Supplemental Data

Requirement of Myosin Vb/Rab11a/Rab11-FIP2 Complex in Cholesterol-Regulated Translocation of Niemann-Pick C1 Like 1 Protein to the Cell Surface

Supplemental Figures:

Figure S1. The integrity and dynamics of microfilament are essential for the endocytosis of NPC1L1.

A. Diagram showing the procedure to treat the cells. Briefly, the cells were incubated in cholesterol-depleting medium for 60 min. Then the cells were treated with the indicated drugs for 30 min to disturb cytoskeletons. Cholesterol/CDX was directly added to the medium, and the cells were replenished with cholesterol for different time durations and subsequently fixed and analyzed.

B. CRL-1601/NPC1L1-EGFP cells were treated as shown in (A). At the indicated time points, the cells were fixed and examined by confocal microscopy. Bar, 10 μm.

Figure S2. Expression of the C-terminal tails (CTs) of myosin Va, Vb and Vc analyzed by western blotting.

NPC1L1-RFP was co-expressed with EGFP-tagged myosin Vb-CTs in CRL-1601 cells. 48 hours after transfection, the cells were harvested and western blotting was carried out with indicated antibodies.

Figure S3. Deleting the Rab11a-binding site abolishes the dominant-negative effect of myosin Vb-CT.

A. NPC1L1-RFP was co-expressed with EGFP-tagged myosin Vb-CT or Rab11a-binding site-deleted myosin Vb-CT (Vb-CTΔRBD) individually in CRL-1601 cells. 48 hours after transfection, the cells were either depleted of cholesterol for 60 min (Chol-Dep), or replenished with cholesterol for 60 min (Chol-Rep) following cholesterol depletion. Then the cells were fixed and examined by confocal microscopy. Ctr, control; Bar, 10 μm.

B. Quantification of the intracellular localization of NPC1L1-RFP shown in (A). Error bars represent standard deviations.

Figure S4. Expression of wild type, the GTP-locked (S17V) or the GDP-locked (T22N) forms of Rab8a does not affect the exocytosis of NPC1L1.

A. CRL-1601 cells were co-expressed with NPC1L1-RFP and the EGFP tagged wild type

Rab8a and its two mutants. 48 hours after transfection, the cells were either depleted of cholesterol for 60 min (Chol-Dep), or replenished with cholesterol for 60 min (Chol-Rep) following cholesterol depletion. Then the cells were fixed and examined by confocal microscopy. Ctr, control; Bar, 10 μm.

B. Quantification of the intracellular localization of NPC1L1-RFP shown in (A). Error bars represent standard deviations.

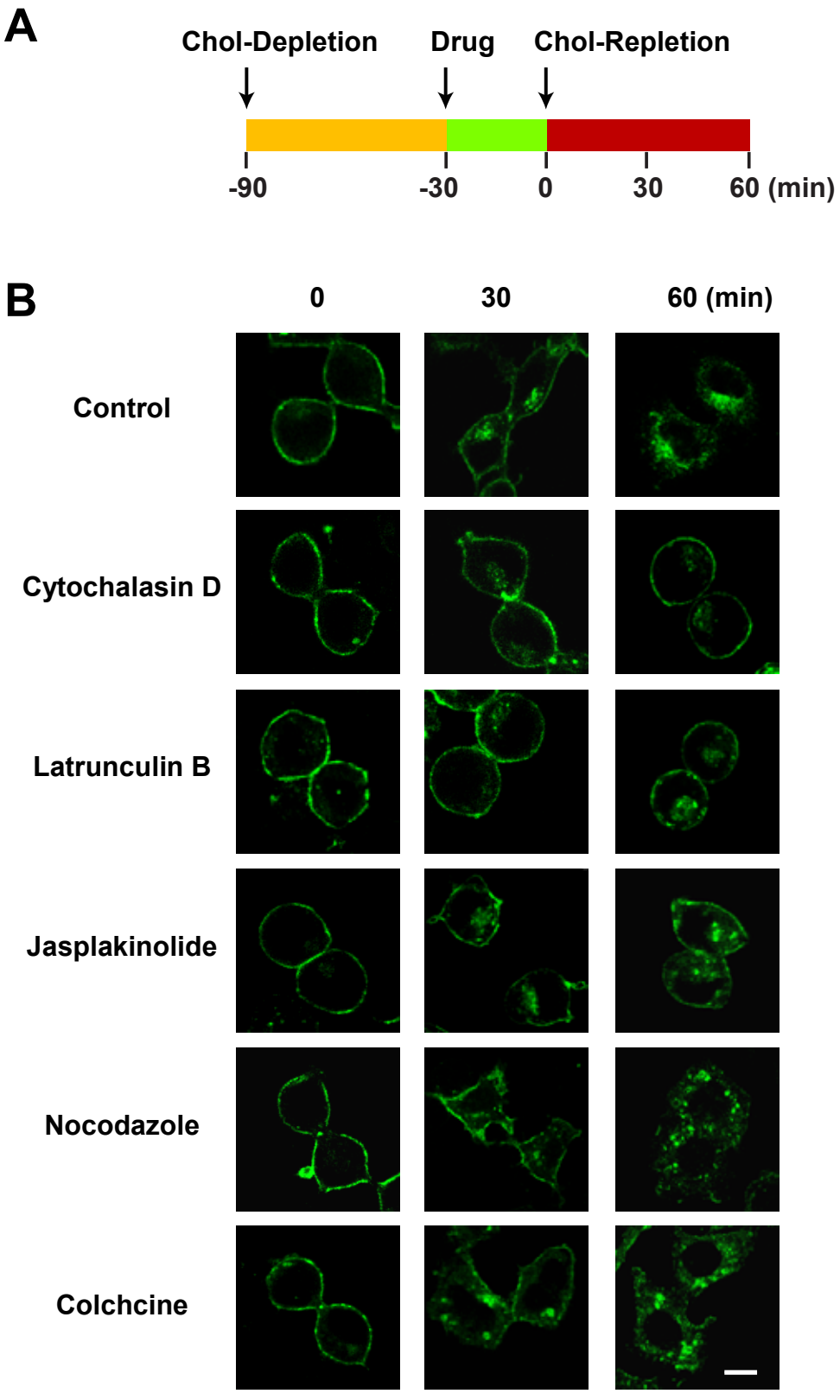
A
Chol-Depletion
Drug
Chol-Repletion
-90
-30
0
30
60 (min)
B
0
30
60 (min)
Control
Cytochalasin D
Latrunculin B
Jasplakinolide
Nocodazole
Colchcine

Figure S1

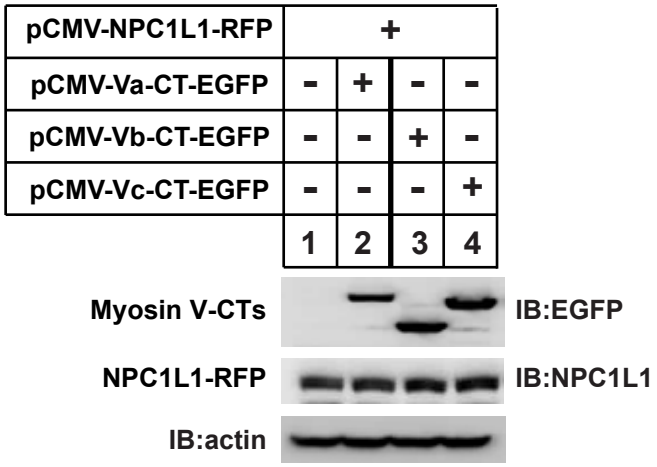
pCMV-NPC1L1-RFP
+
pCMV-Va-CT-EGFP
-
+
-
-
pCMV-Vb-CT-EGFP
-
-
+
-
pCMV-Vc-CT-EGFP
-
-
-
+
1
2
3
4
Myosin V-CTs
IB:EGFP
NPC1L1-RFP
IB:NPC1L1
IB:actin

Figure S2

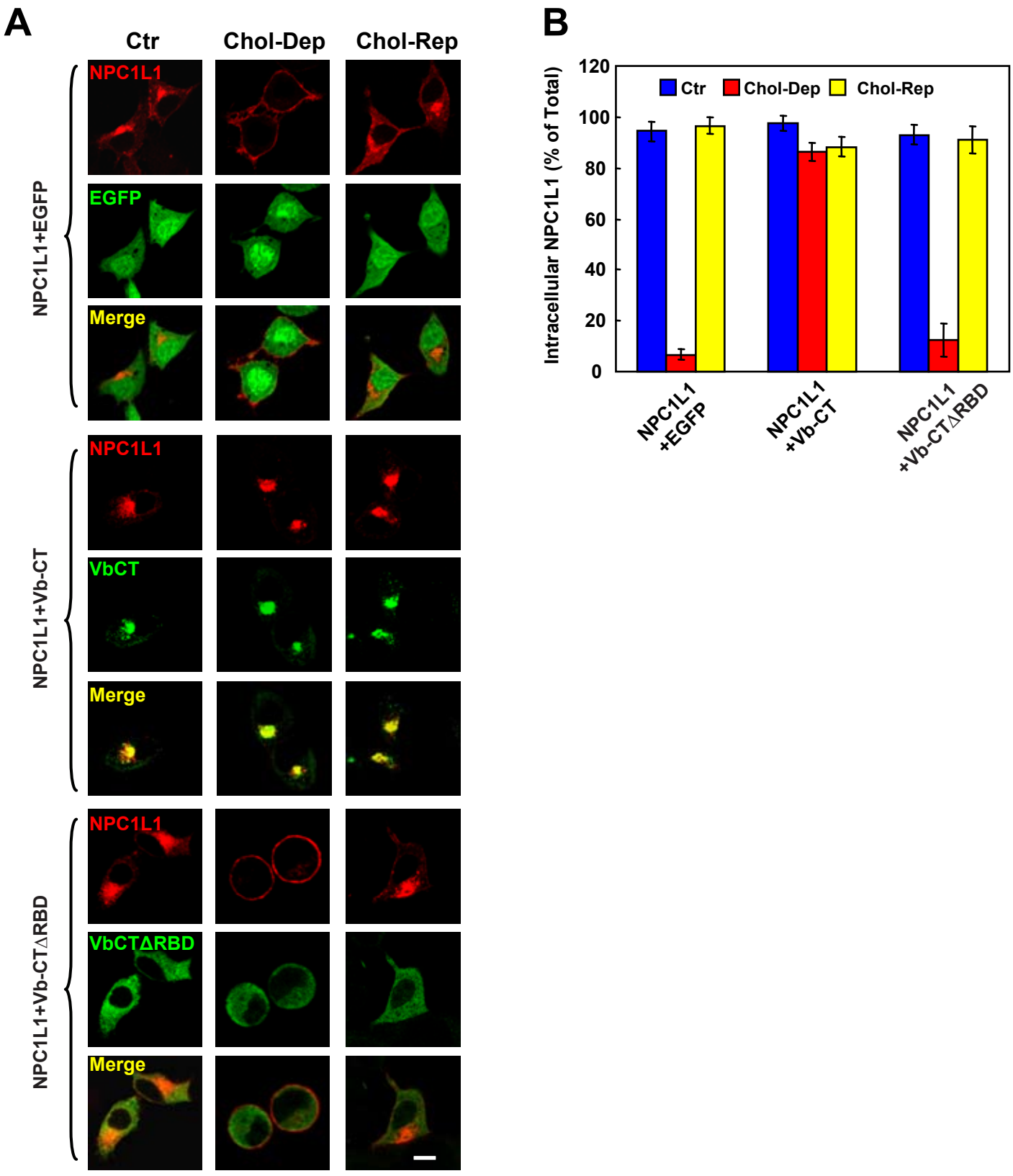
A
Ctr
Chol-Dep
Chol-Rep
NPC1L1+EGFP
NPC1L1
EGFP
Merge
NPC1L1+Vb-CT
NPC1L1
VbCT
Merge
NPC1L1+Vb-CTΔRBD
NPC1L1
VbCTΔRBD
Merge
B
Intracellular NPC1L1 (% of Total)
120
100
80
60
40
20
0
Ctr
Chol-Dep
Chol-Rep
NPC1L1 +EGFP
NPC1L1 +Vb-CT
NPC1L1 +Vb-CTΔRBD

Figure S3

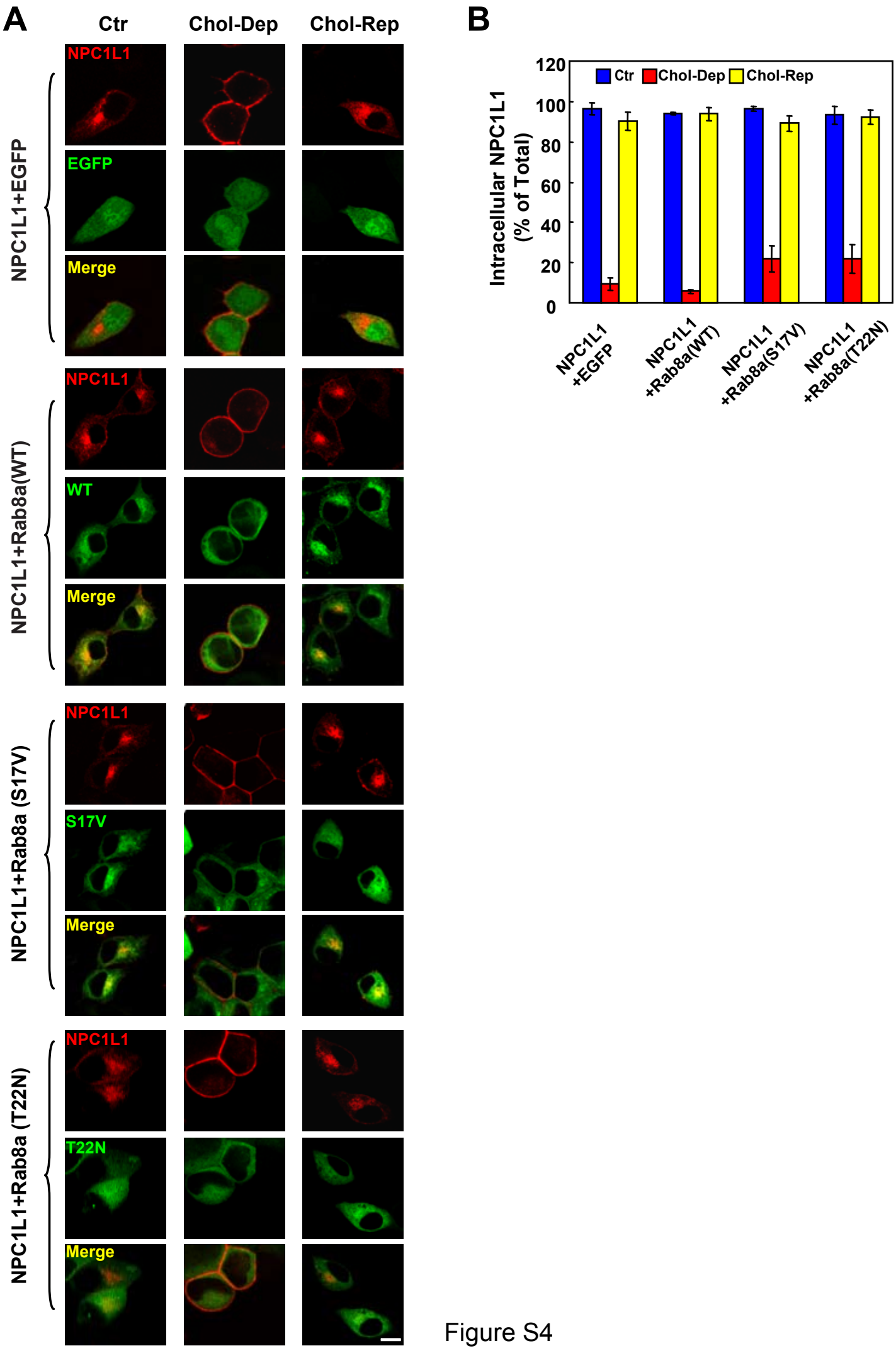
A
Ctr
Chol-Dep
Chol-Rep
NPC1L1+EGFP
NPC1L1
EGFP
Merge
NPC1L1+Rab8a(WT)
NPC1L1
WT
Merge
NPC1L1+Rab8a (S17V)
NPC1L1
S17V
Merge
NPC1L1+Rab8a (T22N)
NPC1L1
T22N
Merge
B
Intracellular NPC1L1 (% of Total)
120
100
80
60
40
20
0
Ctr
Chol-Dep
Chol-Rep
NPC1L1 +EGFP
NPC1L1 +Rab8a(WT)
NPC1L1 +Rab8a(S17V)
NPC1L1 +Rab8a(T22N)

Figure S4