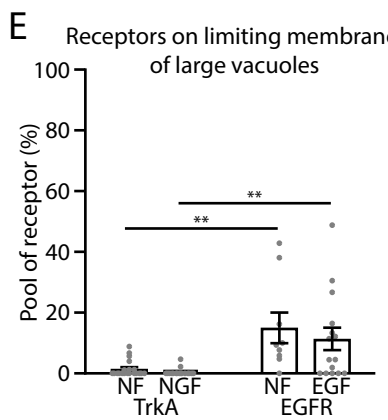

E
Receptors on limiting membrane
of large vacuoles
Pool of receptor (%)
100
80
60
40
20
0
**
**
NF NGF
TrkA
NF EGF
EGFR

Fig. S3. Q67L induces TrkA localization within large vacuolar structures. (**A, B**) Representative TIRF microscopy images of MEFs co-transfected with GFP-tagged Rab7 Q67L and RFP-tagged receptor (TrkA or EGFR), in the presence or absence of its respective ligand (100ng/ml NGF, EGF). Line histograms show receptors being localized within Rab7 vacuoles, on the rim of Rab7 vacuoles and on small Rab7 structures; scale bar= 2µm. (**C**) Quantification of proportion of receptors (TrkA, EGFR) localized within large vacuolar Rab7-Q67L structures. TrkA NF vs EGFR NF: $p<0.0001$; TrkA NGF vs EGFR EGF: $p=0.0001$. (**D**) Quantification of proportion of receptors (TrkA, EGFR) localized to small structures. (**E**) Quantification of proportion of receptors localized on the limiting membrane of large vacuolar Rab7 structures. TrkA NF vs EGFR NF: $p=0.0022$; TrkA NGF vs EGFR EGF: $p=0.0058$. Significance was determined by one-way ANOVA with post hoc Sidak's; n= 7 videos per condition in three independent experiments; error= SEM, $^{**}p<0.01$, $^{***}p<0.001$.

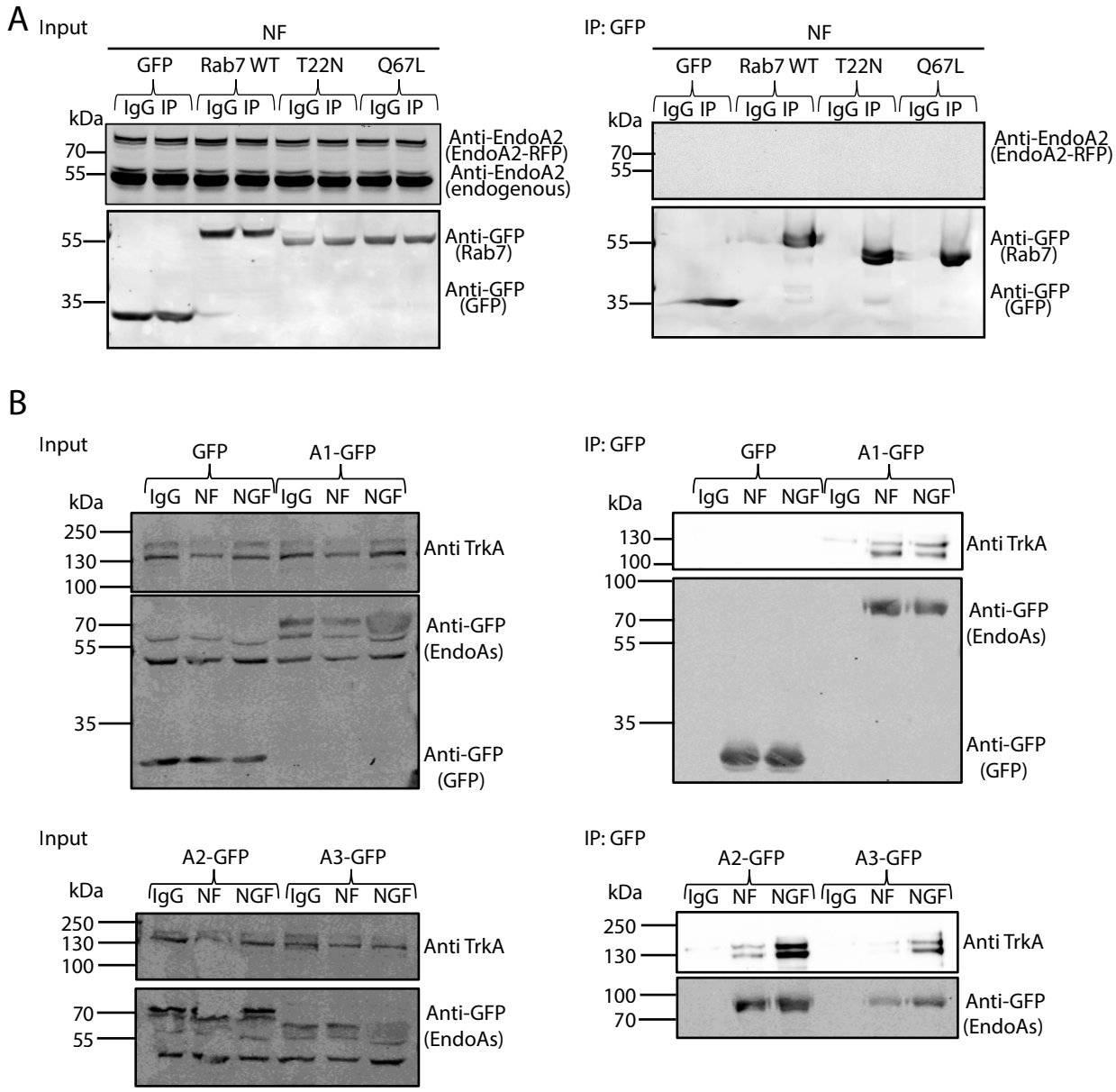


Fig. S4. EndophilinA2 does not associate with late Rab7 in non-stimulated conditions. (**A**) Input is shown on the left, IP on the right. GFP-conjugated beads (or IgG control beads) were used to pull down GFP-Rab7 (WT, T22N, Q67L) showing no interaction with EndophilinA2-RFP in non-stimulated Hek293 cells. (**B**) GFP-conjugated beads (or IgG control beads) were used to pull down GFP, EndophilinA1, A2, or A3-GFP in with TrkA-RFP co-transfected Hek293 cells (Input is IP on the right) in the presence or absence 100 ng/ml NGF.

Suppl. 5

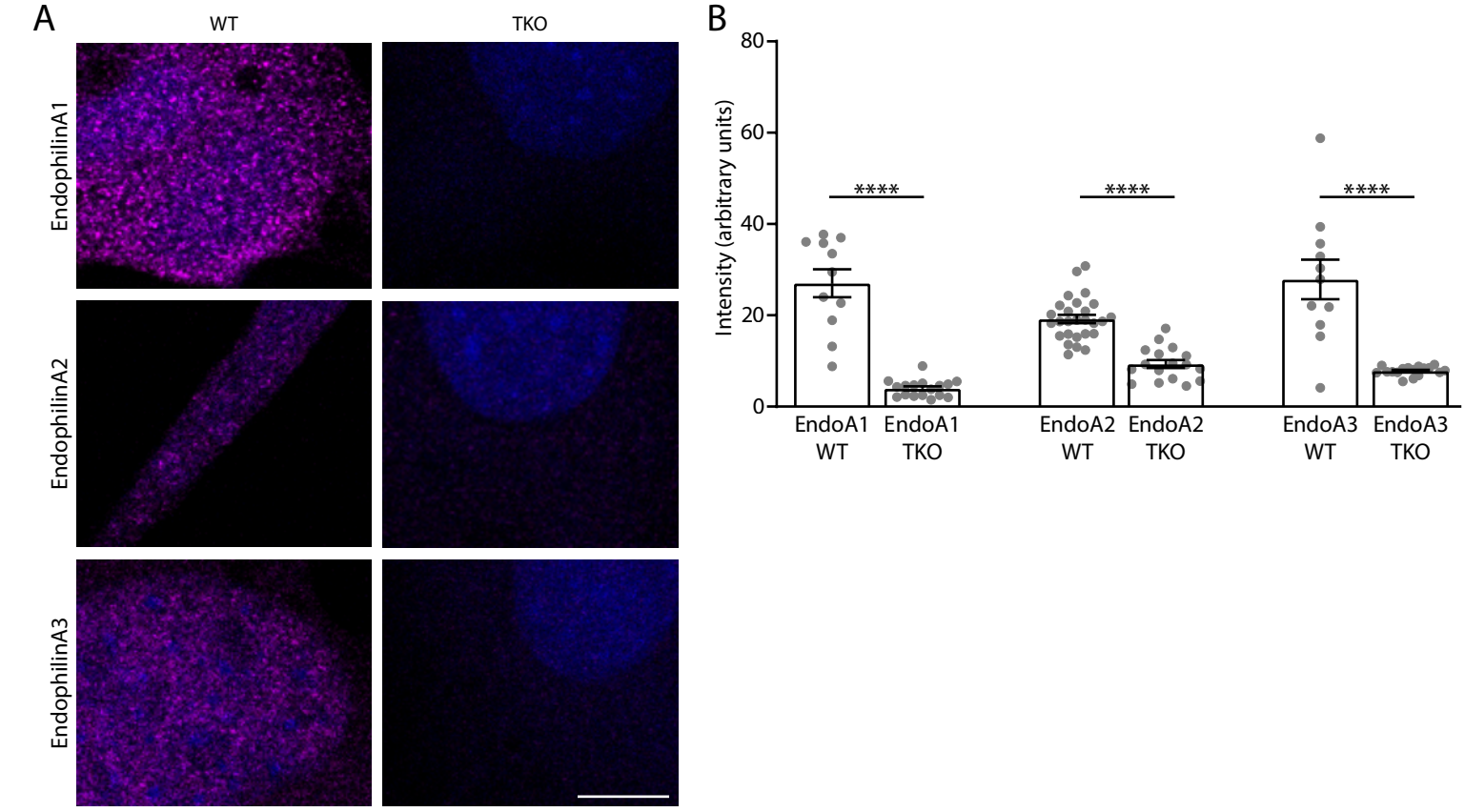


Fig. S5. EndophilinA TKO MEFs do not stain for EndophilinAs. (**A**) WT and EndophilinA TKO MEFs stained against EndophilinA1,2, and 3, scale bar= 10 µm. (**B**) Quantification of staining intensity in WT and TKO MEFs. Significance was determined by student's t-test, ****p<0.0001.

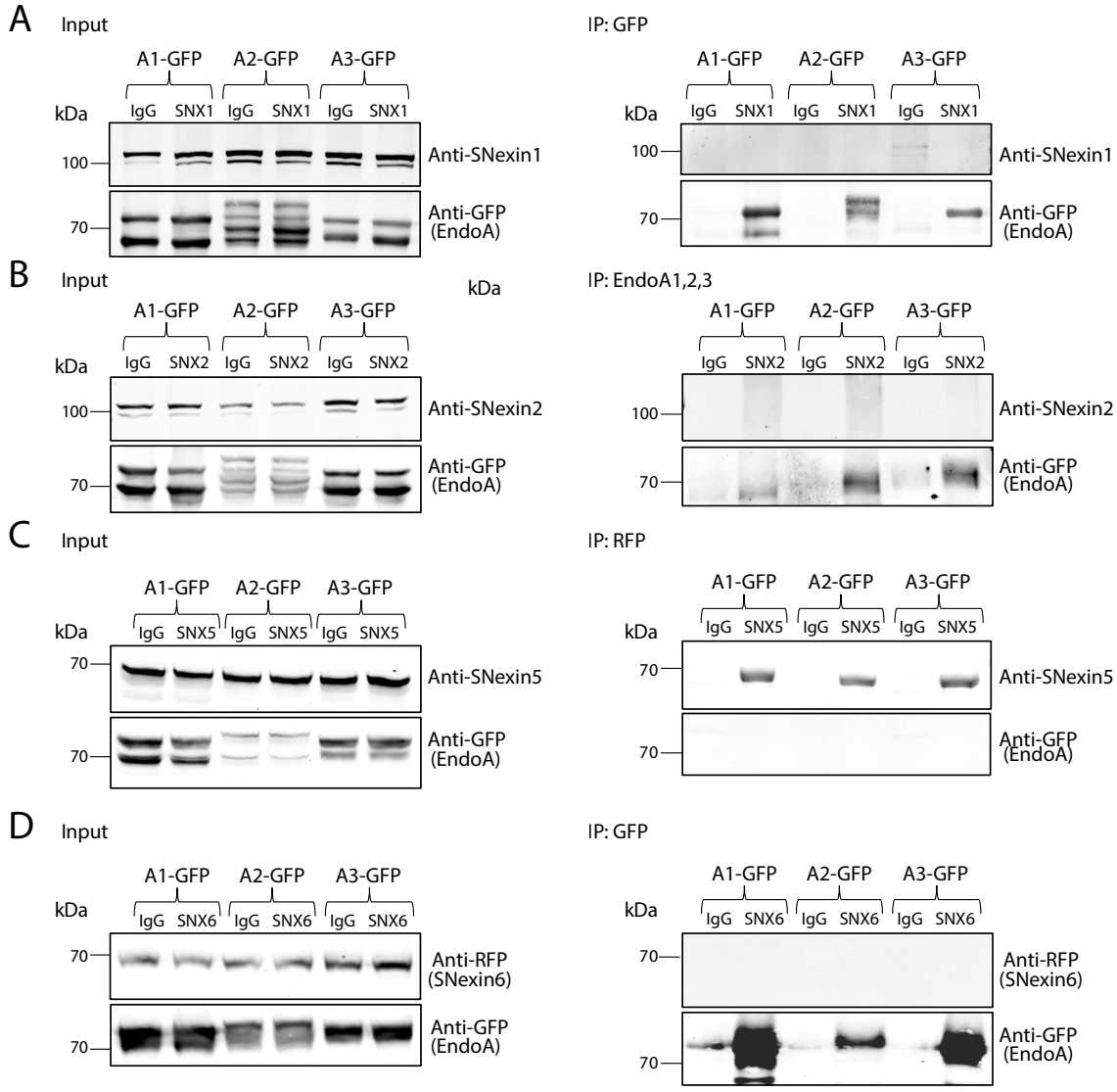


Fig. S6. EndophilinAs do not bind Snxs of retromer complex co-transfected in HEK293 cells. (**A**) EndophilinAs do not co-immunoprecipitate with Snx1. (**B**) EndophilinAs do not co-immunoprecipitate with Snx2. (**C**) EndophilinAs do not co-immunoprecipitate with Snx5. (**D**) EndophilinAs do not co-immunoprecipitate with Snx6.

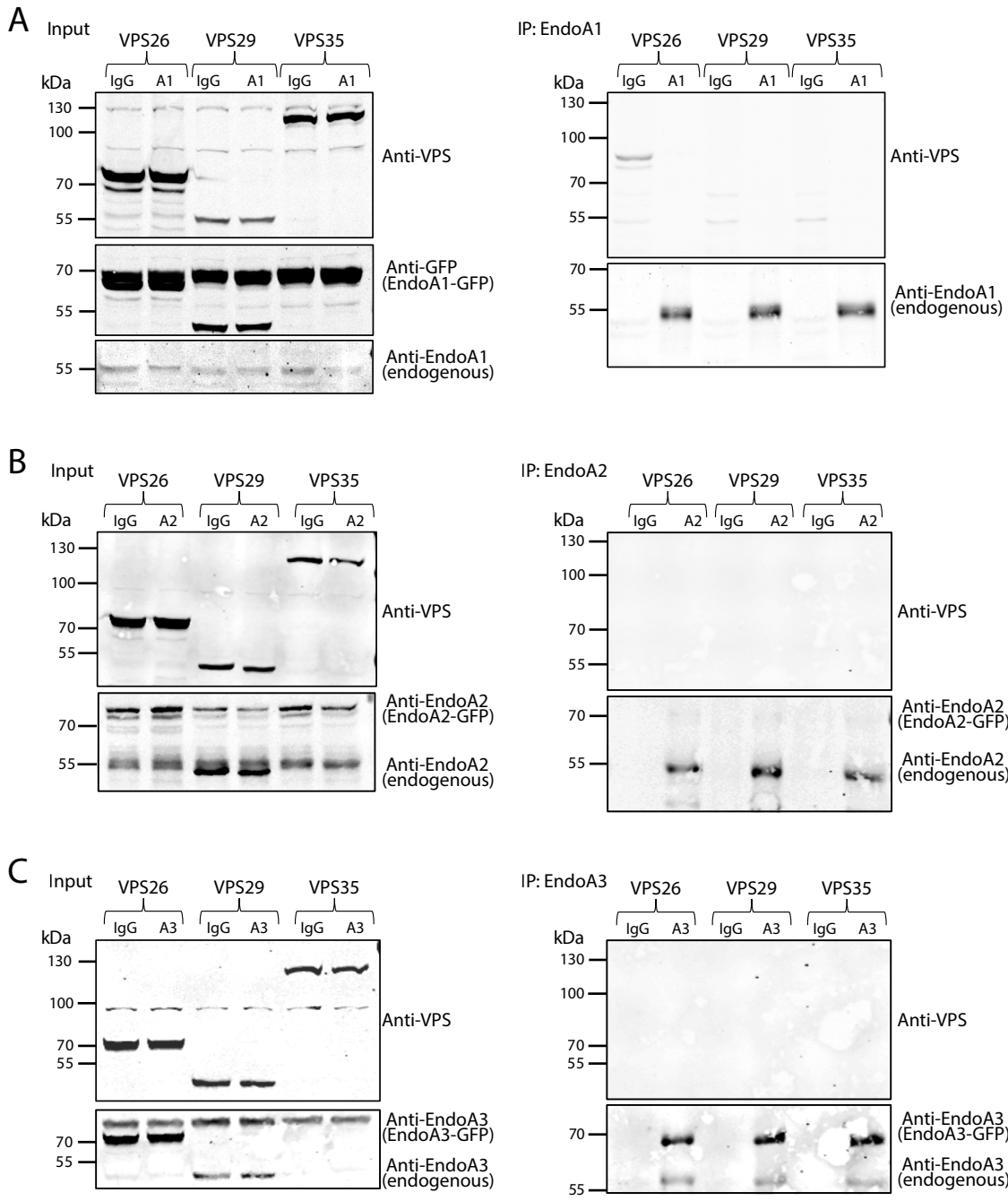


Fig. S7. EndophilinAs do not bind the cargo recognition unit of retromer complex co-transfected in HEK293 cells. (**A**) EndophilinAs do not co-immunoprecipitate with VPS26. (**B**) EndophilinAs do not co-immunoprecipitate with VPS29. (**C**) EndophilinAs do not co-immunoprecipitate with VPS35.

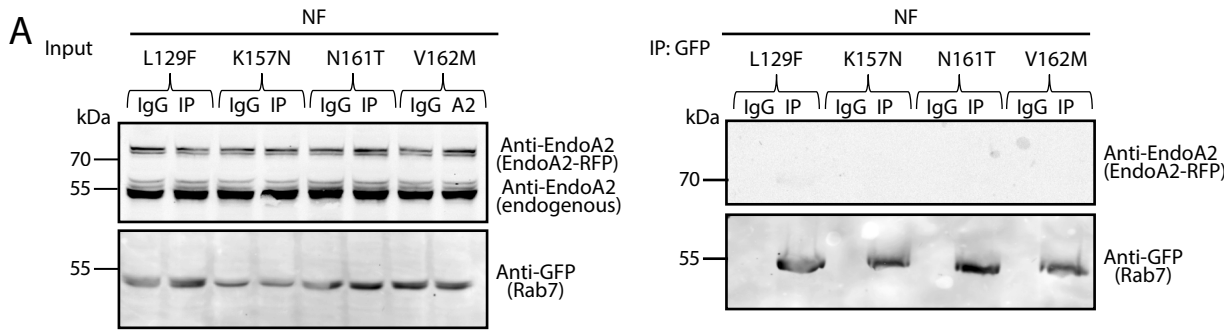
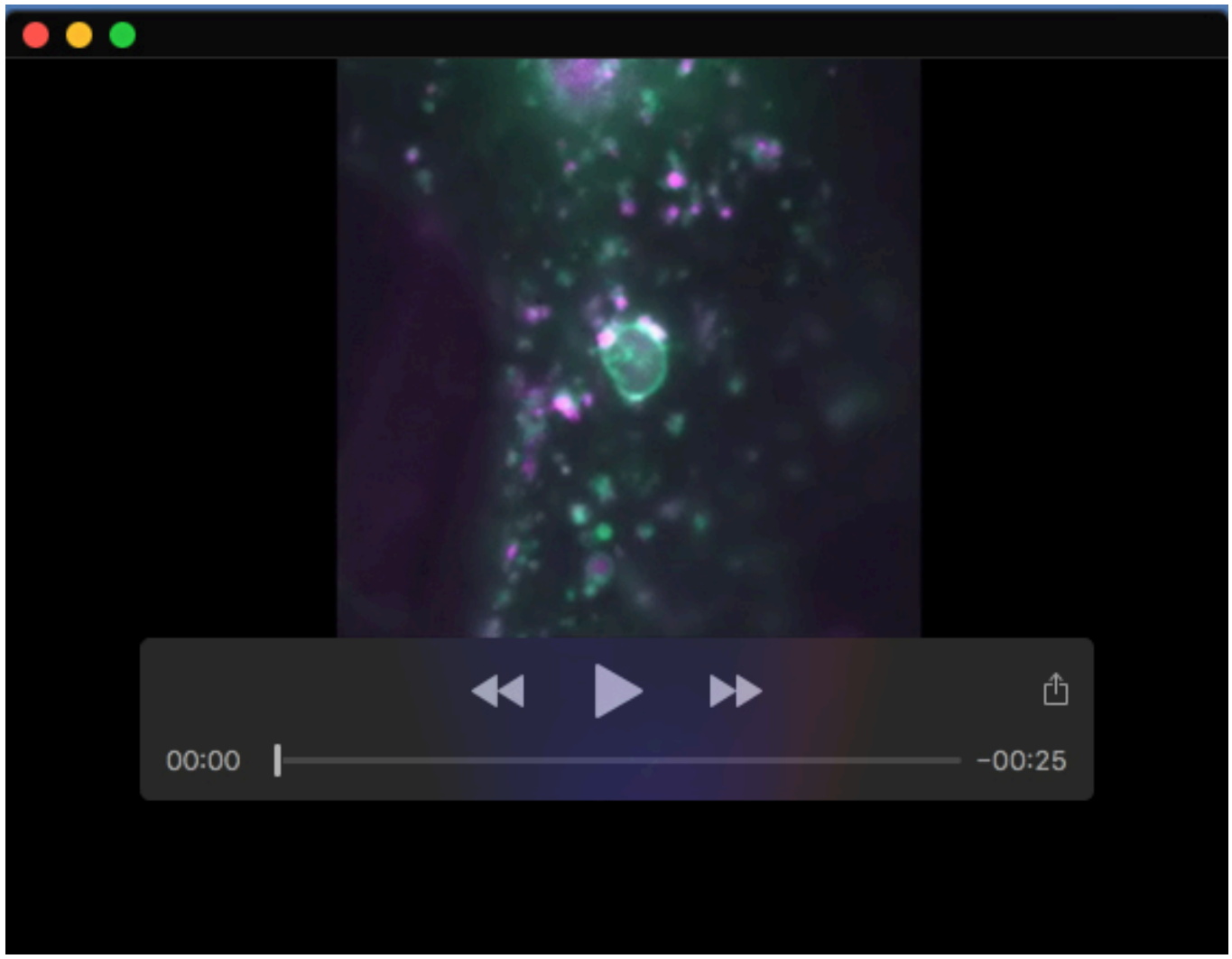
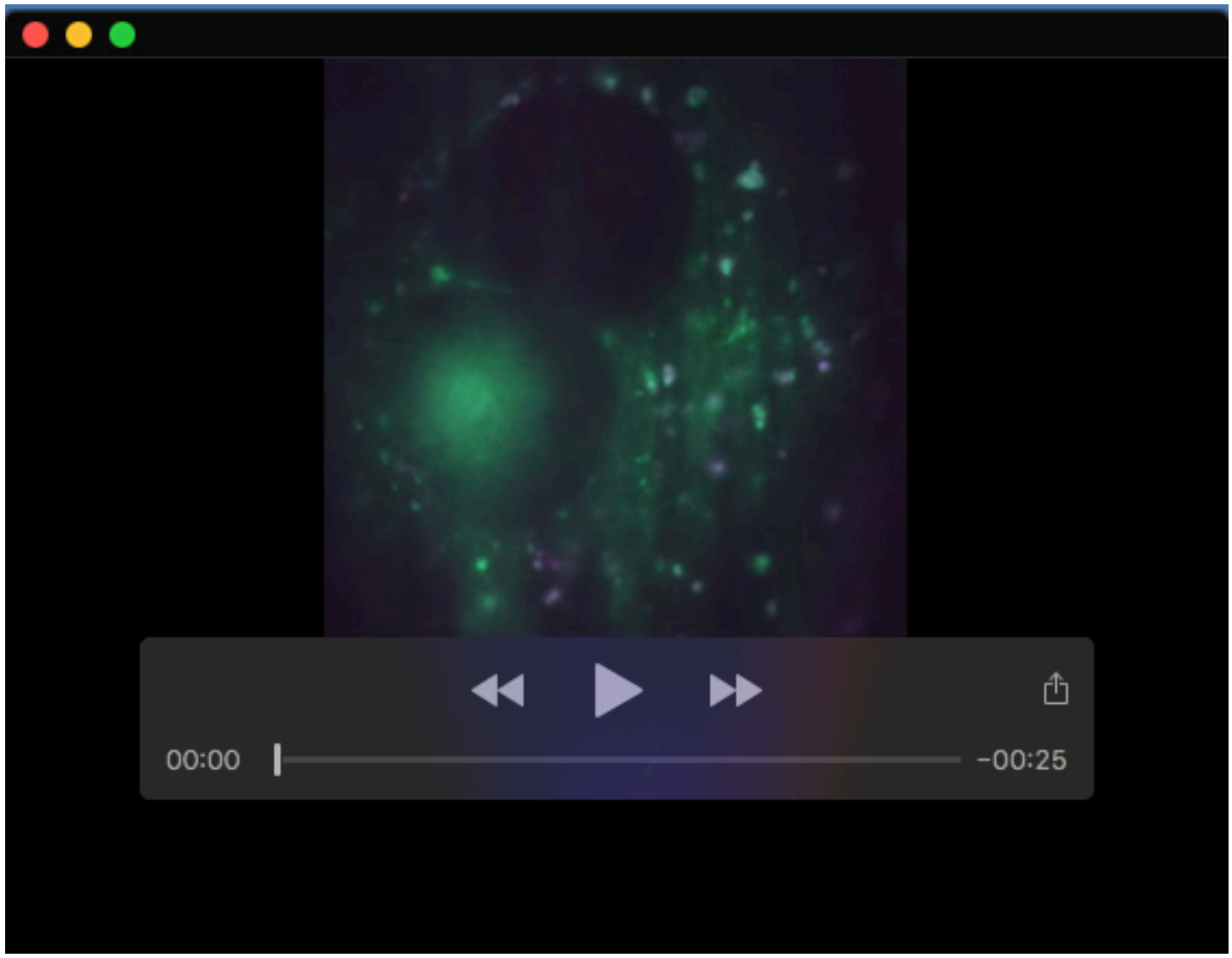


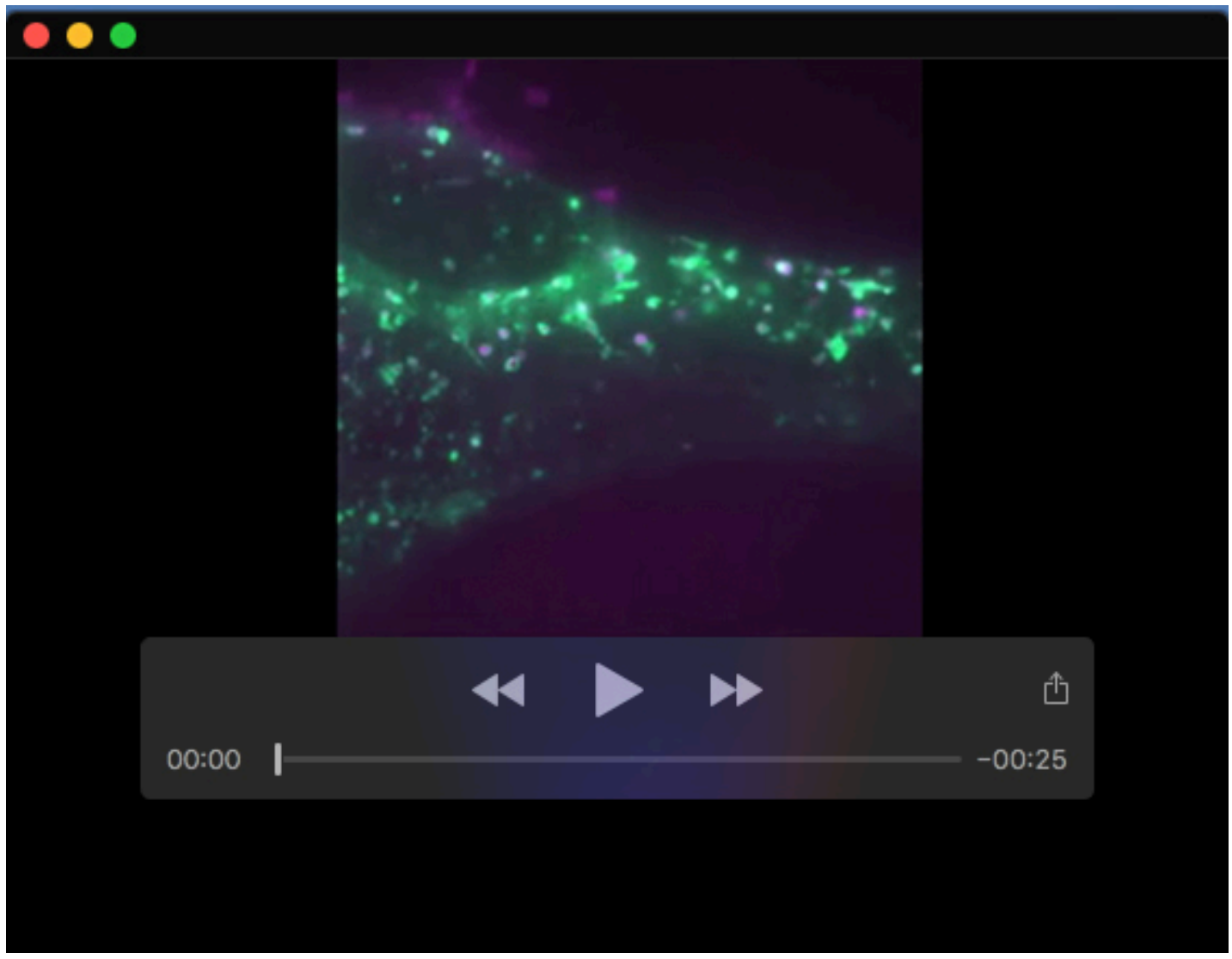
Fig. S8. CMT2B-Rab7 mutations do not bind EndophilinA2 in non-stimulated HEK cells. (**A**) GFP-conjugated beads (or IgG control beads) were used to pull down GFP-Rab7 WT and the CMT2B mutants L129F, K157N, N161T, V162M with EndophilinA2-RFP in non-stimulated Hek293 cells. Input is shown on the left, IP on the right.



Movie 1. Intraluminal Rab7 is bouncing in Rab7 vacuole. (**A**) Live-TIRF acquisition of MEF co-transfected with GFP-Rab7 and TrkA-RFP.



Movie 2. No Rab7 tubulation without NGF. (**A**) Live-TIRF acquisition of MEF co-transfected with GFP-Rab7 and TrkA-RFP in the absence of NGF shows a lack of tubulating events.



Movie 3. Rab7 tubulations in response to NGF. (**A**) Live-TIRF acquisition of MEF co-transfected with GFP-Rab7 and TrkA-RFP stimulated with NGF shows tubulating events.

Table S1. Original data for results in Fig. 1H.

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