Supplementary figure legends

Figure S1 Identification of the MICAL-L1 region that binds to Syndapin2. (**A**) HeLa cells on cover-slips were mock-treated or treated with either MICAL-L1-, Syndapin2-, or Bin1-SiRNA for 72 h, and transfected with GFP-Myc-EHD1 for the last 24 h. Bar, 10 μm. (**B**) HeLa cells were mock-treated or treated with Bin1- or Syndapin2-SiRNA for 72 h and subjected to immunoblot analysis with antibodies against Bin1, Syndapin2 and actin (control). (**C-F**) Yeast two-hybrid studies were performed to assess Syndapin2 binding to: (**C**) MICAL-L1, EHD1, EHD2, EHD3 and EHD4**.** (**D**) MICAL-L1 and a series of truncation mutants, as indicated. (**E**) MICAL-L1 residues 1-368, 368-468, 468-863 and MICAL-L1 residues 368-468 with PXXP-to-AXXA mutations at each PXXP motif. (**F**) MICAL-L1 residues 468-863 and its mutant counterpart with PRAP mutated to ARAA beginning at residue 480. (**G**) Yeast two-hybrid studies were performed with MICAL-L1 as prey and bait. (**H**) Sequence comparison of the PXXP Syndapin-binding motifs of MICAL-L1 and dynamin1. (**I**) HeLa cells were transfected with both GFP-Myc-EHD1 and HA-Syndapin2 for 48 h. Cells were lysed and lysate was subjected to immunoprecipitation using anti-MICAL-L1 antibodies. The cell lysate and the immunoprecipitate were then subjected to immunoblot analysis with antibodies against GFP, the HA-tag and actin. (**J**) HeLa cell lysates were transfected with either GFP or GFP-Myc-EHD3 for 48 h, and subjected to GST pull-down with GST-Syndapin2 as bait. The cell lysate and precipitate were then subjected to immunoblot analysis with antibodies against the GFP-tag.

Figure S2 Exogenously expressed Syndapin2 and MICAL-L1 co- localize on tubular endosomes. (**A**) HeLa cells were mock-treated or treated with Bin1-SiRNA for 72 h. Cells were immunostained for endogenous Syndapin2 and MICAL-L1. Bar, 10 μm. (**B**) HeLa cells were transfected with Tomato-Syndapin2, GFP-MICAL-L1 or co-transfected with both. Singly transfected cells were stained for endogenous MICAL-L1 or Syndapin2 as indicated. Yellow arrowheads mark co-localization to tubular endosomes. Bar, 10 μm.

Figure S3 Syndapin2 and MICAL-L1 dynamically associate with tubular recycling endosomes. FRAP experiments were performed on HeLa cells transfected with Tomato-Syndapin2 and GFP-MICAL-L1. Images were taken before and after photobleaching at the indicated times. Bar, 10 μm.

Figure S4 Loss of Syndapin2 affects organization of early endosomes, lysosomes and golgi. (**A**) HeLa cells were mock-treated or Syndapin2-SiRNA-treated for 72 h and immunostained for either EEA1, Giantin or Lamp1. (**B**) HeLa cells were mock-treated or Syndapin2-SiRNA-treated for 72 h and immunostained for endogenous SNX1. Insets are shown below each image. (**C**) HeLa cells were mock-treated or Syndapin2-SiRNA-treated for 72 h and immunostained for endogenous Rabankyrin-5. Insets are shown below the mock images. Bar, 10 μm.

Figure S5 Purification of His-tagged MICAL-L1-CC domain. (**A**) Samples were collected at various stages of purification of the His-tagged MICAL-L1-CC domain: before and after IPTG induction, after cell lysis, and after cell lysate precipitation to pellet inclusion bodies. Inclusion bodies were then resuspended and precipitated in 1 M urea buffer. Pellets were further solubilized in 8 M urea buffer and both soluble and insoluble fractions were collected. These samples were run on SDS page and stained with Coomassie Blue. (**B**) Unfolded protein in 8 M urea buffer was bound to Ni-NTA column and eluted. Proteins from the eluted fractions were separated SDS-PAGE and stained with Coomassie Blue. (**C**) The fractions rich in His-tagged MICAL-L1-CC domain were pooled together (eluted protein) and dissolved in ten times the volume of refolding buffer. The protein was then concentrated using centrifugal filtration devices. Samples were separated on SDS-PAGE and analyzed by Coomassie staining. (**D**) The refolded and concentrated His-tagged-MICAL-L1-CC domain was subjected to circular dichroism.

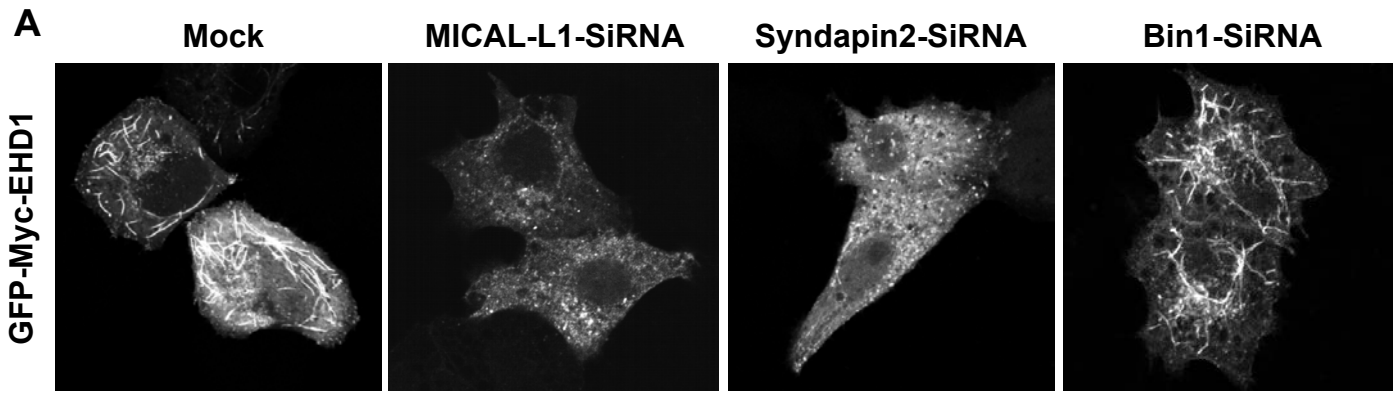
Figure S6 Lipid overlay assay of MICAL-L1 and Syndapin2. (**A-B**) Lipid overlay assays were performed with His-tagged MICAL-L1-CC domain, GST-Syndapin2 (full length) or GST-

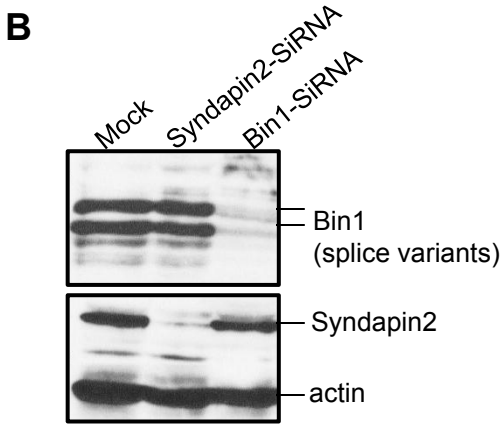
Syndapin2-F-BAR domain. The lipid strips were then immunoblotted for the respective tags. (**C**) Phosphatidic acid-binding probes localize at the tubular endososmes. HeLa cells were transfected with Spo20p phosphatidic acid-binding domain (PABD) probe or its non-interacting mutant (L67P) as indicated by immunostaining for endogenous MICAL-L1. Bar, 10 μm. (**D**) HeLa cells tranfected with the HA-MICAL-L1-CC domain for 16 h were co-stained with antibodies against the HA-tag together with either endogenous MICAL-L1 or Syndapin2. Arrows indicate partial co-localization of the MICAL-L1-CC domain and Syndapin2 on tubular membranes.

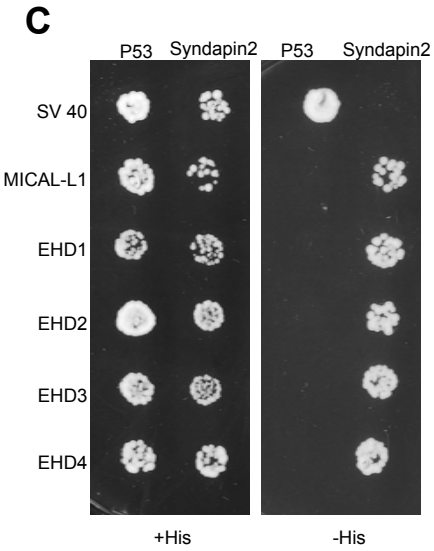
Figure S7 HeLa cells transfected with HA-MICAL-L1-CC domain for 16 h were incubated with Alexa-Fluor 568-labeled transferrin for 5 min. The cells were either fixed (pulse 5 min.) or fixed after 10 min. (Chase 10 min.) or 20 min. (Chase 20 min.) incubations in complete media. Cells were then immunostained with antibodies against the HA-tag. Bar 10 μm.

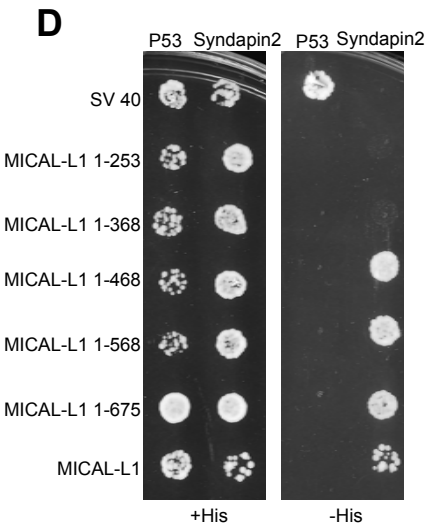
Figure S8 *In vitro* LMV tubulation with liposomes containing phosphatidylserine (PS) and PI(4,5)P2. LMV tubulation was assessed in the absence of protein, GST-Synd2, GST-Synd F-BAR domain or the CC domain of MICAL-L1. LMVs were comprised of a mass ratio of 70% PC, 10% Rhodamine-PE and either 20% PS or PI(4,5)P2. Insets depict the region in the white box. Bar, 10 μm.

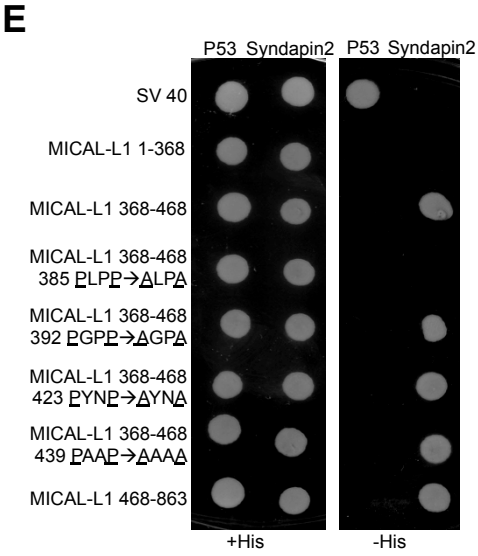
Figure S1

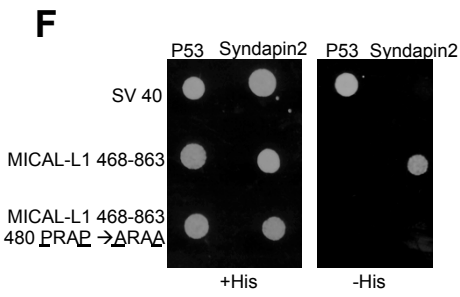

A
Mock
MICAL-L1-SiRNA
Syndapin2-SiRNA
Bin1-SiRNA
GFP-Myc-EHD1

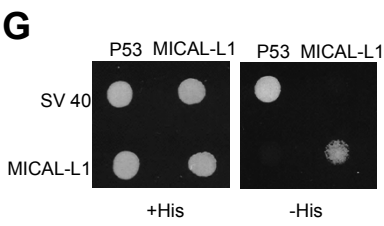

B
Mock
Syndapin2-SiRNA
Bin1-SiRNA
Bin1
(splice variants)
Syndapin2
actin


C
P53
Syndapin2
P53
Syndapin2
SV 40
MICAL-L1
EHD1
EHD2
EHD3
EHD4
+His
-His


D
P53
Syndapin2
P53
Syndapin2
SV 40
MICAL-L1 1-253
MICAL-L1 1-368
MICAL-L1 1-468
MICAL-L1 1-568
MICAL-L1 1-675
MICAL-L1
+His
-His


E
P53
Syndapin2
P53
Syndapin2
SV 40
MICAL-L1 1-368
MICAL-L1 368-468
MICAL-L1 368-468
385 PLPP→ALPA
MICAL-L1 368-468
392 PGPP→AGPA
MICAL-L1 368-468
423 PYNP→AYNA
MICAL-L1 368-468
439 PAAP→AAAA
MICAL-L1 468-863
+His
-His


F
P53
Syndapin2
P53
Syndapin2
SV 40
MICAL-L1 468-863
MICAL-L1 468-863
480 PRAP →ARAA
+His
-His


G
P53
MICAL-L1
P53
MICAL-L1
SV 40
MICAL-L1
+His
-His

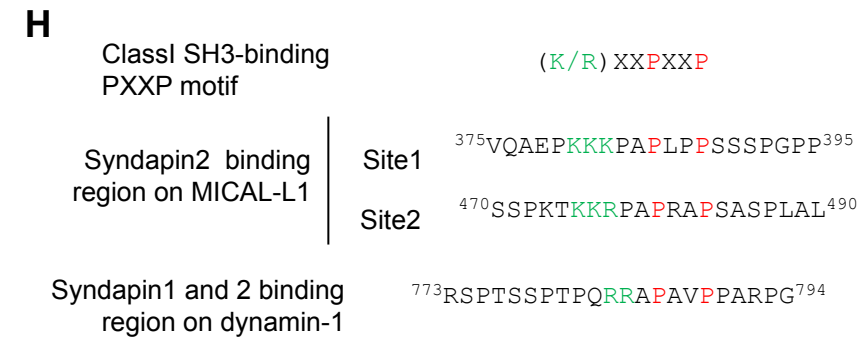
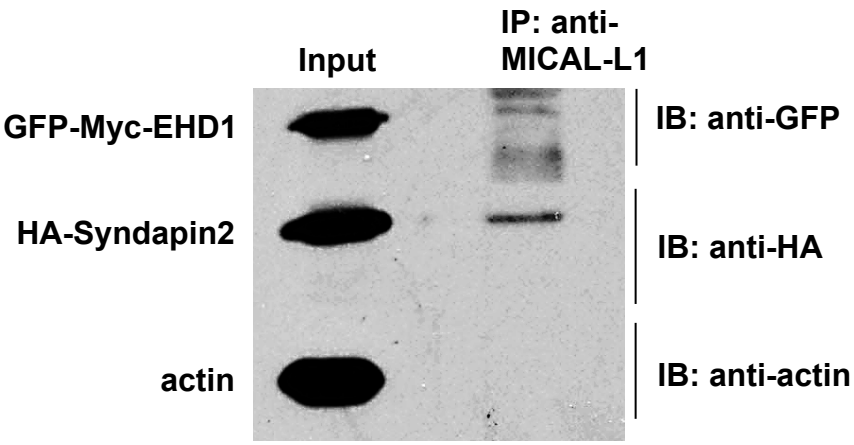

H
ClassI SH3-binding
PXXP motif
(K/R)XXPXXP
Syndapin2 binding
region on MICAL-L1
Site1
375VQAEPKKKPAPLPPSSSPGPP395
Site2
470SSPKTKKRPAPRAPSASPLAL490
Syndapin1 and 2 binding
region on dynamin-1
773RSPTSSPTPQRRAPAVPPARPG794

Figure S1

I


Input
IP: anti-MICAL-L1
GFP-Myc-EHD1
HA-Syndapin2
actin
IB: anti-GFP
IB: anti-HA
IB: anti-actin

J

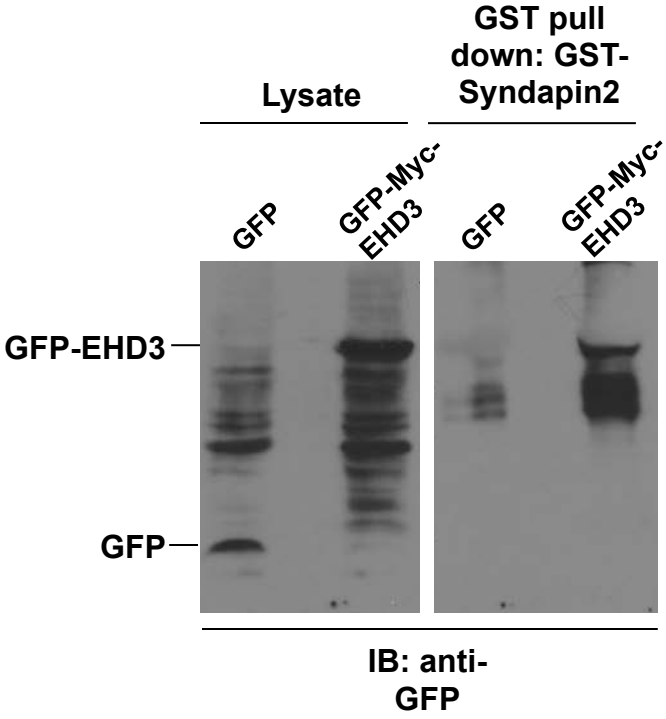

GST pull down: GST-Syndapin2
Lysate
GFP
GFP-Myc-EHD3
GFP
GFP-Myc-EHD3
GFP-EHD3
GFP
IB: anti-GFP

Figure S2

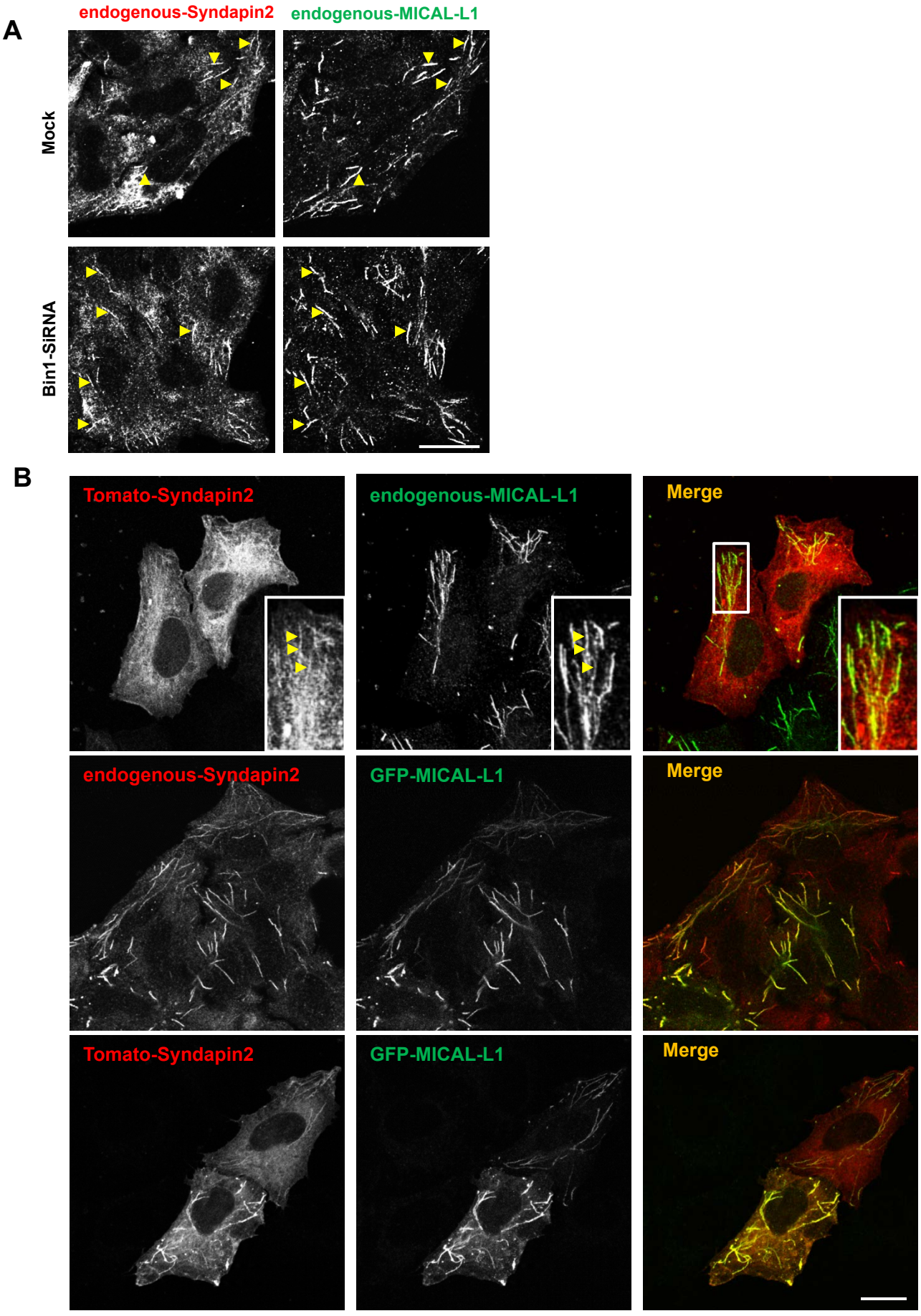

A
endogenous-Syndapin2
endogenous-MICAL-L1
Mock
Bin1-SiRNA
B
Tomato-Syndapin2
endogenous-MICAL-L1
Merge
endogenous-Syndapin2
GFP-MICAL-L1
Merge
Tomato-Syndapin2
GFP-MICAL-L1
Merge

Figure S3

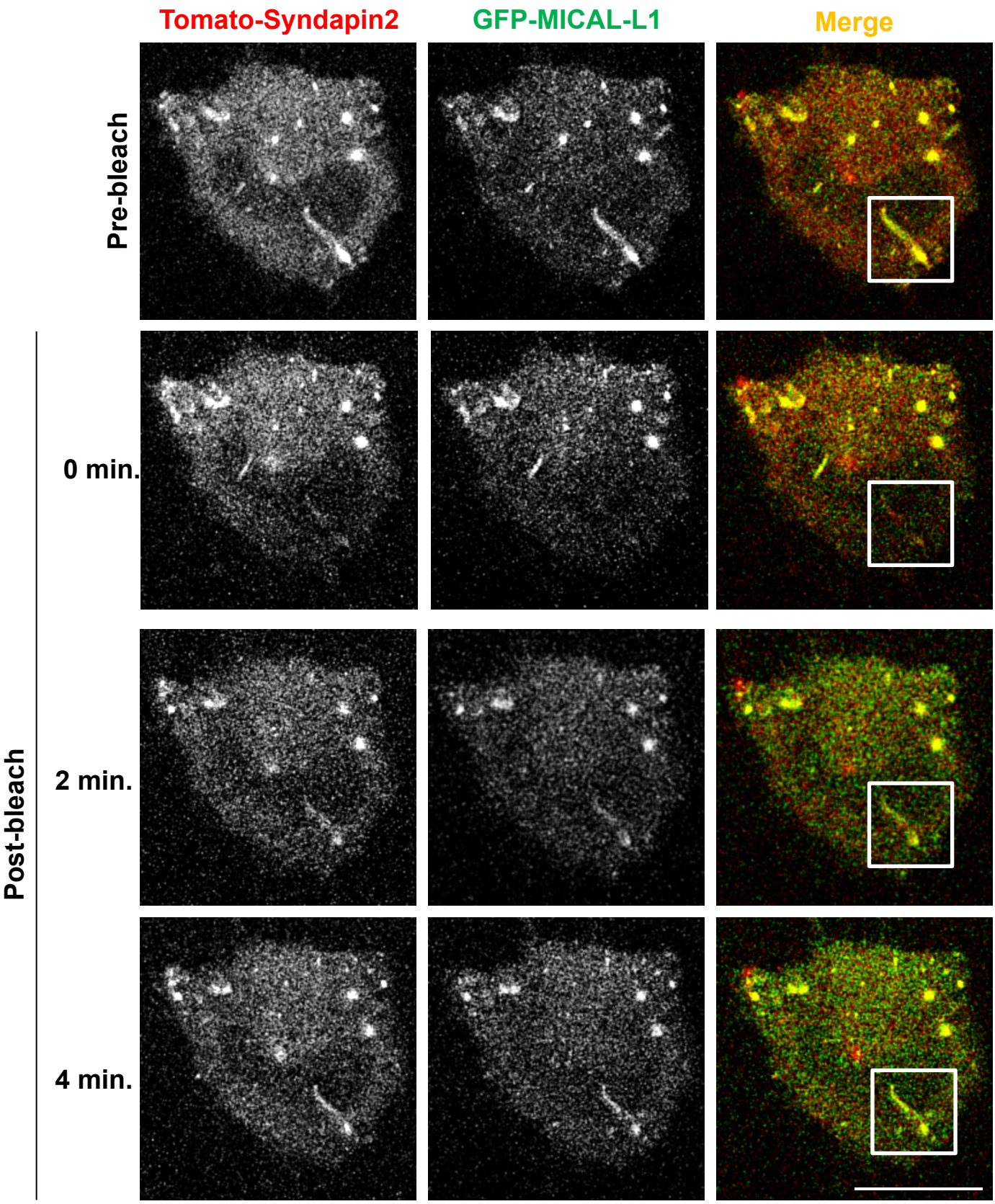

Tomato-Syndapin2
GFP-MICAL-L1
Merge
Pre-bleach
Post-bleach
0 min.
2 min.
4 min.

Figure S4A

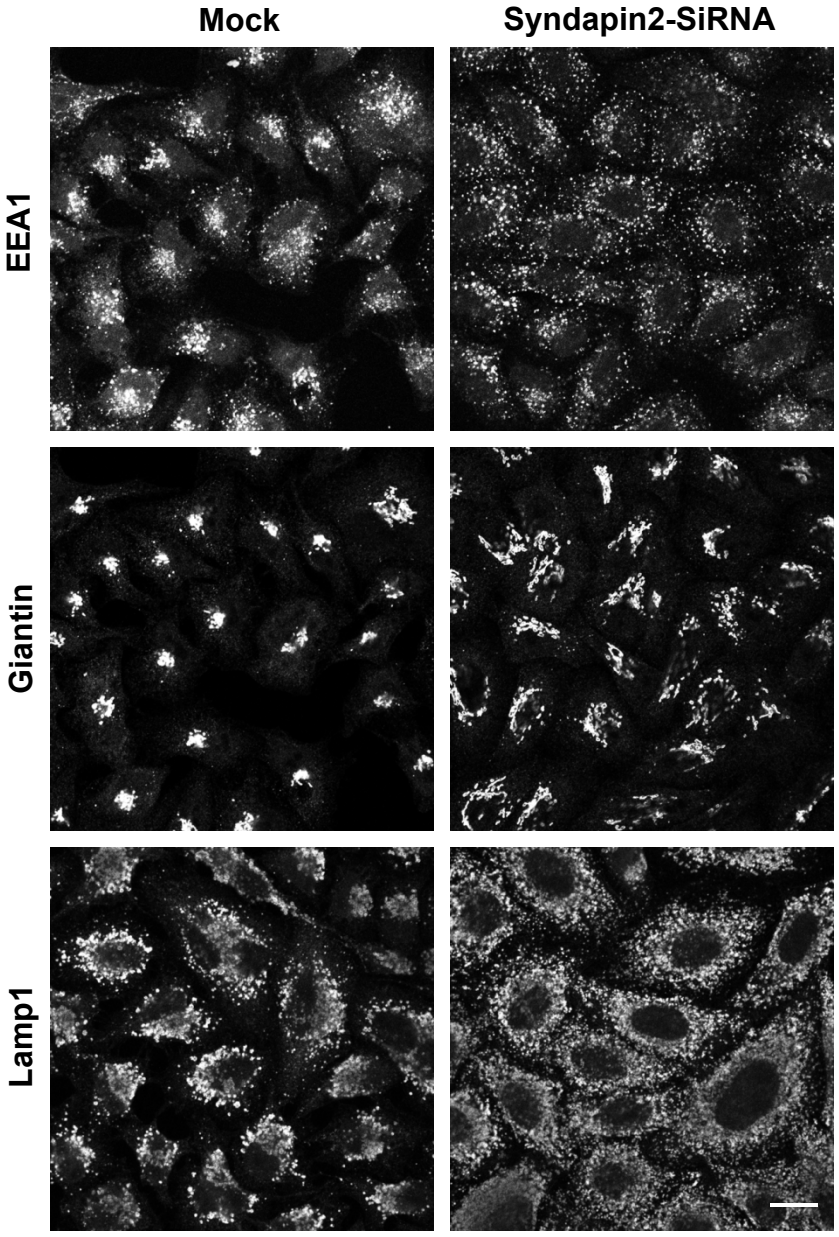


Figure S4B

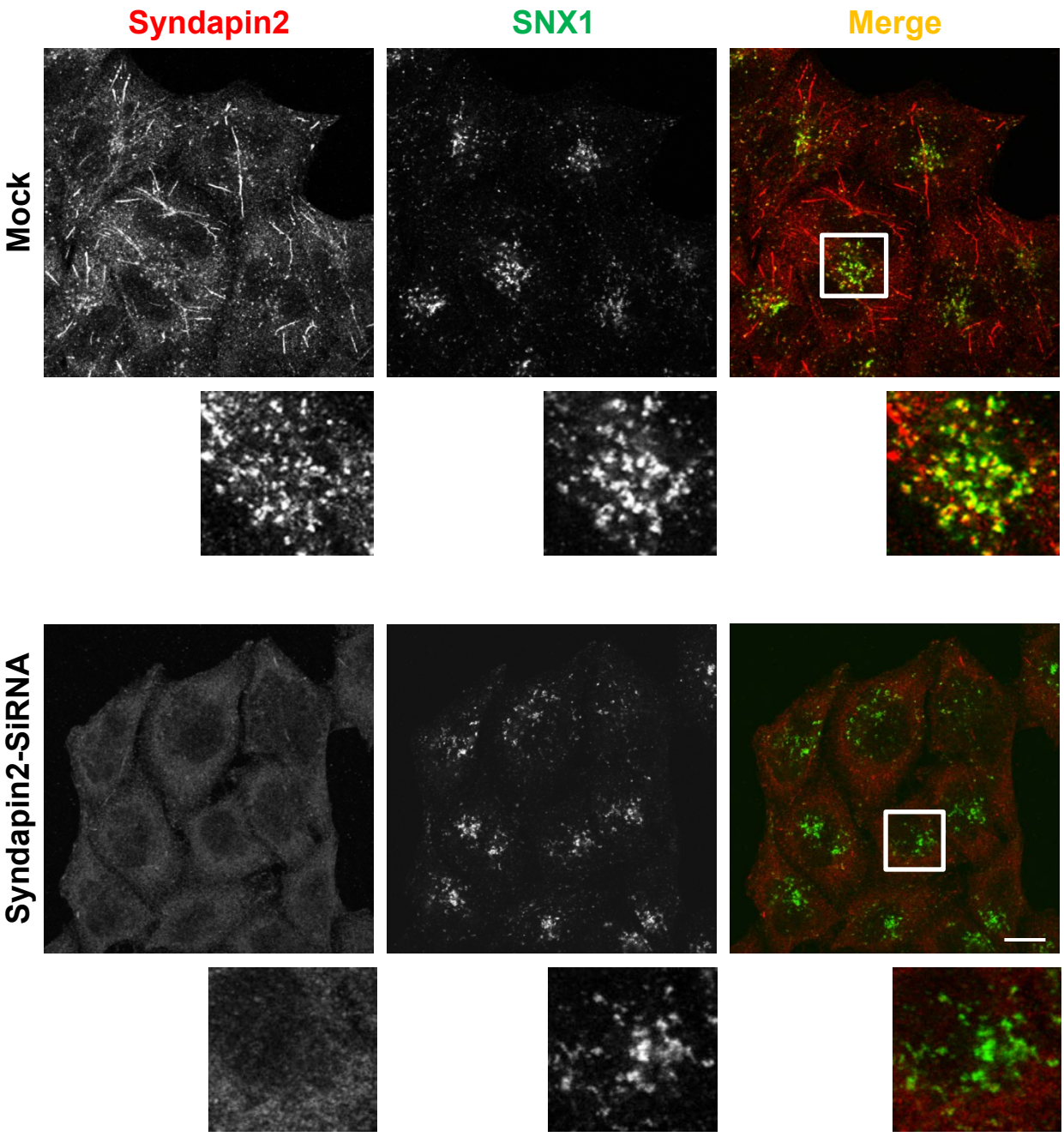


Figure S4C

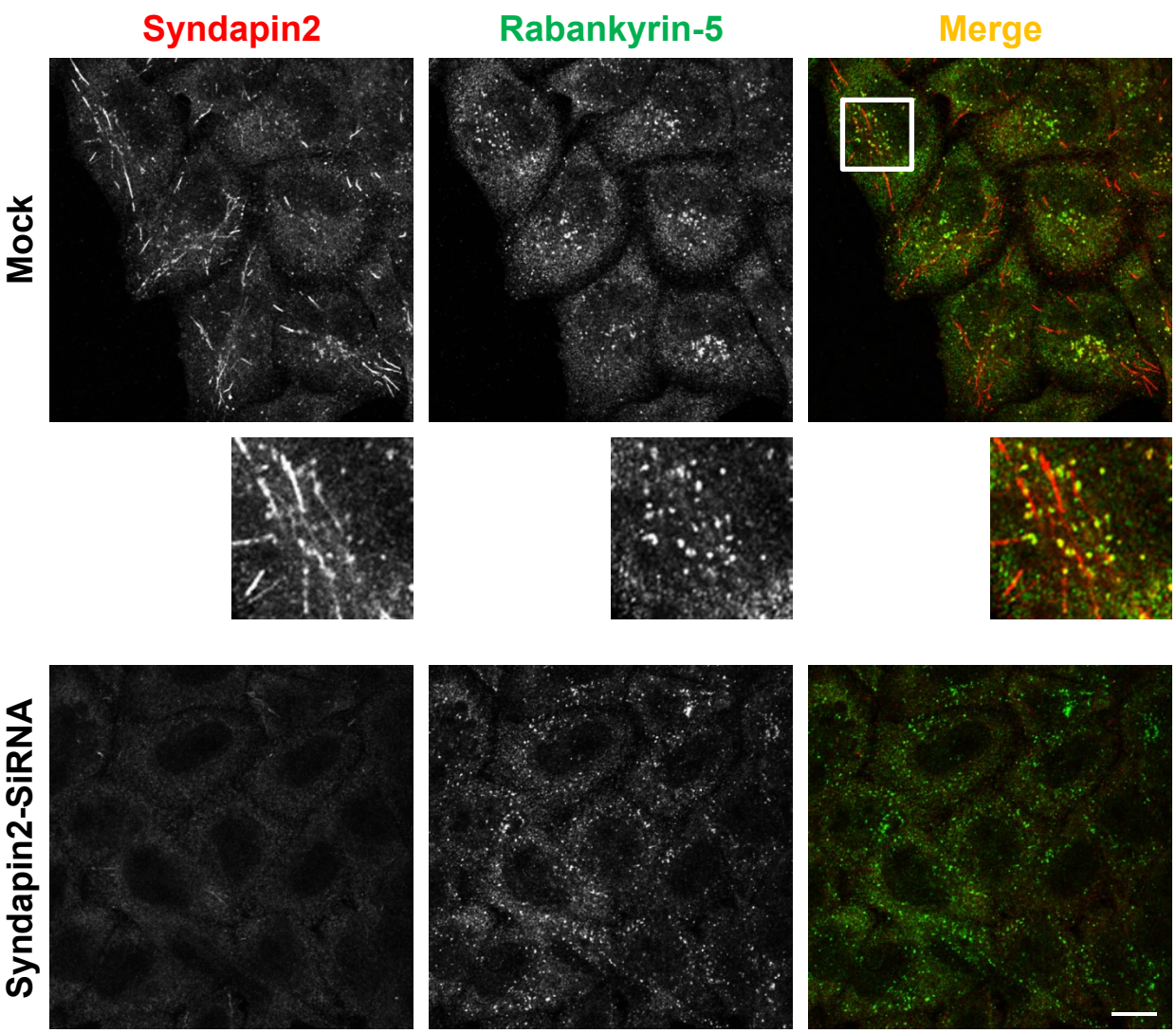


Figure S5

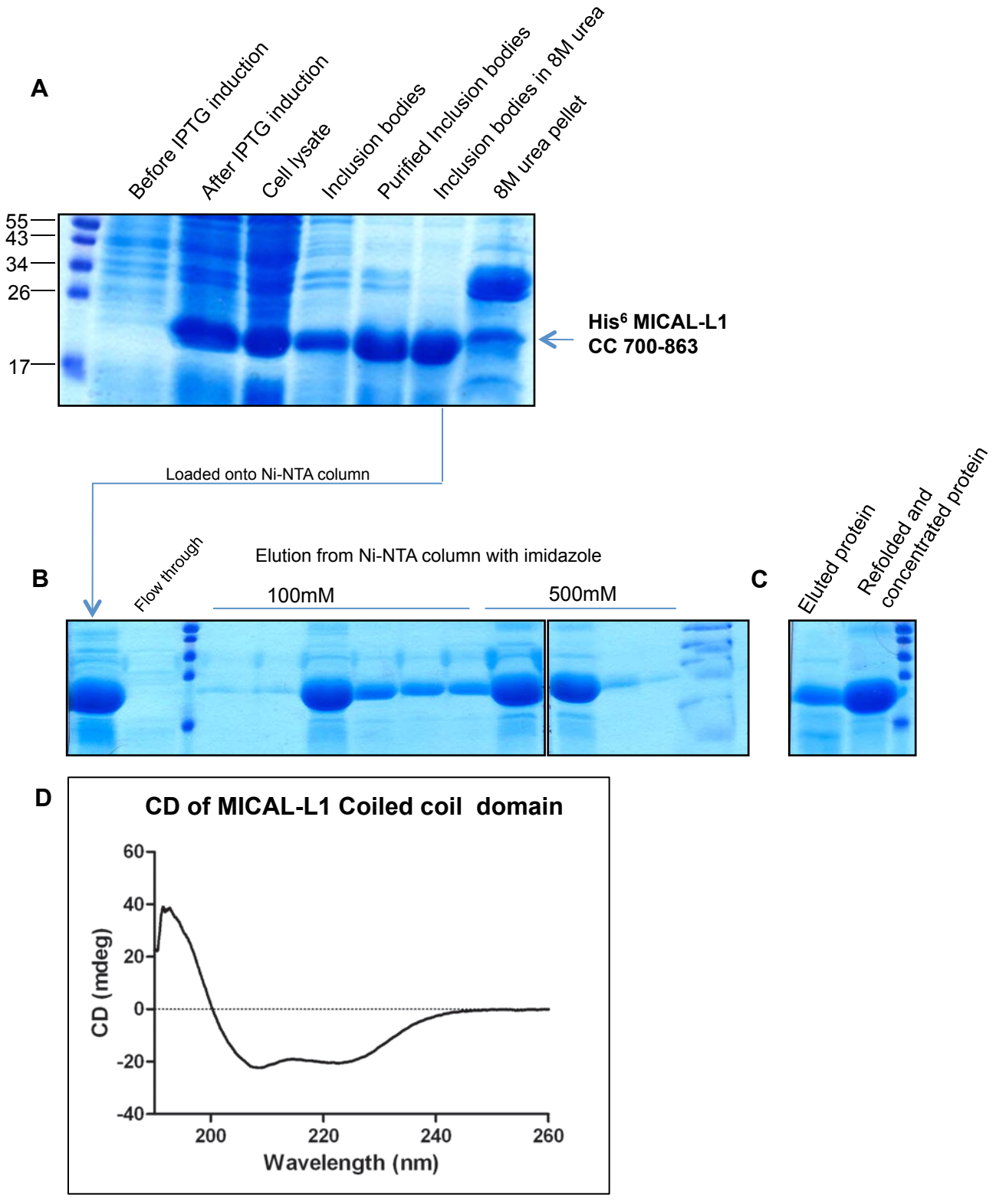

A
Before IPTG induction
After IPTG induction
Cell lysate
Inclusion bodies
Purified Inclusion bodies
Inclusion bodies in 8M urea
8M urea pellet
55
43
34
26
17
His6 MICAL-L1
CC 700-863
Loaded onto Ni-NTA column
B
Flow through
Elution from Ni-NTA column with imidazole
100mM
500mM
C
Eluted protein
Refolded and concentrated protein
D
CD of MICAL-L1 Coiled coil domain
CD (mdeg)
60
40
20
0
-20
-40
200
220
240
260
Wavelength (nm)

Figure S6

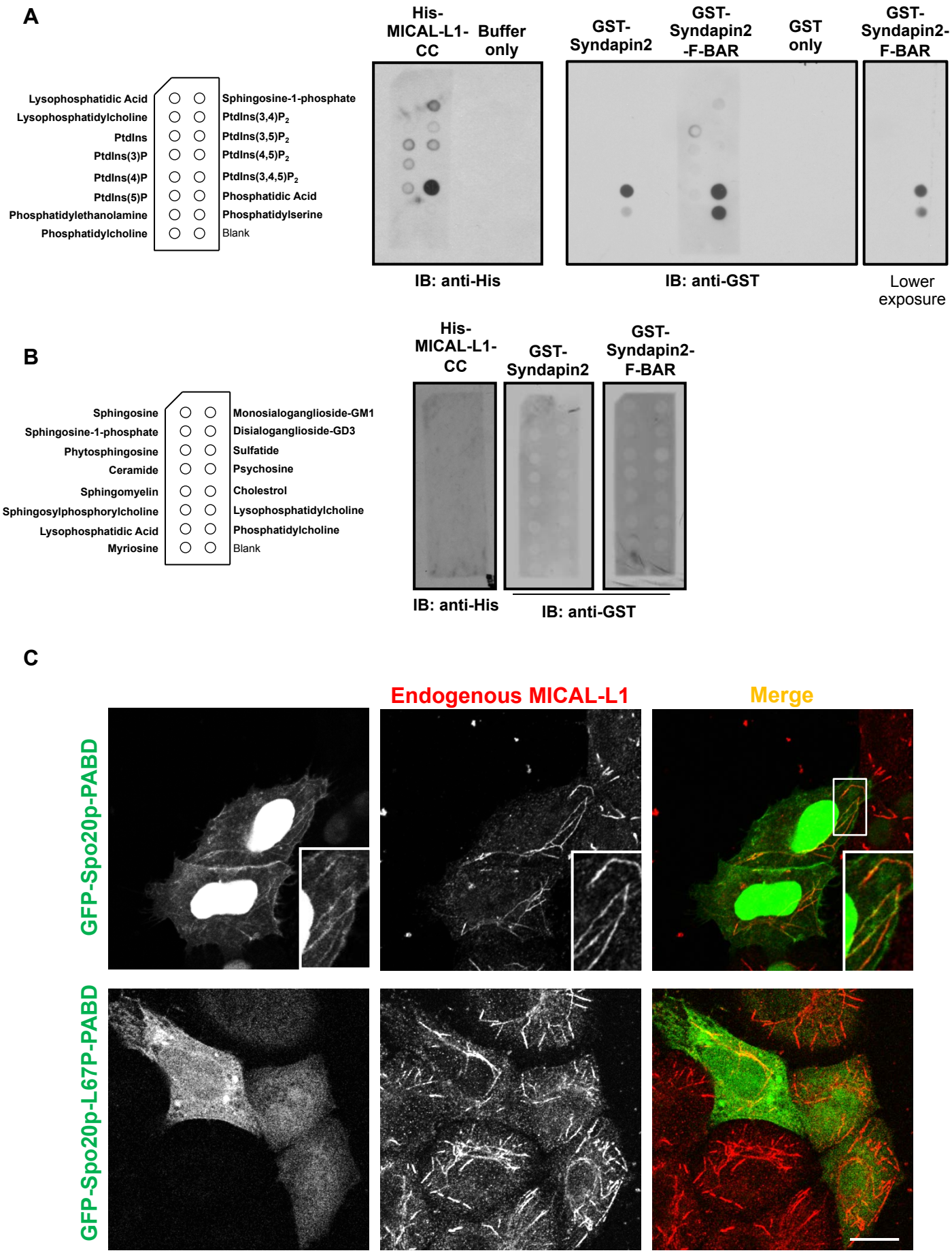

A
His-MICAL-L1-CC
Buffer only
GST-Syndapin2
GST-Syndapin2-F-BAR
GST only
GST-Syndapin2-F-BAR
Lysophosphatidic Acid
Lysophosphatidylcholine
PtdIns
PtdIns(3)P
PtdIns(4)P
PtdIns(5)P
Phosphatidylethanolamine
Phosphatidylcholine
Sphingosine-1-phosphate
PtdIns(3,4)P2
PtdIns(3,5)P2
PtdIns(4,5)P2
PtdIns(3,4,5)P2
Phosphatidic Acid
Phosphatidylserine
Blank
IB: anti-His
IB: anti-GST
Lower exposure
B
His-MICAL-L1-CC
GST-Syndapin2
GST-Syndapin2-F-BAR
Sphingosine
Sphingosine-1-phosphate
Phytosphingosine
Ceramide
Sphingomyelin
Sphingosylphosphorylcholine
Lysophosphatidic Acid
Myriosine
Monosialoganglioside-GM1
Disialoganglioside-GD3
Sulfatide
Psychosine
Cholestrol
Lysophosphatidylcholine
Phosphatidylcholine
Blank
IB: anti-His
IB: anti-GST
C
Endogenous MICAL-L1
Merge
GFP-Spo20p-PABD
GFP-Spo20p-L67P-PABD

Figure S6

D

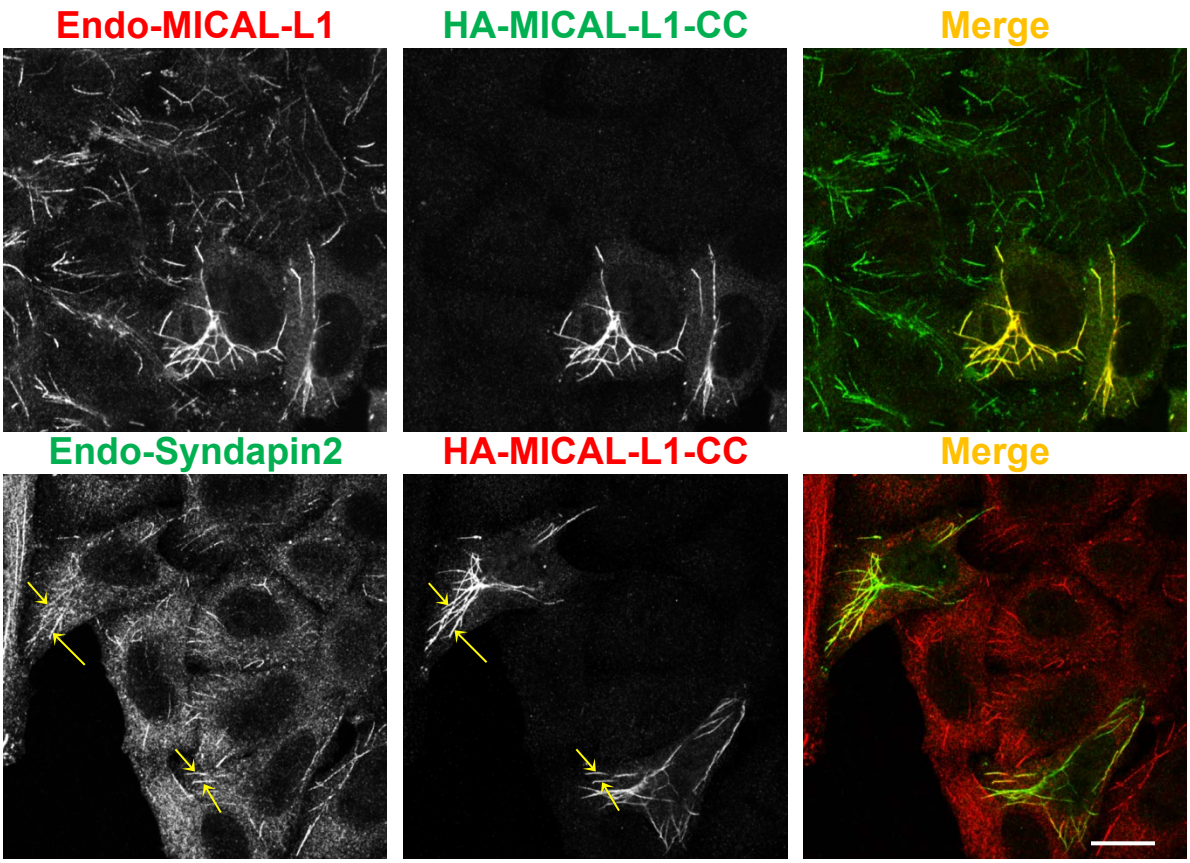

Endo-MICAL-L1
HA-MICAL-L1-CC
Merge
Endo-Syndapin2
HA-MICAL-L1-CC
Merge

Figure S7

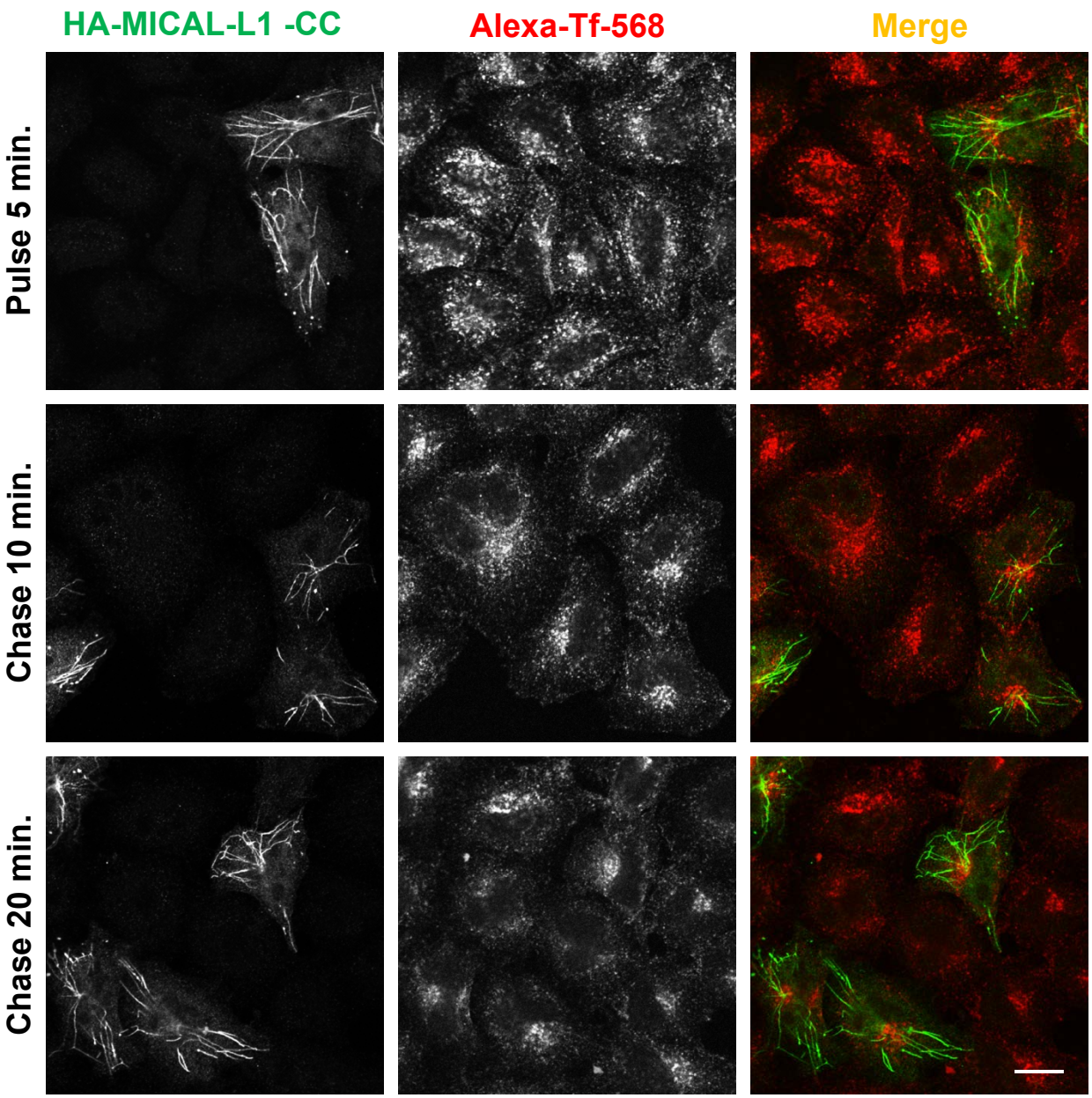
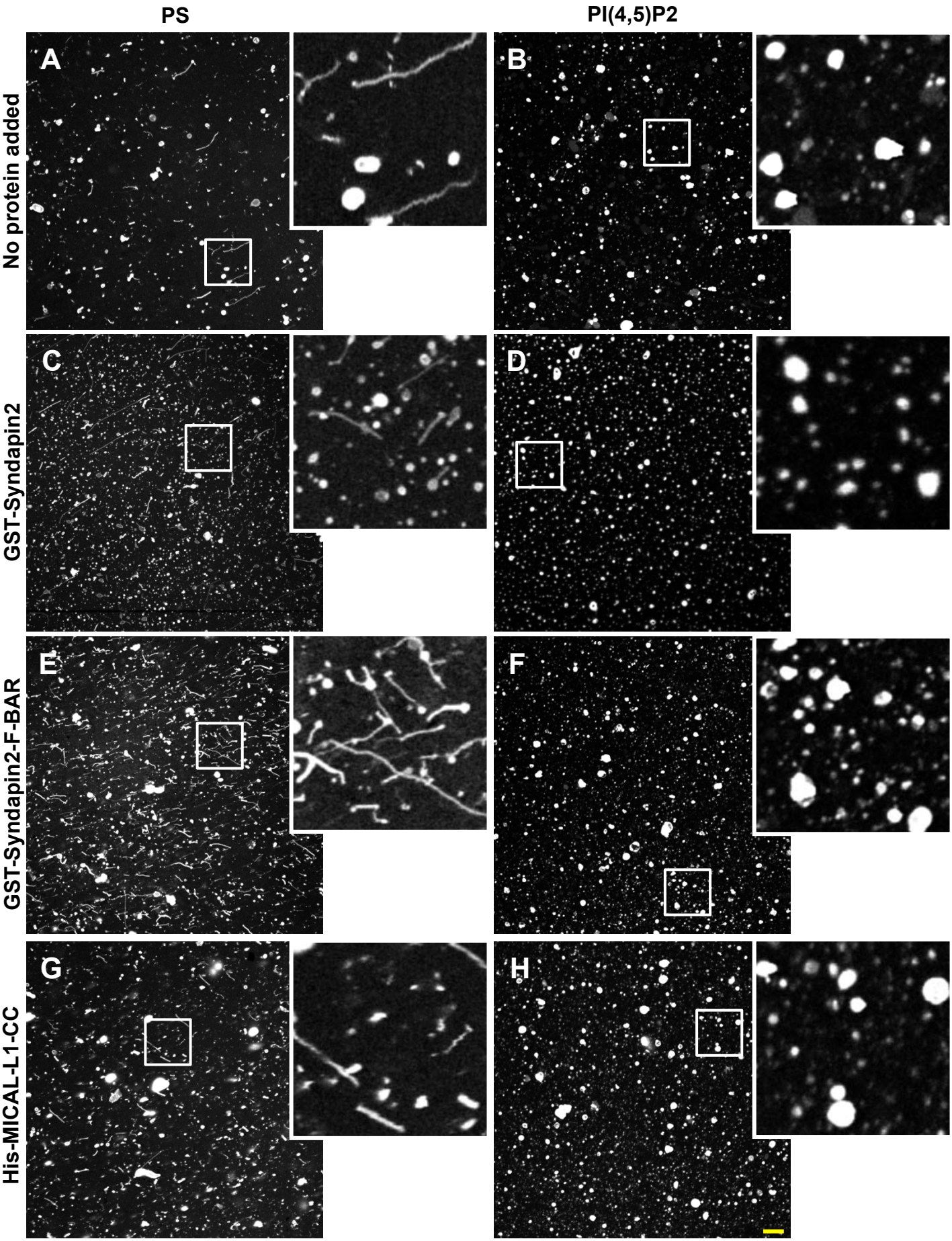

HA-MICAL-L1 -CC
Alexa-Tf-568
Merge
Pulse 5 min.
Chase 10 min.
Chase 20 min.

Figure S8


PS
PI(4,5)P2
No protein added
GST-Syndapin2
GST-Syndapin2-F-BAR
His-MICAL-L1-CC
A
B
C
D
E
F
G
H