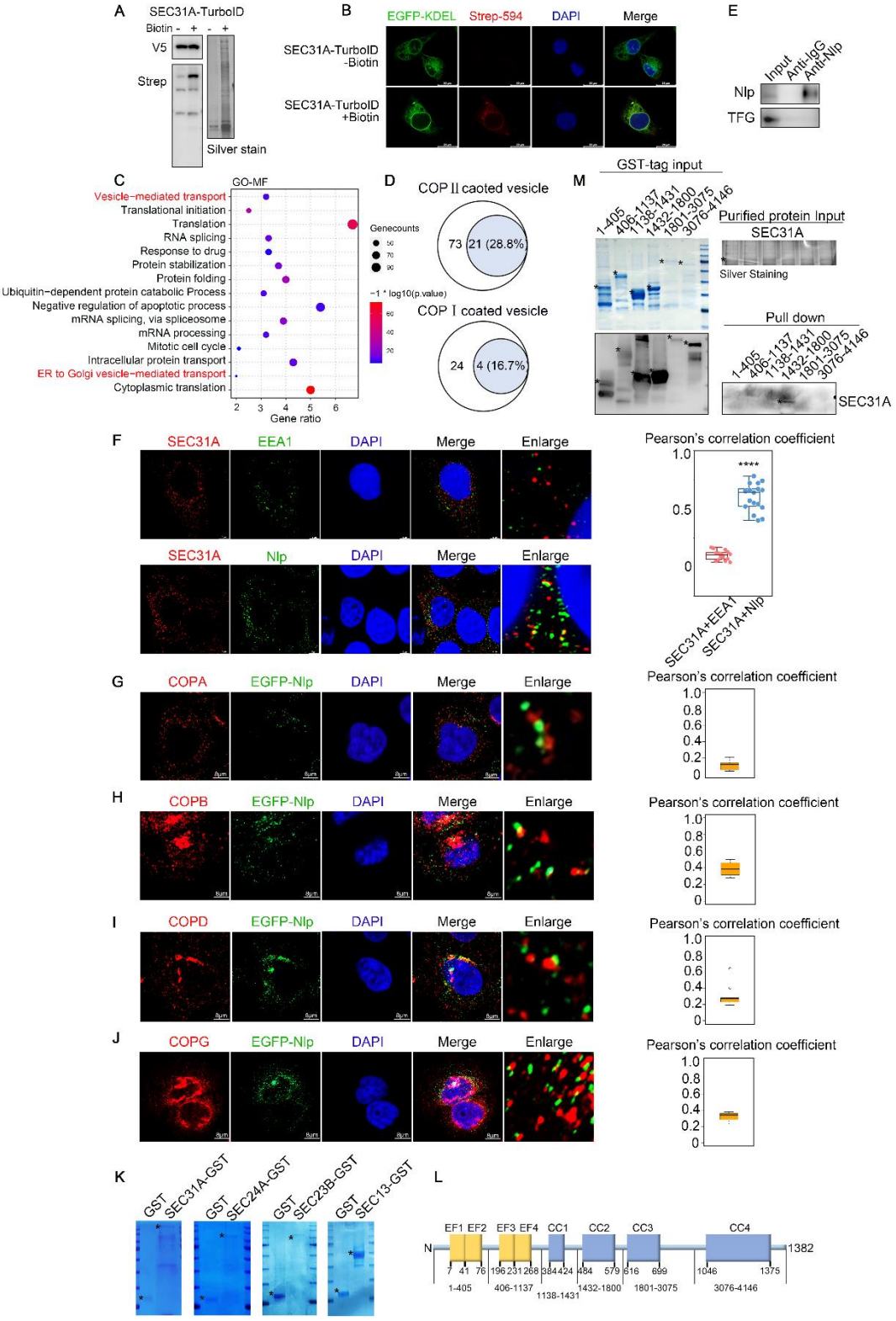


Figure S1. Nlp interacted with ER-to-Golgi vesicles and altered their stability.

(A) Immunoblotting and silver staining assay in HeLa cells that have been transfected with SEC31A-TurboID-V5 plasmids for 36 hours. Cells were labeled with or without 50μM biotin for 10 min before washing and collecting.

(B) Confocal of SEC31A-TurboID-V5 system. HeLa-EGFP-KDEL Cells transfected with SEC31A-TurboID-V5 plasmids were treated with or without 50 μM biotin for 10 min before fixing. (Scale bar, 20 μm.).

(C-D) Enrichment analysis of Molecular Functions in Nlp-interacted proteins using two different Nlp antibodies. Ranked by p-value and cut off by minimal percentage of 2%.

(E) Co-IP assay using anti-Nlp or anti-IgG in HeLa cells.

(F) Colocalization of SEC31A and EEA1 or EGFP-Nlp by Leica. Pearson's correlation coefficient was analyzed. (Scale bar, 5 μm. ****$p<0.0001$.).

(G-J) Colocalization of Nlp and (G) COPA, (H) COPB, (I) COPD or (J) COPG by LSM980. Pearson's correlation coefficient was analyzed. (Scale bar, 8 μm.).

(K) Coomassie stain gel of GST, SEC31A-GST, SEC24A-GST SEC23B-GST or SEC13-GST purified protein related to Figure 2 A-D.

(L) Indicated truncates of Nlp were constructed according to its functional domains.

(M) SEC31A purified protein was obtained from SEC31A-GST using Thrombin. After verifications by Coomassie brilliant blue, silver staining and Western blot, GST pull-down assay was performed using GST-tag purified proteins of Nlp truncates and SEC31A purified protein.

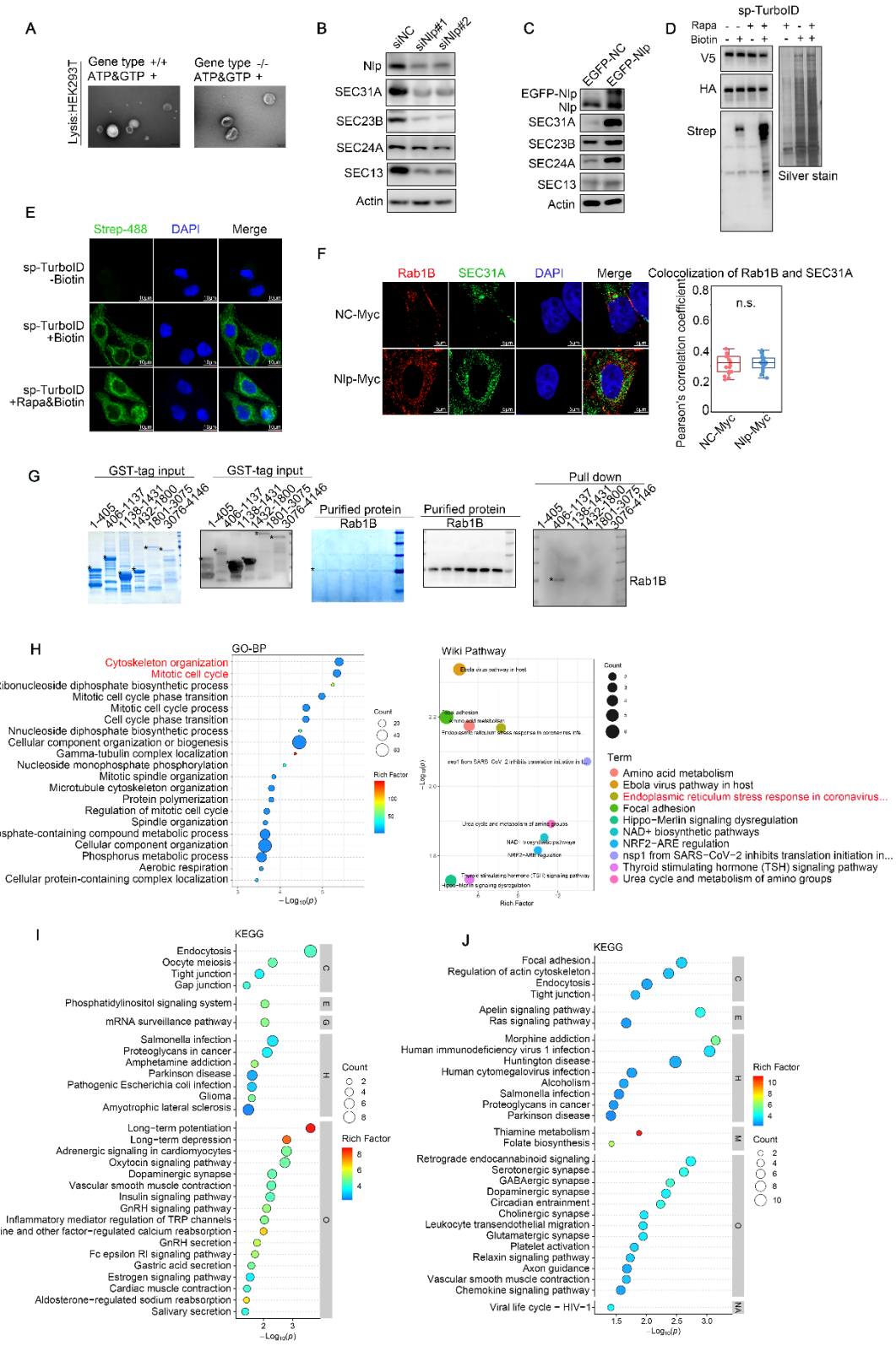


Figure S2. TurboID and Split-TurboID system involved to analyze Nlp-associated transporting cargoes.

(A) Negative staining TEM visualization of the morphology of the vesicle membrane structures formed by *in vitro* vesicle formation assay. Membrane source was from MEF-*Nlp*$^{-/-}$ or MEF-*Nlp*$^{+/+}$ cells, and cytosolic protein source was HEK293T cell lysis. (Scale bar,100 nm.).

(B) Immunoblotting of HeLa cells that have been transfected with siRNA for 48 hours.

(C) Immunoblotting of stable EGFP-NC HeLa cells and EGFP-Nlp HeLa cells.

(D) Immunoblotting and silver staining assay in HeLa cells that have been transfected with Split-TurboID system plasmids for 36 hours. Cells were labeled without Biotin, with 50 μM biotin or with 50 μM biotin & 100 nM rapamycin for 4 hours before washing and collecting.

(E) Confocal of Split-TurboID system. HeLa cells transfected with Split-TurboID system for 36 hours were treated with or without 50 μM biotin for 1 hour before fixing. (Scale bar, 10 μm.).

(F) Colocalization of SEC31A and Rab1B by LSM980 in HeLa cells that have been transfected with plasmids for 48 hours. (Scale bar, 5 μm. n.s. represented no significance.).

(G) Rab1B purified protein was obtained from Rab1B-GST using Thrombin. After verifications by Coomassie brilliant blue and Western blot, GST pull-down assay was performed using GST-tag purified proteins of Nlp truncates and Rab1B purified protein.

(H) Results of SEC31A-TurboID and Split-TurboID were grouped into three parts: the intersection of the TurboID and split-TurboID as Nlp-specific sorting/transporting proteins; the remaining part of TurboID as non-Nlp-specific sorting/transporting proteins; the remaining part of split-TurboID as Nlp-associated proteins. Figures represented enrichment analysis of Biology Process and WiKi Pathway of Nlp-associated proteins.

(I-J) Results of SEC31A-TurboID and Split-TurboID system. Figures represented enrichment analysis of KEGG Pathway of (I) non-Nlp specific sorting/transporting proteins, (J) Nlp-specific sorting/transporting proteins.

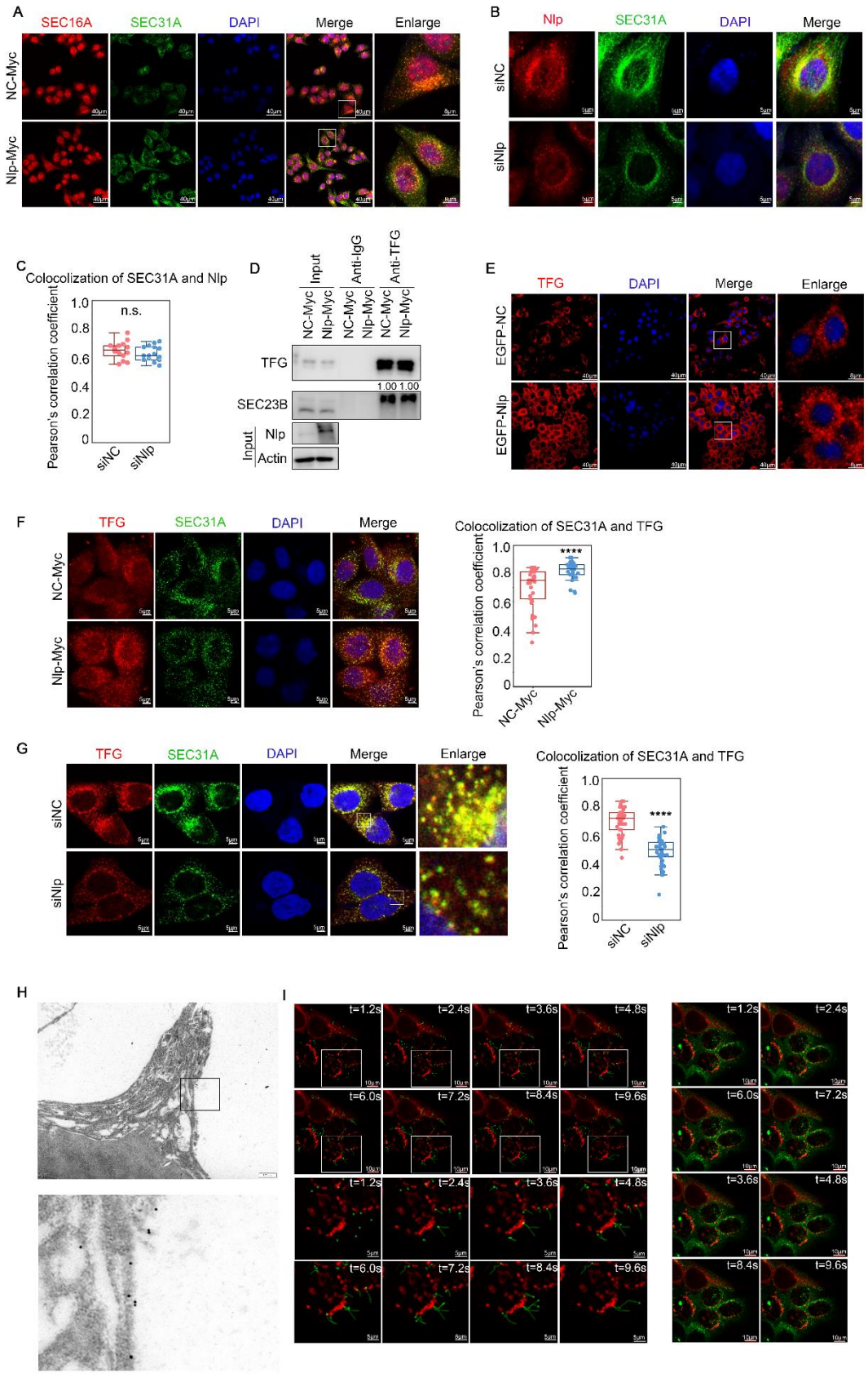


Figure S3. Nlp regulated budding and uncoating of COPII-coated vesicles.

(A) Colocalization of Nlp and SEC16A by LSM780 in HeLa cells that have been transfected with siRNA for 48 hours. (Scale bar, 40 μm or 8 μm.).

(B-C) Colocalization of Nlp and SEC31A by LSM780 in HeLa cells that have been transfected with siRNA for 48 hours. (Scale bar, 5 μm. n.s. represented no significance.).

(D) Co-IP assay using anti-TFG or anti-IgG in HeLa cells that have been transfected with plasmids for 48 hours.

(E) Confocal assay represented the aggregation ability of TFG in HeLa cells that have been transfected with plasmids for 48 hours. (Scale bar, 40 μm or 8 μm.).

(F-G) Colocalization of TFG and SEC31A by LSM780 in HeLa cells that have been transfected with plasmids or siRNA for 48 hours. (Scale bar, 5 μm. ****$p<0.0001$.).

(H) Immunogold TEM labeled by anti-Nlp in HeLa cells. (Scale bar, 500 nm.).

(I) Time lapse of EGFP-Nlp and Golgi-mCherry. (Scale bar, 10 μm or 5 μm.).

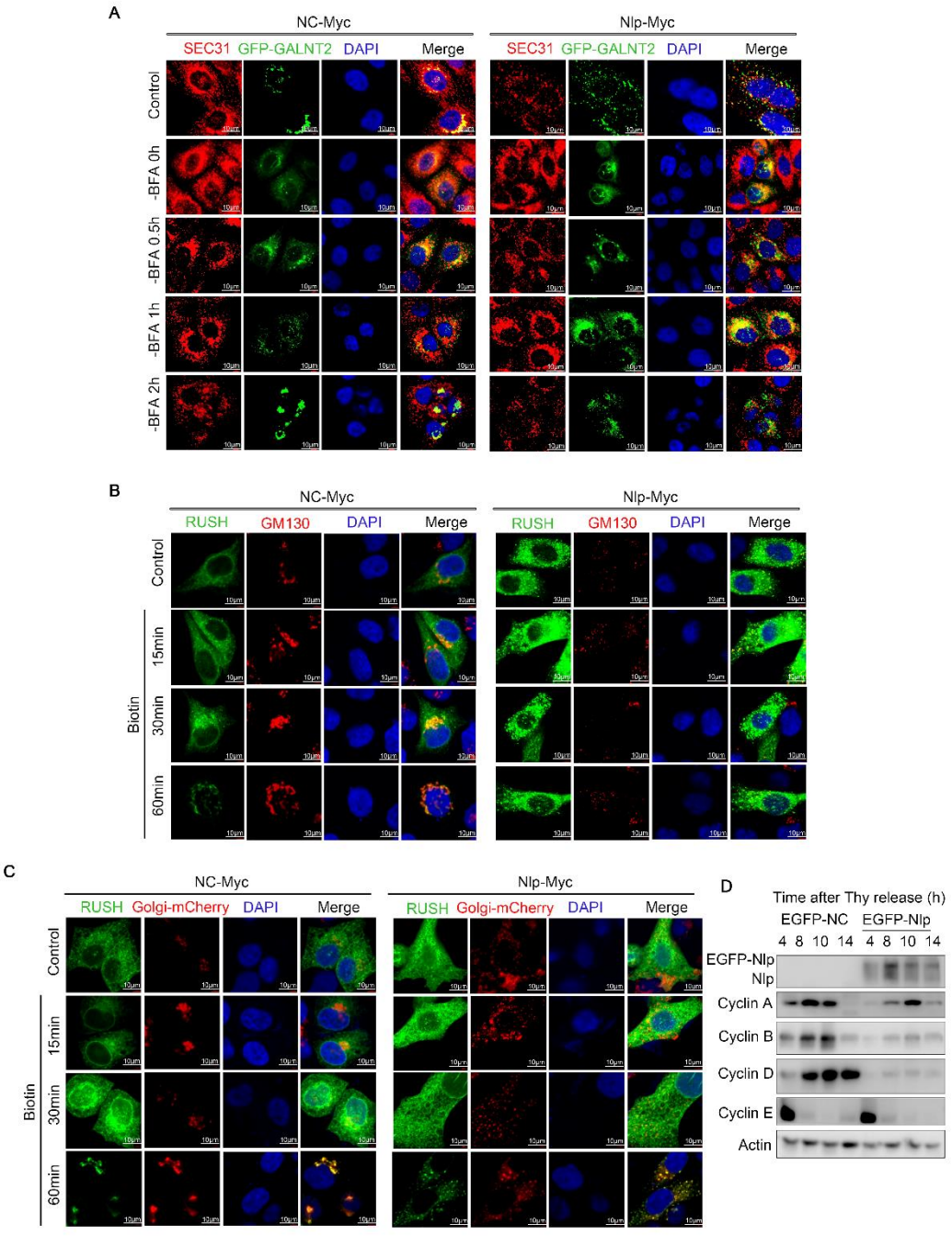


Figure S4. Overexpression of Nlp induced fragmentation of Golgi and changed ER-Golgi vesicle trafficking manner.

(A) Colocalization of GFP-GALNT2 and SEC31A by LSM780 in HeLa cells that have been transfected with GFP-GALNT2 and Nlp-Myc or NC-Myc for 36 hours. After the transfection, cells were treated with 5 μg/mL BFA for 30 min, and removed BFA for different times to capture the movements of vesicles. (Scale bar, 10 μm.).

(B-C) Confocal for RUSH assay. (B) HeLa cells or (C) stable Golgi-mCherry HeLa cells were transfected with Str-KDEL-SBP-GFP plasmid for 36 hours, and then incubated with 80 μM of biotin for different time (0~60 min) for confocal assay. (Scale bar, 10 μm.).

(D) Stable control HeLa-C3 or HeLa-EGFP-Nlp cells were arrested in G1-S boundary and released into fresh medium. Cells were collected every 4 hours for confocal (Figure 6F), and verified by immunoblotting.

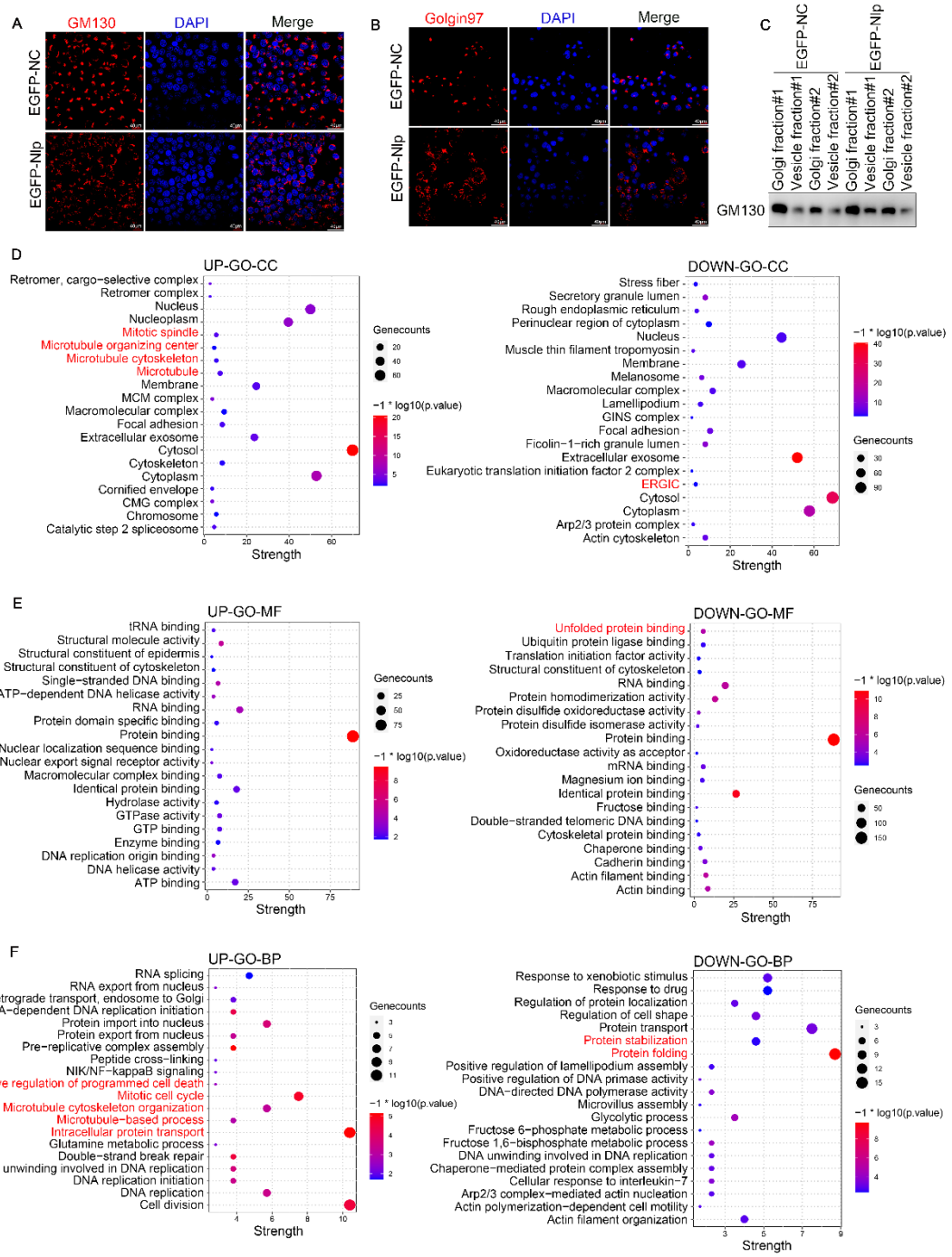


Figure S5. Overexpression of Nlp induced functional fragmentation of Golgi reduced ERGIC component as well as protein stabilization.

(A-B) Confocal of (A) GM130 and (B) Golgin97 in stable EGFP-NC HeLa cells and EGFP-Nlp HeLa cells. (Scale bar, 40 μm.).

(C) Golgi fractions and secretory vesicle fractions were purified from stable control HeLa-C3 or HeLa-EGFP-Nlp cells, and verified by immunoblotting.

(D-F) Golgi apparatus was purified from stable control HeLa-C3 or HeLa-EGFP-Nlp cells for LC-MS/MS. Figures represented the enrichment analysis of upregulated proteins and downregulated protein when Nlp was overexpressed compared to control group (FC=2).

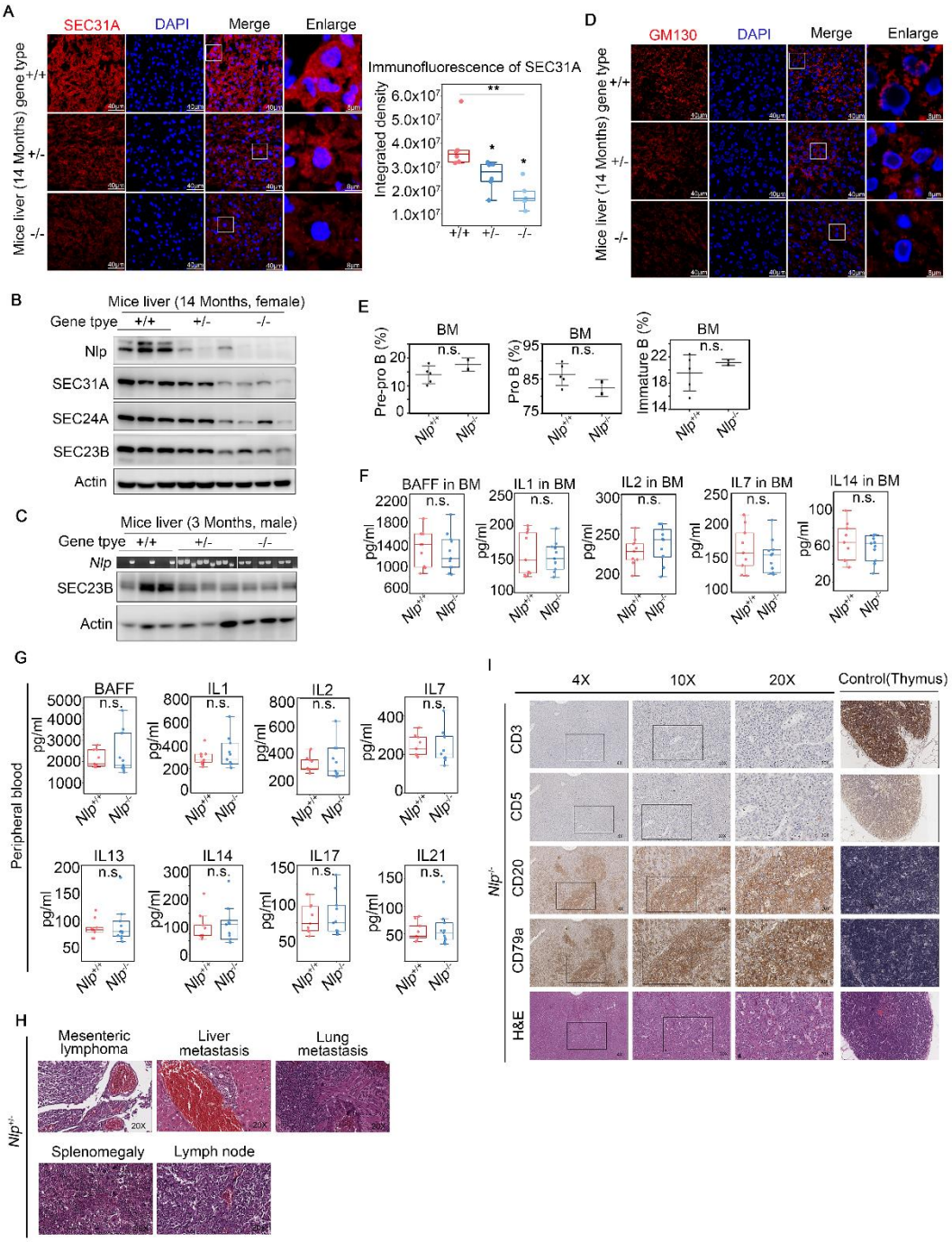


Figure S6. *Nlp* deficient mice displayed spontaneous lymphoma.

(A) Confocal of SEC31A in livers of 14 months $Nlp^{+/+}$ (n=6), $Nlp^{+/-}$ (n=6) and $Nlp^{-/-}$ mice (n=5). (Scale bar, 40 μm or 8 μm. *p<0.05, **p<0.01.).

(B) Immunoblotting of 14 months $Nlp^{+/+}$, $Nlp^{+/-}$ and $Nlp^{-/-}$ female mice livers.

(C) Immunoblotting of 3 months $Nlp^{+/+}$, $Nlp^{+/-}$ and $Nlp^{-/-}$ male mice livers.

(D) Confocal of GM130 in livers of 14 months $Nlp^{+/+}$, $Nlp^{+/-}$ and $Nlp^{-/-}$ mice. (Scale bar, 40 μm or 8 μm.).

(E) Hematologic phenotype of $Nlp^{-/-}$ (n=2) mice and wildtype mice (n=5). (n.s. represented no significance.).

(F-G) Interleukin levels of $Nlp^{-/-}$ mice (n=11) and wildtype mice (n=10). (n.s. represented no significance.).

(H) Representative images of spontaneous lymphoma in $Nlp^{+/-}$ mice.

(I) H&E and IHC images of spontaneous lymphoma in representative $Nlp^{-/-}$ mice. Control group was same with Figure 8K.

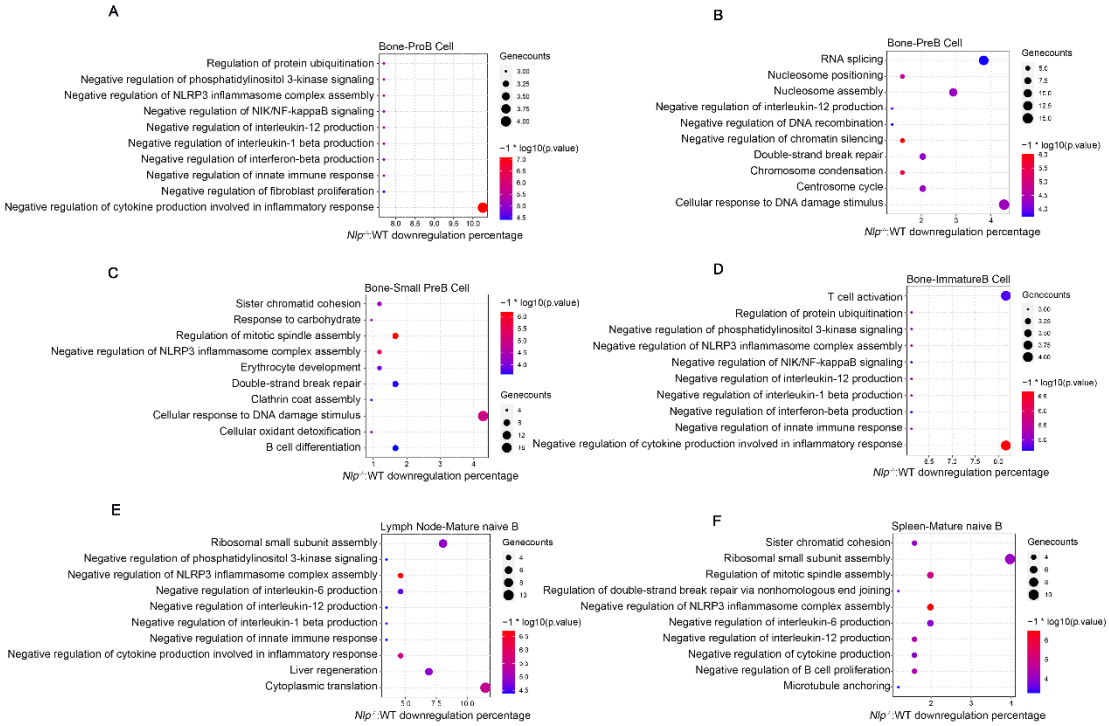


Figure S7. Single cell analysis of *Nlp* deficient mice and wildtype mice.

(A-D) GO analysis of downregulation of (A) Pro-B cells, (B) Pre-B cells, (C) small Pre-B cells and (D) immature B cells in bone marrow of *Nlp*$^{-/-}$ mice compared to wildtype mice.

(E) GO analysis of downregulation of mature naive B cells in lymph node of *Nlp*$^{-/-}$ mice compared to wildtype mice.

(F) GO analysis of downregulation of mature naive B cells in spleen of *Nlp*$^{-/-}$ mice compared to wildtype mice.

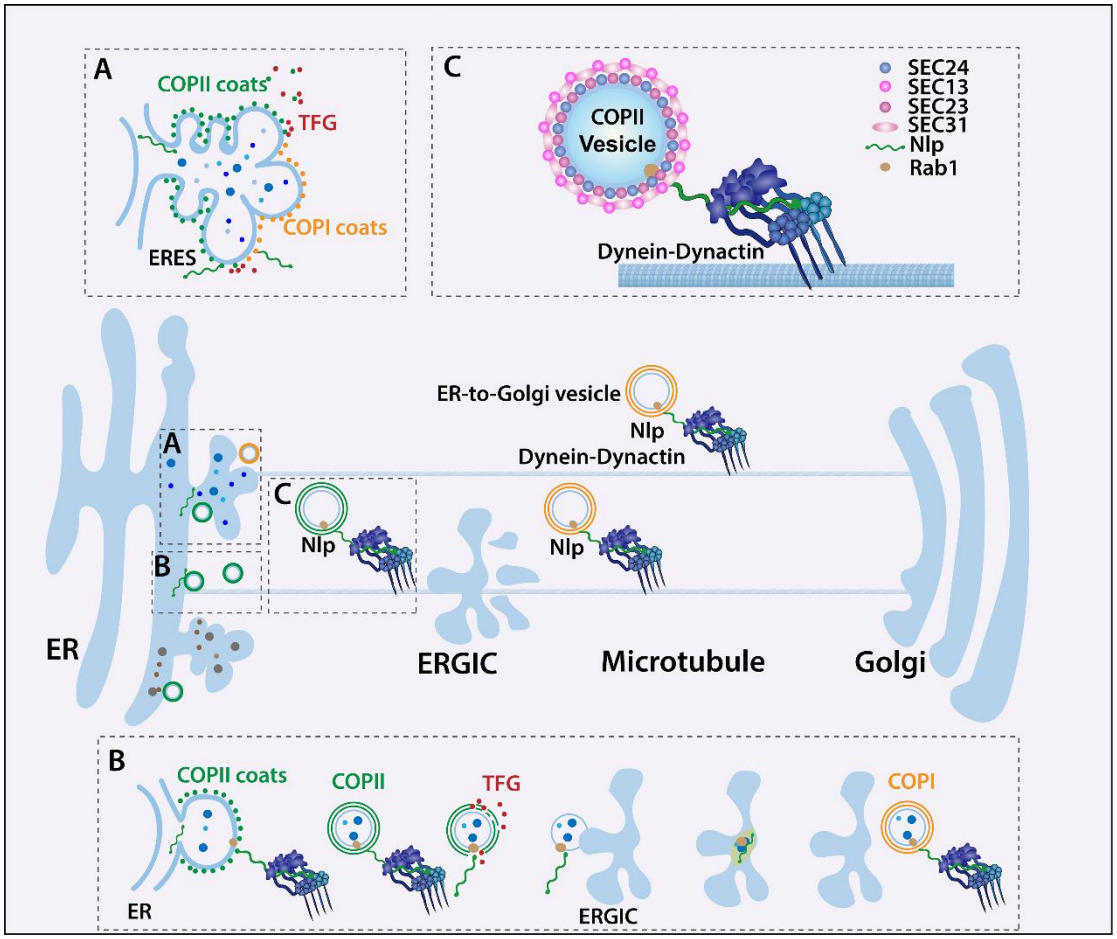
Movie S1. Two-color three-dimensional N-STORM images of Nlp (red) and SEC31A (green) localizations in the HeLa cells.

Movie S2. Two-color three-dimensional N-STORM images of Nlp (red) and SEC24A (green) localizations in the HeLa cells.

Movie S3. FRAP of EGFP-TFG in HeLa cells. Cells that have been transfected with NC-Myc or Nlp-Myc plasmids for 36 hours. The intensity of EGFP-TFG was decreased to 20% using photobleaching and observed its recovery ability for 200s.

Movie S4. Time lapse movement recording of RUSH in HeLa cells. Cells that have been transfected with Str-KDEL-SBP-GFP and NC-Myc or Nlp-Myc plasmids for 36 hours, and then incubated with 80 μM of biotin to detect movement.

Movie S5. Time lapse movement recording of RUSH in HeLa cells. Cells that have been transfected with Str-KDEL-SBP-GFP plasmids and siNC or siNlp for 36 hours, and then incubated with 80 μM of biotin to detect movement.



Graphical abstract. Adapter integrated ER-to-Golgi vesicle transport model. Particular adapter integrated the whole biological processes of cargoes as an invariable code or marker to maintain the specificity, continuity and accuracy of cargoes. Among the adapters, Nlp participated in the forming and transporting of partial ER-to-Golgi vesicles. At the beginning of the vesicle formation, Nlp acted as a platform for the assembly of COPII-coated vesicles, and might participate in cargo selection. During transporting, Nlp cooperated with Rab1 to regulate the continuity of COPII and COPI-coated vesicles transformation, the direction of partial ER-to-Golgi vesicles, as well as the stability of vesicles and transport system.

Supplementary Table 1. Key resources table.

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		

Sec31A rabbit polyclonal antibody	Proteintech	Cat#17913-1-AP
Sec31A mouse monoclinal antibody	Santa Cruz	Cat#sc-376587
Sec24A rabbit polyclonal antibody	Proteintech	Cat#15958-1-AP
Sec24A mouse monoclinal antibody	Santa Cruz	Cat#sc-517155
Sec13 rabbit polyclonal antibody	Proteintech	Cat#15397-1-AP
Sec13 mouse monoclinal antibody	Santa Cruz	Cat#sc-514308
Sec23B rabbit polyclonal antibody	Abcam	Cat#ab151258
TFG mouse monoclinal antibody	proteintech	Cat#66916-1-Ig
TFG rabbit polyclonal antibody	Proteintech	Cat#11571-1-AP
GALNT rabbit polyclonal antibody	Sigma-Aldrich	Cat#HPA011222
GM130 rabbit polyclonal antibody	Abcam	Cat#ab52649
GM130 mouse monoclinal antibody	Abcam	Cat#ab169276
Golgin97 mouse monoclinal antibody	Abcam	Cat#ab169287
Erp72 rabbit polyclonal antibody	Proteintech	Cat#14712-1-AP
Erp72 mouse monoclinal antibody	BD	Cat#610970
Sec61B rabbit polyclonal antibody	CST	Cat#14648S
Sec16A rabbit polyclonal antibody	Proteintech	Cat#20025-1-AP
NINL rabbit polyclonal antibody	Sigma-Aldrich	Cat#HPA000686
Nlp rabbit polyclonal antibody	MBL	N/A
COPG rabbit polyclonal antibody	Proteintech	Cat#12393-1-AP
beta COP rabbit polyclonal antibody	Abcam	Cat#ab2899
COPA mouse monoclinal antibody	Santa Cruz	Cat#sc-398099
COPB mouse monoclinal antibody	Santa Cruz	Cat#sc-393615

COPD mouse monoclinal antibody	Santa Cruz	Cat#sc-515549
STX5 mouse monoclinal antibody	Santa Cruz	Cat#sc-365124
Rab1B rabbit polyclonal antibody	Proteintech	Cat#17824-1-AP
DYNC1H1 Polyclonal Antibody	Proteintech	Cat#12345-1-AP
P150 Glued Polyclonal Antibody	Proteintech	Cat#55182-1-AP
PERK rabbit polyclonal antibody	Proteintech	Cat#20582-1-AP
eIF2α rabbit polyclonal antibody	CST	Cat#5324S
CHOP rabbit polyclonal antibody	Proteintech	Cat#15204-1-AP
peIF2α rabbit polyclonal antibody	CST	Cat#3398S
ATF6 rabbit polyclonal antibody	Proteintech	Cat#24169-1-AP
FAM134B rabbit polyclonal antibody	Proteintech	Cat#21537-1-AP
IRE1α rabbit polyclonal antibody	Proteintech	Cat#27528-1-AP
Sec62 rabbit polyclonal antibody	abcam	Cat#ab244335
CD3e rabbit monoclonal antibody	ThermoFisher	Cat#MA5-14524
CD5 rat monoclonal antibody	ThermoFisher	Cat#MA5-17781
CD20 rabbit monoclonal antibody	ThermoFisher	Cat#PA5-16701
CD79a rabbit monoclonal antibody	ThermoFisher	Cat#MA5-14556
α-Tubulin mouse monoclonal antibody	Proteintech	Cat#66031-1-Ig
β-Actin mouse monoclonal antibody	CST.	Cat#3700
HA-Tag rabbit polyclonal antibody	CST	Cat#3724S
V5-Tag rabbit polyclonal antibody	CST	Cat#13202S
His mouse monoclonal antibody	Santa Cruz	Cat#sc-8036
GST mouse monoclonal antibody	Santa Cruz	Cat#sc-138

TMEM173/STING polyclonal antibody	Proteintech	Cat#19851-1-AP
Phospho-STING (Ser366) antibody	CST	Cat#85735
TBK1/NAK (D1B4) antibody	CST	Cat#3504
Phospho-TBK1/NAK (Ser172) antibody	CST	Cat#5483
IRF-3 (D83B9) antibody	CST	Cat#4302
Phospho-IRF-3 (Ser396) antibody	CST	Cat#4947
SREBP1 rabbit polyclonal antibody	abcam	Cat#ab28481
VEGFA rabbit polyclonal antibody	abcam	Cat#ab52917
beta Catenin polyclonal antibody	Invitrogen	Cat#71-2700
Streptavidin, Alexa Fluor 488 conjugate	ThermoFisher	Cat#S11223
Goat-anti-Rabbit, Alexa Fluor 647	Beyotime Bio.	Cat#A0468
Goat-anti-Mouse, Alexa Fluor 647	Beyotime Bio.	Cat#A0473
Goat-anti-Rabbit, Alexa Fluor 488	Beyotime Bio.	Cat#A0428
Goat-anti-Mouse, Alexa Fluor 488	Beyotime Bio.	Cat#A0423
Goat-anti-Rabbit, Alexa Fluor 594	ZSGB-BIO	Cat#ZF-0516
Goat-anti-Rabbit IgG HRP-polymer	ZSGB-BIO	Cat#PV-6001
Goat-anti-Mouse IgG HRP-polymer	ZSGB-BIO	Cat#PV-6002
Bacterial and virus strains		
BL21(DE3) Chemically Competent Cell	TransGen Biotech	Cat#CD601-02
Trans5α Chemically Competent Cell	TransGen Biotech	Cat#CD201-01
Chemicals, peptides, and recombinant proteins		
Fetal Bovine Serum	Gemini	Cat#A87F82H
OptiPrep™ Density Gradient Medium	Sigma-Aldrich	Cat#D1556

ATP (100mM, Nuclease free)	Beyotime Bio.	Cat#D7378
GTP (100mM, Nuclease free)	Beyotime Bio.	Cat#D7380
Creatine phosphate	Solarbio	Cat#C9921
Creatine phosphokinase	Solarbio	Cat#C9470
HEPES	ThermoFisher	Cat#15630106
PIPES	Beyotime Bio.	Cat#ST470
EGTA	Beyotime Bio.	Cat#ST068
0.25M $CaCl_2$	Beyotime Bio.	Cat#ST365
Digitonin	Sigma-Aldrich	Cat#D141
Paclitaxel	Selleck	Cat#S1150
Polybrene	Genechem	Cat#REVG0001
Lipofectamine 2000	ThermoFisher	Cat#11668019
Neofect	Neofect	Cat#TF201201
Biotin	Sigma-Aldrich	Cat#B4501
Tunicamycin	MCE	Cat#HY-A0098
GSK2606414	Selleck	Cat#S7307
Nocodazole	MCE	Cat#HY-13520
TPE-MI	MCE	Cat# HY-143218
Trimethoprim	MCE	Cat#HY-B0510
Brefeldin A	Selleck	Cat#S7046
Thermo Scientific Pierce Beads	ThermoFisher	Cat#88816
Glutathione High Capacity Magnetic Agarose Beads	Sigma-Aldrich	Cat#G0924
Protein A/G Magnetic Beads	MCE	Cat#HY-K0202

Experimental models: Cell lines		
HeLa cells	This paper	N/A
KYSE450 cells	This paper	N/A
Nlp$^{+/+}$ MEF cells	This paper	N/A
Nlp$^{+/-}$ MEF cells	This paper	N/A
Nlp$^{-/-}$ MEF cells	This paper	N/A
Experimental models: Organisms/strains		
Nlp$^{+/+}$ C57BL/6	In house	N/A
Nlp$^{+/-}$ C57BL/6	In house	N/A
Nlp$^{-/-}$ C57BL/6	In house	N/A
Oligonucleotides		
siRNA, see Table S2	This paper	N/A
qPCR primer, see Table S2	This paper	N/A
Recombinant DNA		
Str-KDEL_ST-SBP-EGFP	(Franck Perez., 2012)	Addgene#65264
C1(1-29)-TurboID-V5_Pcdna3	(Alice Ting., 2018)	Addgene#107173
pLX304 CMV FKBP-V5-sTurboID (N)	(Alice Ting., 2020)	Addgene#153002
pLX304 CMV HA-HaloTag-FRB-sTurboID	(Alice Ting., 2020)	Addgene#153003
TOP-H2B-YFP-DD	(Han et al., 2014)	Addgene#96891
SEC31A-TurboID-V5_Pcdna3	This paper	N/A
pLX304 CMV HA-HaloTag-Sec31A-FRB-split-TurboID(C)	This paper	N/A

pLX304 CMV NINL-FKBP-split-TurboID(N)	This paper	N/A
pEGFP-C3-Nlp	This paper	N/A
pEGFP-C3-NC	This paper	N/A
Golgi-mCherry	Inovogen	Cat#KLV3500
pCS+MT-Nlp-Myc	This paper	N/A
pCS+MT-NC-Myc	This paper	N/A
pEGFP-TFG	This paper	N/A
GFP-GalNAc-T2	SinoBio	Cat#HG13764
pET28A-Nlp-6*His	This paper	N/A
pGEX-4T-1-SEC31A	This paper	N/A
pGEX-4T-1-SEC24A	This paper	N/A
pGEX-4T-1-SEC23B	This paper	N/A
pGEX-4T-1-SEC13	This paper	N/A
pGEX-4T-1-Rab1B	This paper	N/A
Software and algorithms		
GraphPad Prism	GraphPad Software	
R	R project	
ImageJ (Fiji)	NIH	
FlowJo	FlowJo	
Nikon Elements	Nikon	
ZEN Zeiss	Zeiss	
OriginPro	OriginLab	

Supplementary Table 2. List of oligonucleotides used in this study.

Name	Sequence (5'-3')
si-h-NINL_101	CGCGCTACTTCTAGAGTCT
si-h-NINL_102	GGAGTTCCATAGACTTTCT
si-h-NINL_103	AGTTCGCGCTCTCAACAAA
si-h-SEC31A_101	GATGCAACATTTAGTACGA
si-h-SEC31A_102	GCATGGCCGATGCCATTAT
si-h-SEC31A_103	GGACACTAGTACTGTAGGA
m-NINL-1-F	CTCCCCTCCCCATTTTCTTAC
m-NINL-2-R	CAGTGGTCCAGGCTCTAGTTTTG
m-NINL-3-F	GAGGGTTTATTGGATACACGG
m-NINL-4-R	AACAGCACCTTTGATGCCTAC
m-NINL-1a-F	TGGGATCTGATTCCCTCTTCT