SUPPLEMENTAL FIGURE LEGENDS

Figure S1. Low doses of Mn induce the degradation of GPP130 in HeLa and AF-5 cells.

A. HeLa cells were treated with 100 μM $MnCl_2$ for 4 h or left untreated. Cells were then co-immunostained to detect giantin and GPP130. Scale bar, 10 μm.

B. Immunoblot analyses showing that treatment of AF-5 cells with 100 μM $MnCl_2$ induces degradation of GPP130. This degradation was blocked when cells were treated with $MnCl_2$ and chloroquine (100 μM) simultaneously.

Figure S2. GPP130 is present inside the lumen of Rab5 Q79L-induced giant MVBs while EEA1 is present on the limiting membrane.

HeLa cells, transfected with Rab5 Q79L-GFP and treated with 500 μM $MnCl_2$ for 4 h, were co-immunostained to detect GPP130 and EEA1. Scale bar, 10 μm, inset 2.5X.

Figure S3. Dominant negative Dynamin II does not inhibit the degradation of GPP130.

A. HeLa cells were transfected with HA-tagged Dyn II WT or Dyn II K44A and 24 h post transfection were either treated with 500 μM $MnCl_2$ for 8 h or left untreated. The cells were then fixed and stained using anti-GPP130 and anti-HA antibodies. Scale bar, 10 μm.

B. Quantitation of mean GPP130 fluorescence per cell with GPP130 levels in the absence of Mn as 100% (mean ± SE, n > 12 cells at each time point, $p > 0.05$).

C. HeLa cells transfected with Dyn II WT or Dyn II K44A were loaded with fluorescent EGF for 15 min and subsequently fixed, stained and imaged to detect HA and GFP. Asterisks indicate transfected cells in the K44A panels. These did not endocytose EGF whereas neighboring nontransfected cells did. Scale bar, 10 μm.

D. Quantitation of the mean EGF fluorescence per cell with levels in Dyn II WT expressing cells normalized to 100% (mean ± SE, n > 20 cells for each construct, $p < 0.05$).

Figure S4. Dominant negative Rab5 does not affect Mn-induced degradation of GPP130.

A. HeLa cells were transfected with GFP-tagged Rab5 WT or Rab5 S34N and 24 h post transfection they were treated with 500 μM $MnCl_2$ for 8 h or left untreated. Cells were then fixed, stained and imaged to detect GPP130 and GFP. Scale bar, 10 μm.

B. Quantitation of mean GPP130 fluorescence per cell with levels in the absence of Mn expressed as 100% (mean ± SE, n > 12 cells at each time point, $p > 0.05$).
C. HeLa cells were transfected with Rab5 WT or Rab5 S34N and 24 h post transfection the EGF degradation assay was performed. Asterisks indicate cells expressing Rab5 S34N, which yielded lower, but still evident, levels due to Rab5 S34N being cytoplasmic and hence extracted during the saponin permeabilization step of the EGF degradation assay. Scale bar, 10 μm.
D. Quantitation of the mean EGF fluorescence per cell with levels at time 0 set to 100% (mean ± SE, n > 12 cells per construct per time point, $p < 0.05$).

Figure S5. Dominant negative Rab7 blocks the degradation of fluorescently labeled EGF.
***A*.** HeLa cells were transfected with Rab7 WT and Rab7 T22N. Twenty four hours post transfection the EGF-degradation assay was performed. Scale bar, 10 μm.
B. Quantitation of the mean EGF fluorescence per cell with levels at time 0 normalized to 100% (mean ± SE, n > 12 cells per construct per time point, $p < 0.05$).

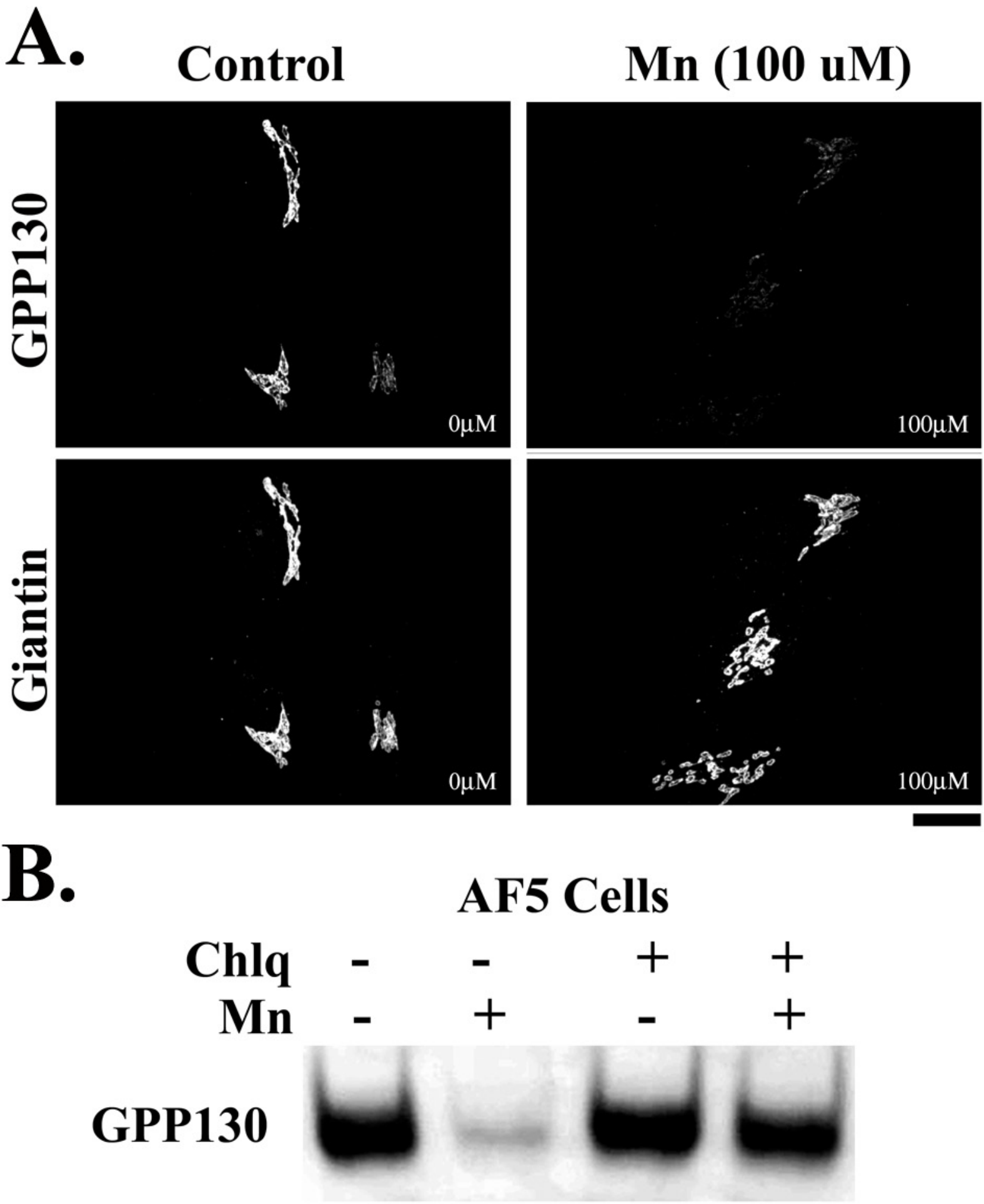
Figure S6. Cytoplasmic domain of GPP130 is not required for its internalization into Rab5 Q79L-induced giant MVBs.

HeLa cells were cotransfected with Rab5 Q79LΔGFP and GPP130Δ2-11-GFP, treated with Mn for 4 h and were processed to detect GFP and EEA1. Scale bar, 10 μm; inset 2.5X.

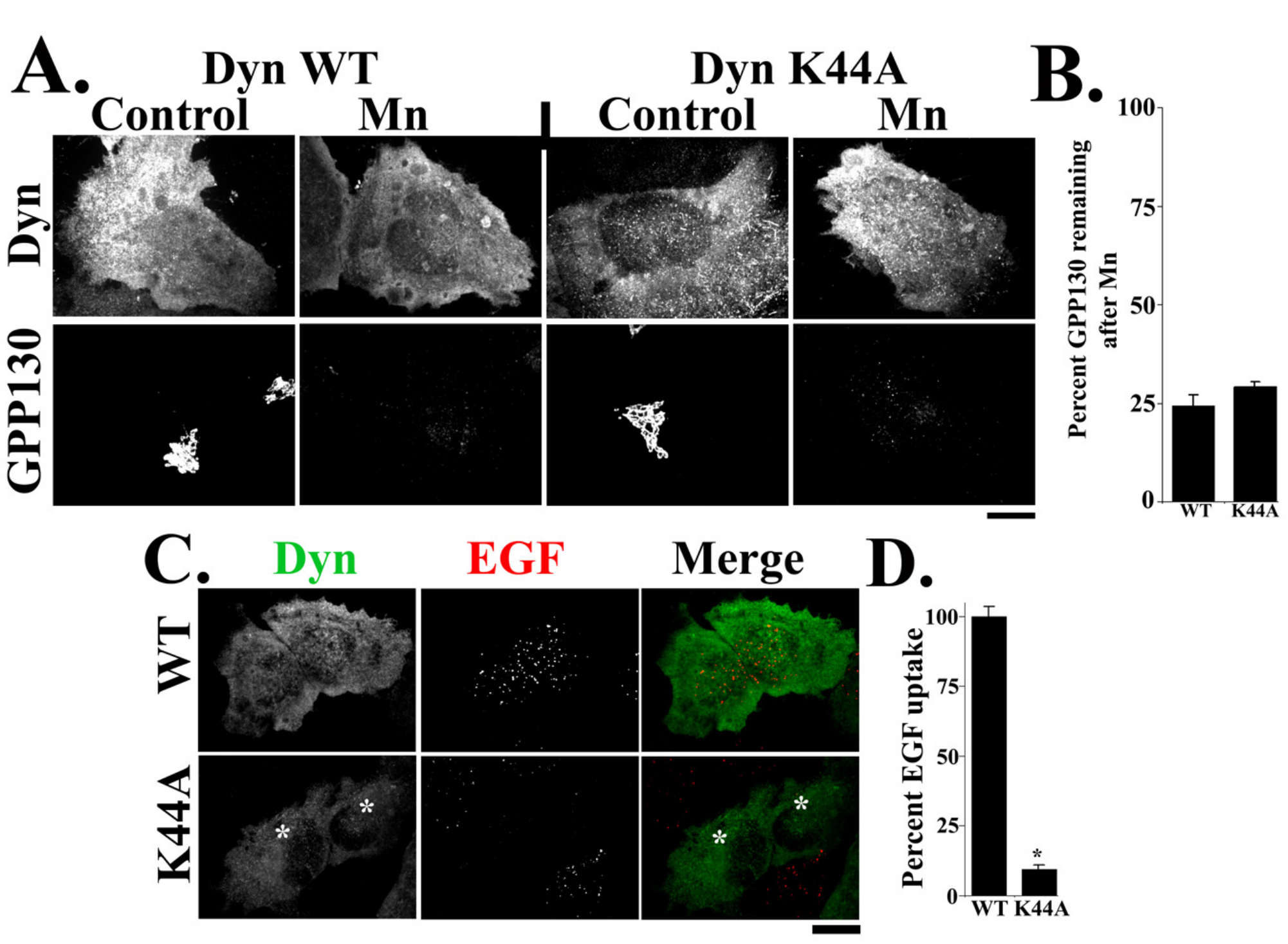
Figure S7. GFP-tagged GPP130 constructs with deletions in the stem domain are targeted to the Golgi.
HeLa cells were transfected with the indicated stem-deleted constructs, treated with cycloheximide for 4 h and processed to detect GPP130 and GFP. Scale bar, 10 μm.

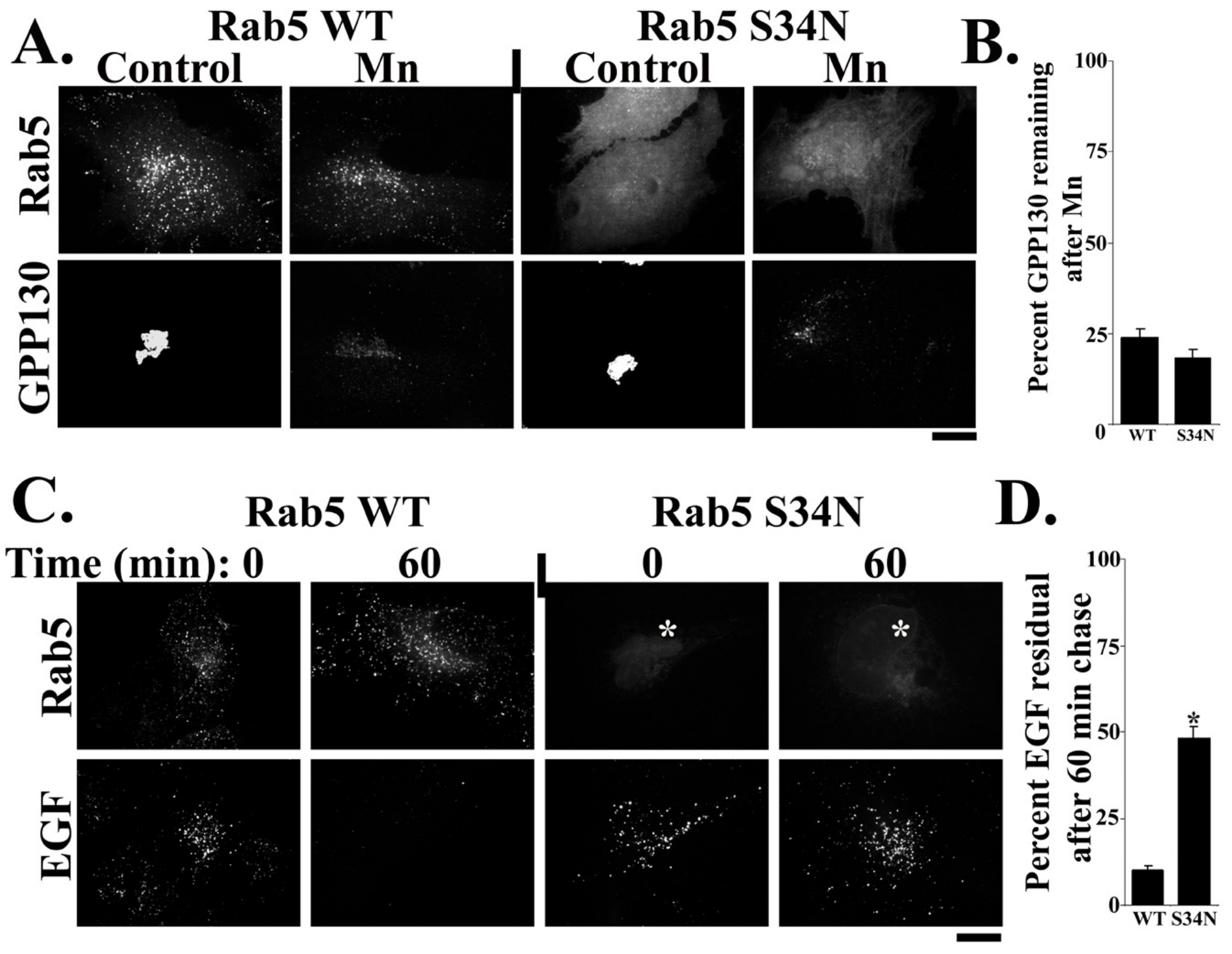
Figure S8. **GPP130$_{\Delta 88\text{-}247}$-GFP remains Golgi localized after 8 h of treatment with Mn.**
HeLa cells were siRNA-transfected to knock down endogenous GPP130. After two days the cells were re-transfected with an RNAi-immune GPP130$_{1\text{-}247}$-GFP and GPP130$_{\Delta 88\text{-}247}$-GFP. Twenty four hours later cells were treated with 500 μM $MnCl_2$ for 8 h or left untreated. Cells were then fixed and imaged to detect endogenous GPP130 (using anti-GPP130 mAb against

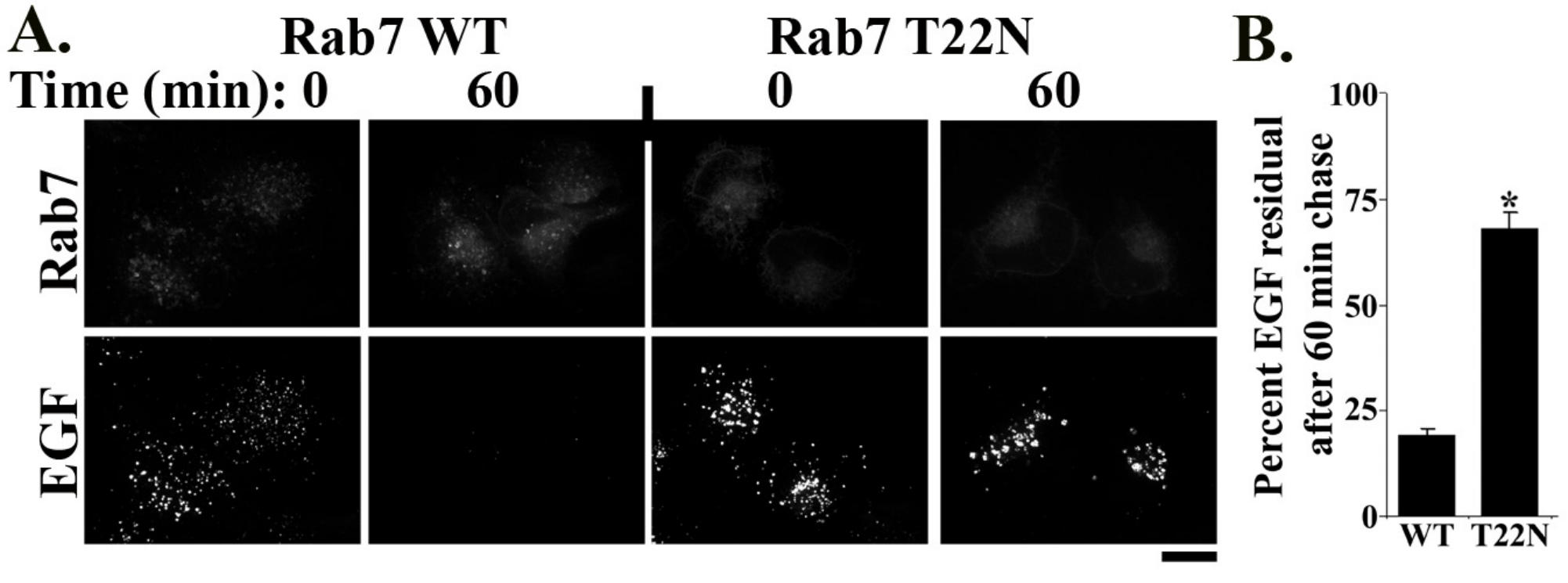
acidic domain) and GFP. The cells shown in the panels in this figure had no detectable endogenous GPP130. Scale bar, 10 μm.

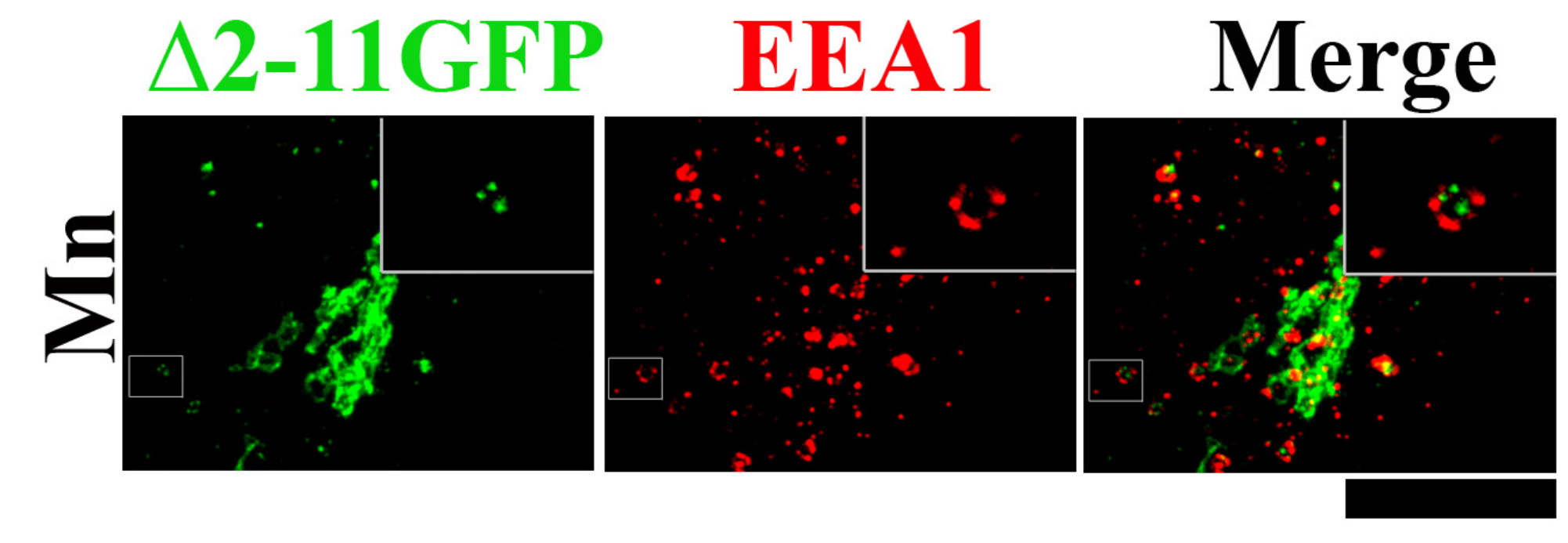

A.
Control
Mn (100 uM)
GPP130
Giantin
0μM
100μM
0μM
100μM
B.
AF5 Cells
Chlq - - + +
Mn - + - +
GPP130

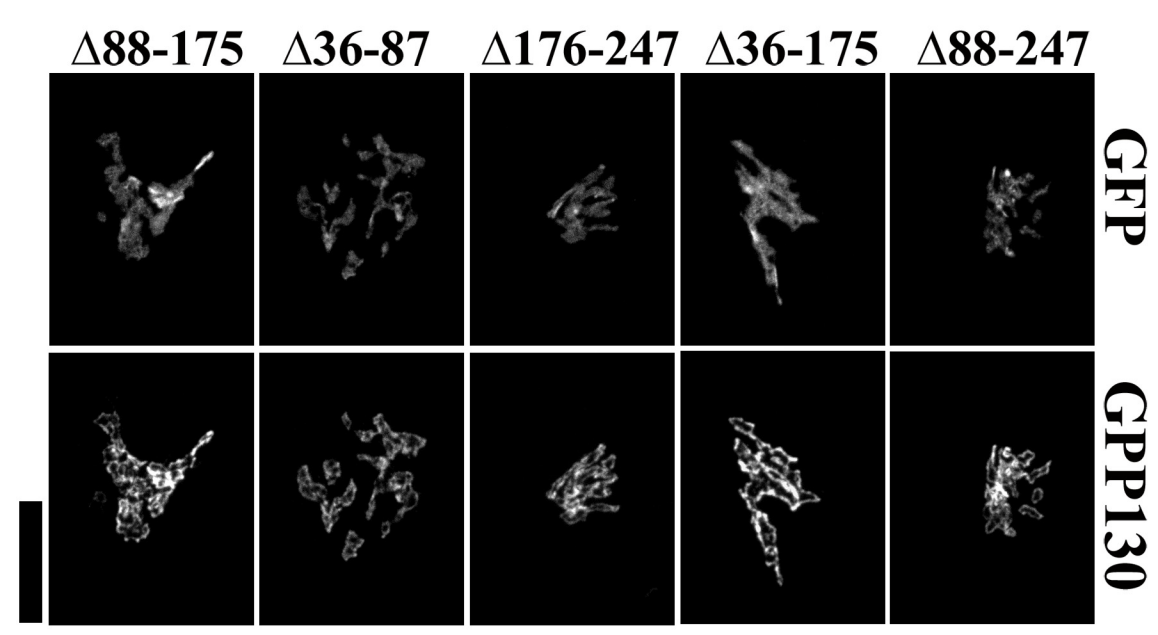
Rab5 Q79L/ GPP130/ EEA1
Rab5
GPP130
EEA1
GPP130
Rab5
EEA1
Merge

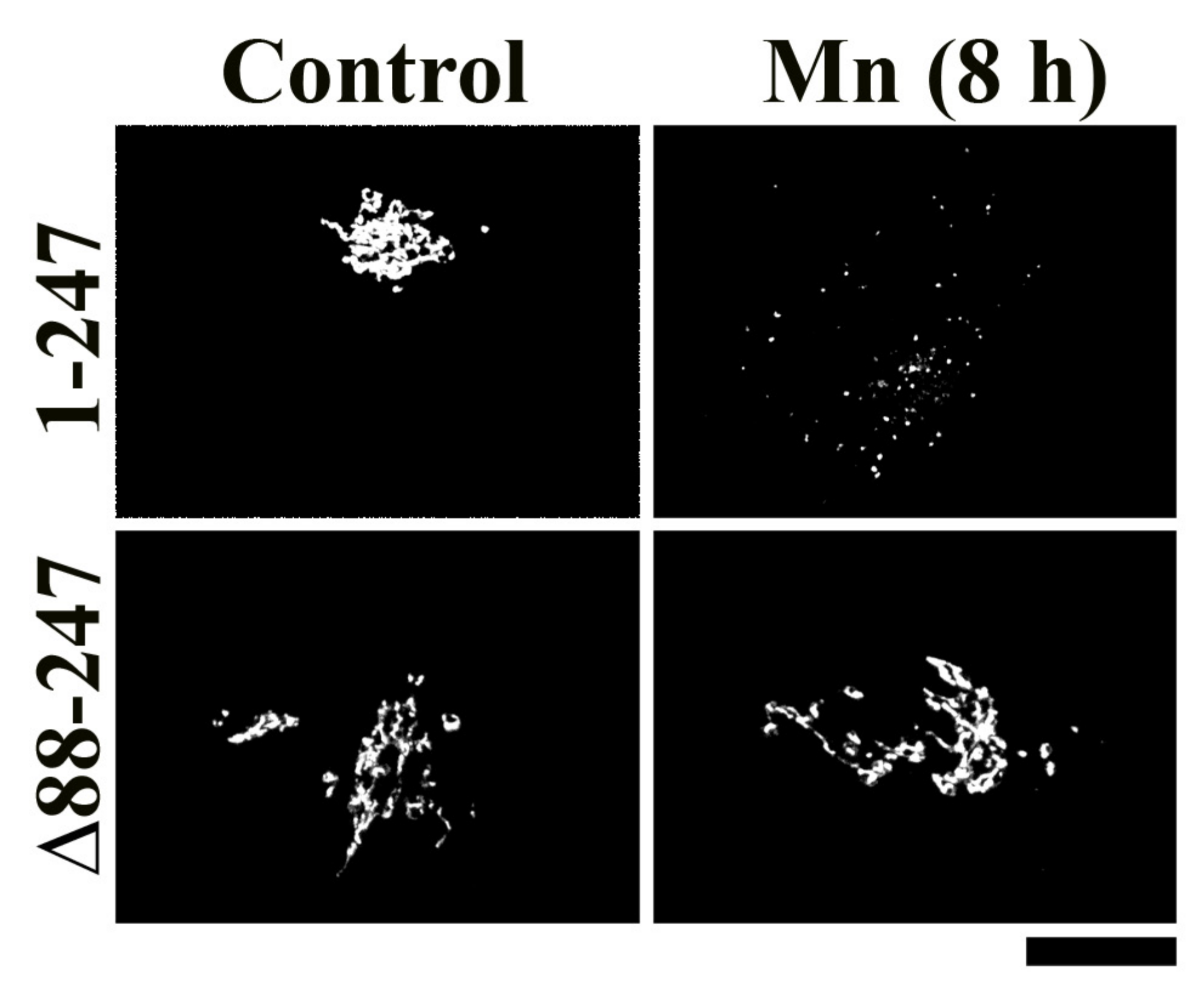

A.
Dyn WT
Dyn K44A
Control
Mn
Control
Mn
Dyn
GPP130
B.
Percent GPP130 remaining after Mn
100
75
50
25
0
WT
K44A
C.
Dyn
EGF
Merge
WT
K44A
D.
Percent EGF uptake
100
75
50
25
0
WT
K44A


A.
Rab5 WT
Rab5 S34N
Control
Mn
Control
Mn
Rab5
GPP130
B.
Percent GPP130 remaining after Mn
100
75
50
25
0
WT
S34N
C.
Rab5 WT
Rab5 S34N
Time (min): 0
60
0
60
Rab5
EGF
*
*
D.
Percent EGF residual after 60 min chase
100
75
50
25
0
*
WT
S34N


A.
Rab7 WT
Rab7 T22N
Time (min): 0
60
0
60
Rab7
EGF
B.
Percent EGF residual after 60 min chase
100
75
50
25
0
*
WT
T22N


Δ2-11GFP
EEA1
Merge
Mn


Δ88-175
Δ36-87
Δ176-247
Δ36-175
Δ88-247
GFP
GPP130


Control
Mn (8 h)
1-247
Δ88-247