# Expanded View Figures

**Figure EV1. TRIM16 mediates IL-1β secretion.**

A Knockdown efficacies as determined by immunoblots.
B LDH release data for samples in Fig 1A.
C, D Levels of (C) IL-1β and (D) LDH release were determined in supernatants from THP-1 cells subjected to knockdowns as indicated, treated with 100 ng/ml LPS overnight, then starved in EBSS for 3 h.
E LDH release results for samples in Fig 1C.
F Co-IP analysis of interactions between flag-TRIM16 with myc-pro-IL-1β in HEK293T cells.
G Immunoblot analyses of knockdown efficacies in THP-1 cells.
H LDH release data for samples in Fig 1E.
I Immunoblot analyses of the levels of mIL-1β in supernatants from THP-1 cells that were subjected to knockdown as indicated and were sequentially treated with LPS and LLOMe.
J LDH release data from samples in Fig 1F.
K, L Levels of (K) IL-1β and (L) LDH release was determined in supernatants from THP-1 cells subjected to knockdown as indicated, treated with 100 ng/ml LPS overnight, then starved in EBSS for 3 h.
M Immunoblot analyses of knockdown efficacies in primary human MDM cells.
N LDH release results for samples in Fig 1G.
O Intracellular localization analysis of TRIM16, pro-IL-1β, and LAMP2 by confocal microscopy. Cells, THP-1. Line tracings correspond to arrows. Scale bars, 5 μm.

Data information: Means ± SEM; $n \geq 5$, except for immunoblot quantifications and TRIM screen where $n \geq 3$. $^{*}P < 0.05$, $^{\dagger}P \geq 0.05$ (*t*-test for N; ANOVA for B, C, D, H, J, K, L).

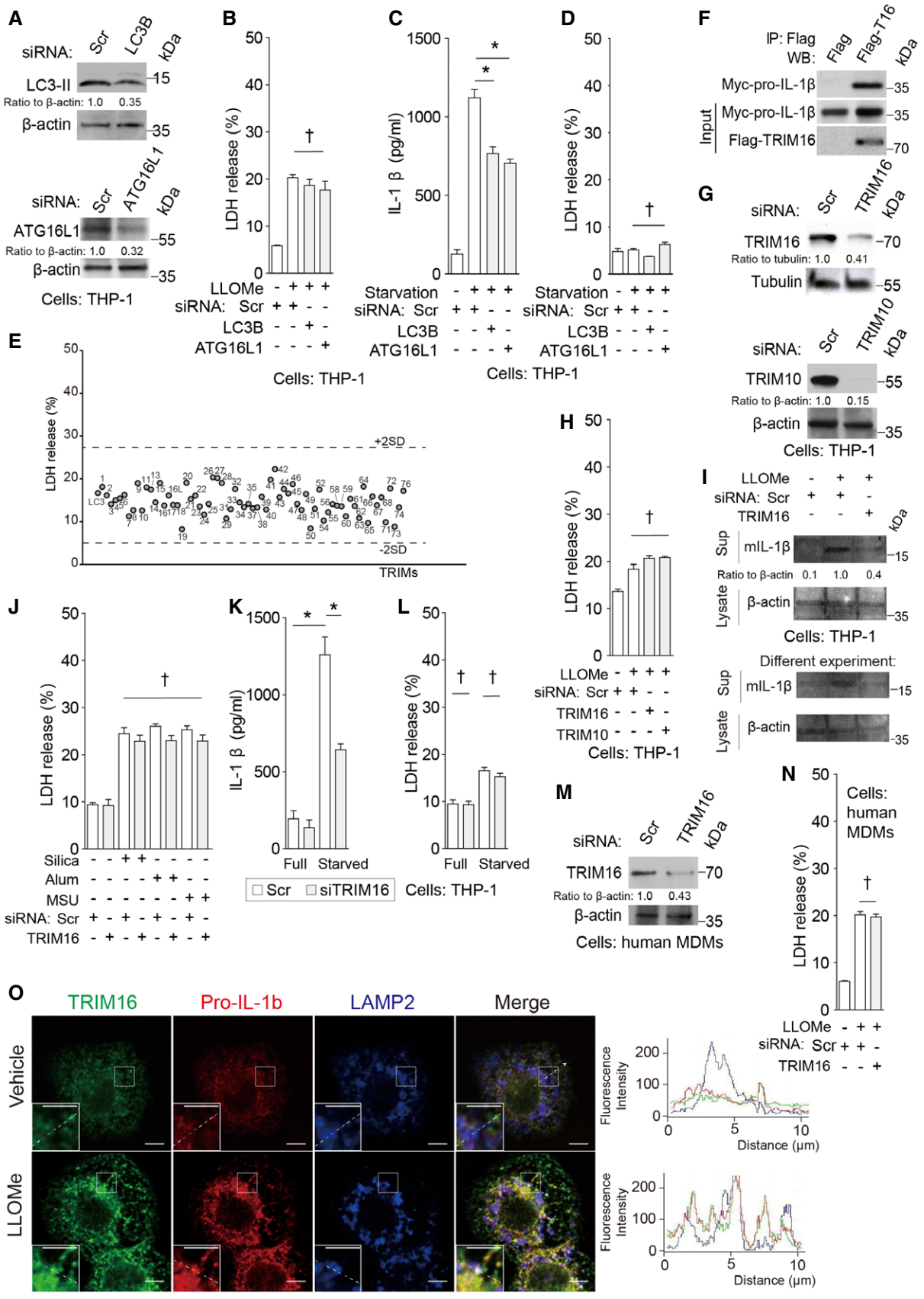
A
siRNA: Scr LC3B kDa
LC3-II
Ratio to β-actin: 1.0 0.35
β-actin
siRNA: Scr ATG16L1 kDa
ATG16L1
Ratio to β-actin: 1.0 0.32
β-actin
Cells: THP-1
B
LDH release (%)
LLOMe
siRNA: Scr
LC3B
ATG16L1
C
IL-1 β (pg/ml)
Starvation
siRNA: Scr
LC3B
ATG16L1
Cells: THP-1
D
LDH release (%)
Starvation
siRNA: Scr
LC3B
ATG16L1
Cells: THP-1
E
LDH release (%)
+2SD
-2SD
TRIMs
Cells: THP-1
F
IP: Flag
WB: Flag Flag-T16 kDa
Myc-pro-IL-1β
Input
Myc-pro-IL-1β
Flag-TRIM16
G
siRNA: Scr TRIM16 kDa
TRIM16
Ratio to tubulin: 1.0 0.41
Tubulin
siRNA: Scr TRIM10 kDa
TRIM10
Ratio to β-actin: 1.0 0.15
β-actin
Cells: THP-1
H
LDH release (%)
LLOMe
siRNA: Scr
TRIM16
TRIM10
Cells: THP-1
I
LLOMe
siRNA: Scr
TRIM16
kDa
Sup
mIL-1β
Ratio to β-actin 0.1 1.0 0.4
Lysate
β-actin
Cells: THP-1
Different experiment:
Sup
mIL-1β
Lysate
β-actin
J
LDH release (%)
Silica
Alum
MSU
siRNA: Scr
TRIM16
K
IL-1 β (pg/ml)
Full Starved
Scr siTRIM16
Cells: THP-1
L
LDH release (%)
Full Starved
Cells: THP-1
M
siRNA: Scr TRIM16 kDa
TRIM16
Ratio to β-actin: 1.0 0.43
β-actin
Cells: human MDMs
N
Cells: human MDMs
LDH release (%)
LLOMe
siRNA: Scr
TRIM16
O
TRIM16
Pro-IL-1b
LAMP2
Merge
Vehicle
LLOMe
Fluorescence Intensity
Distance (μm)

**Figure EV1.**

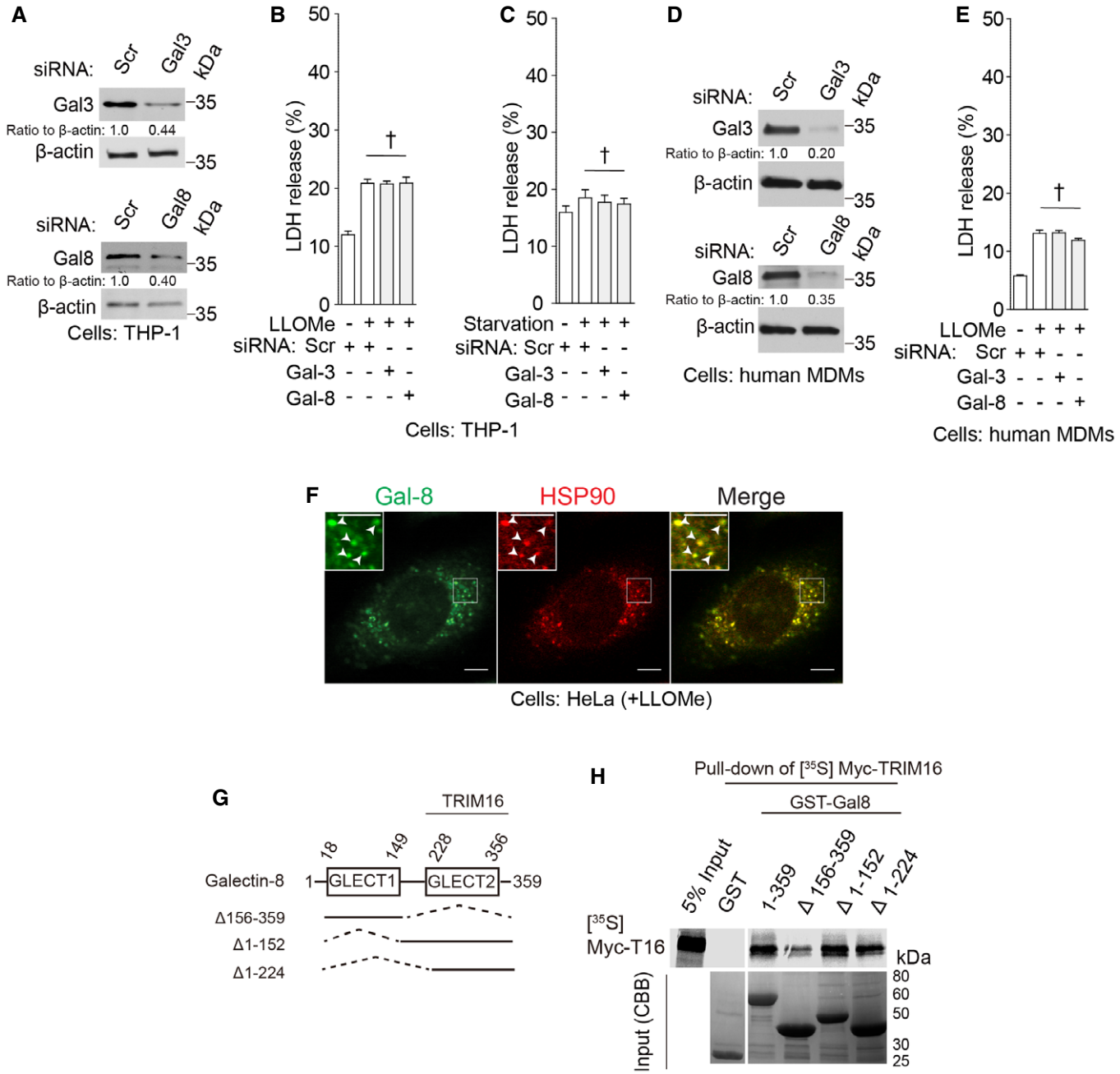


**Figure EV2. Galectin-8 participates in IL-1β secretion and interacts with TRIM16.**

A Immunoblot analyses of knockdown efficacies in THP-1 cells.

B, C LDH release data for samples in (B) Fig 2A and (B) Fig 2B.

D Immunoblot analyses of knockdown efficacies in primary human MDMs.

E LDH release data for samples in Fig 2C.

F Confocal microscopy of HeLa cells treated with LLOMe and stained for galectin-8 and HSP90. Arrowheads in enlarged insets indicate colocalization. Scale bars, 5 μm.

G Galectin-8 domains and deletion constructs used.

H *In vitro* translated and radiolabeled [$^{35}$S] myc-HA-TRIM16 was incubated with full-length- and deletions of GST-galectin-8 in the presence of flag-ULK1 and cold ATP, and GST pull downs were performed and [$^{35}$S] radiolabeled Myc-HA-TRIM16 detected by SDS-PAGE and autoradiography. Amounts of GST fusion proteins are shown in Coomassie brilliant blue (CBB)-stained gels.

Data information: Means ± SEM; $n \geq 5$, except for immunoblot quantifications where $n \geq 3$. $^{\dagger}P \geq 0.05$ (ANOVA for B, C, E).

**Figure EV3. TRIM16 recruits IL-1β to LC3-positive carrier membranes for secretion and motifs in TRIM16 and Sec22b.**

A, B IL-1β levels in supernatants of HeLa cells reconstituted with flag-pro-IL-1β and myc-caspase-1 and treated as indicated.

C CRISPR TRIM16 mutant A9 in HeLa cells (Chauhan *et al*, 2016).

D Processing of pro-IL1β detected by immunoblotting of lysates from HeLa cells (wild type and TRIM16$^{KO}$) reconstituted with pro-IL-1β and pro-caspase-1 by transfection and treated with LLOMe in the presence of autolysosomal/lysosomal degradation inhibitors E64D plus pepstatin. Ratios of mIL-1β to tubulin levels are shown below the blots.

E Overlapping area (high content microscopy and processing) of GFP-Sec22b and LC3B in HeLa cells fed or starved in EBSS.

F Alignments of the SNC1 domain of TRIM16, shown as they are annotated in the NCBI entry NP_006461, where TRIM16 SNC1 domain is compared with R-SNAREs, including longin-containing SNAREs, in NCBI CDD:227472 (http://www.ncbi.nlm.nih.gov/Structure/cdd/cddsrv.cgi?uid=227472). Note that the SNC1 domain CDD: 227472 includes sequences from both longin and SNARE domains and that only the SNARE domains contain typical "0" ionic layers (the boxed R residues). Underlined residues, features of the SNARE motif in *Saccharomyces cerevisiae* (Sc) Snc1, with flanking layers maintaining hydrophobic interactions when this SNARE is in the four-helix bundle SNARE fusion complex; the N-terminal flanking parts encompass all layers of the SNARE motif of SNC1, whereas the C-terminal part shown encompasses a half of C-terminally positioned hydrophobic layers.

G Domain organization of TRIM16 and Sec22b. The relationship between the NCBI-annotated domains (boxes) and predicted coiled-coil regions of TRIM16 (overlined regions; as annotated in UniProtKB – O95361) are indicated. The relationships between NCBI-annotated SNC1 motif (underlined region) and the NCBI-annotated longin and SNARE domains of Sec22b are shown.

H, I LDH release controls corresponding to IL-1β secretion complementation experiments of TRIM16$^{KO}$ cells with TRIM16 Sec22b-interacting and its Sec22b-nonbinding mutant shown in Fig 3M and N.

J Sub-regions selected for cross-correlation analysis of flag-TRIM-16 and GFP-Sec22b super-resolution data.

Data information: Means ± SEM; $n \geq 5$, except for immunoblot quantifications and image analyses where $n \geq 3$. $^{*}P < 0.05$, $^{\dagger}P \geq 0.05$ (*t*-test for A, B, E; ANOVA for H, I).

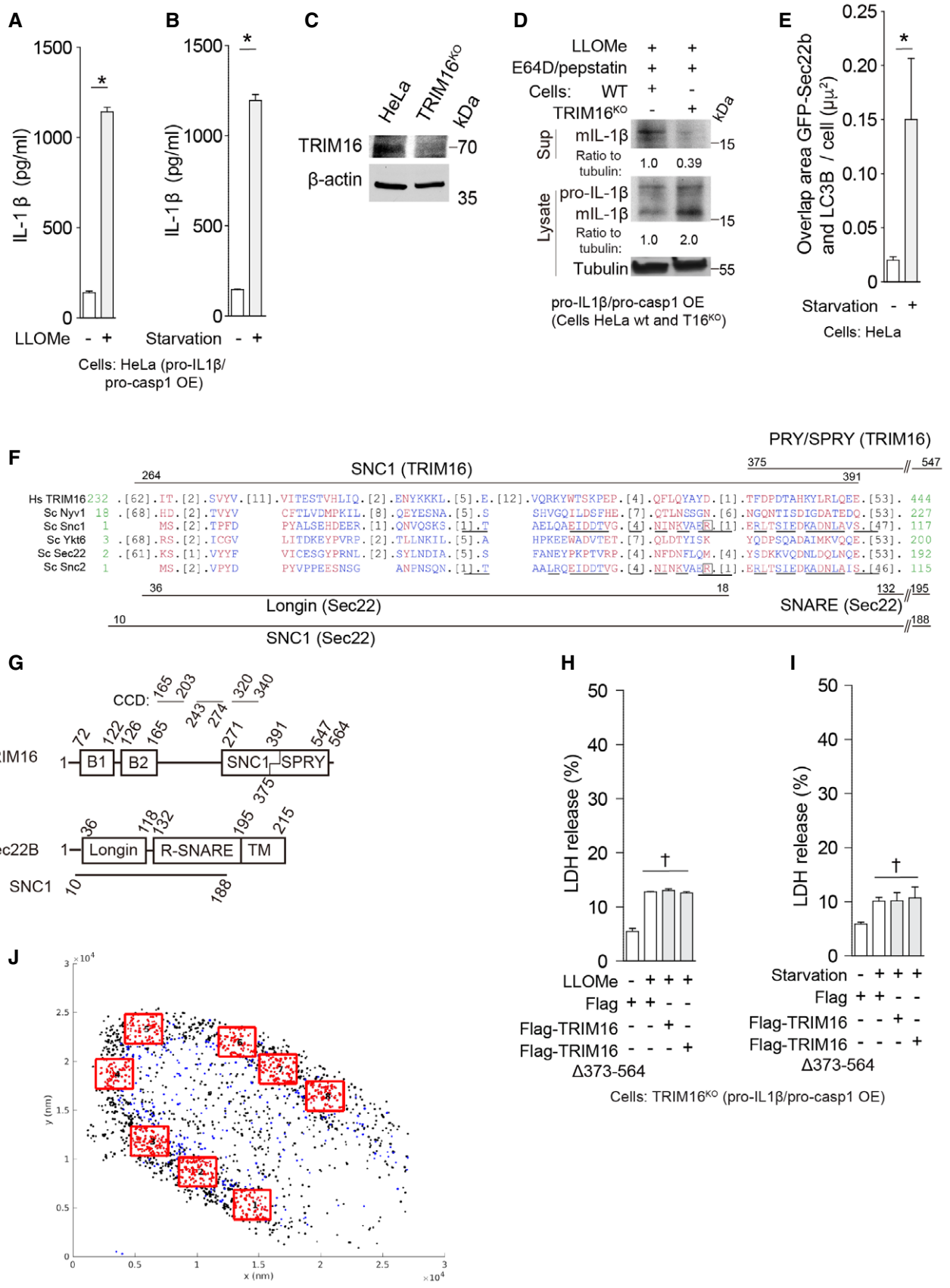


**Figure EV3.**

**Figure EV4. R-SNARE Sec22b participates in IL-1β secretion.**

A HeLa cells subjected to knockdown as indicated were treated with LLOMe, and high content analysis of LC3 puncta formation in response to LLOMe was performed using a Cellomics HCS scanner (epifluorescence) and iDEV software. Masks, software-defined objects (primary objects, cell outlines; internal secondary objects, LC3 puncta). Scale bar, 5 μm.

B Average count of LC3 puncta per cell illustrated in (A), carried out in 96-well plates with 49 fields per well and 500 primary objects per field.

C Immunoblot analyses of knockdown efficacies in THP-1 cells.

D, E LDH release data for samples in (D) Fig 4A and (E) Fig 4B.

F Immunoblot analyses of knockdown efficacies in human primary macrophages (MDM).

G LDH release data for samples in Fig 4C.

H–K IL-1β levels or LDH release in supernatants of either wild-type or ATG9 knockout MEFs reconstituted with flag-pro-IL-1β and myc-caspase-1 and treated as indicated.

L, M HeLa cells transfected with mature IL-1β (mIL-1β) were subjected to siRNA knockdowns and IL-1β release measured after LLOMe stimulation; blot shows Sec22b knockdown efficiency.

N–Q IL-1β or LDH release from HeLa cells overexpressing GFP-sec22b or GFP and mature mIL-1β upon stimulation by LLOMe or starvation.

R Immunoblot analyses of knockdown efficacies in HeLa cells stably expressing mRFP-GFP-LC3 (tandem HeLa) configured for simultaneous IL-1β secretion measurements by expressing flag-pro-IL-1β and myc-caspase-1.

Data information: Means ± SEM; $n \geq 5$, except for immunoblot quantifications where $n \geq 3$. $^{*}P < 0.05$, $^{\dagger}P \geq 0.05$ (*t*-test for G–L, N–Q; ANOVA for B, D).

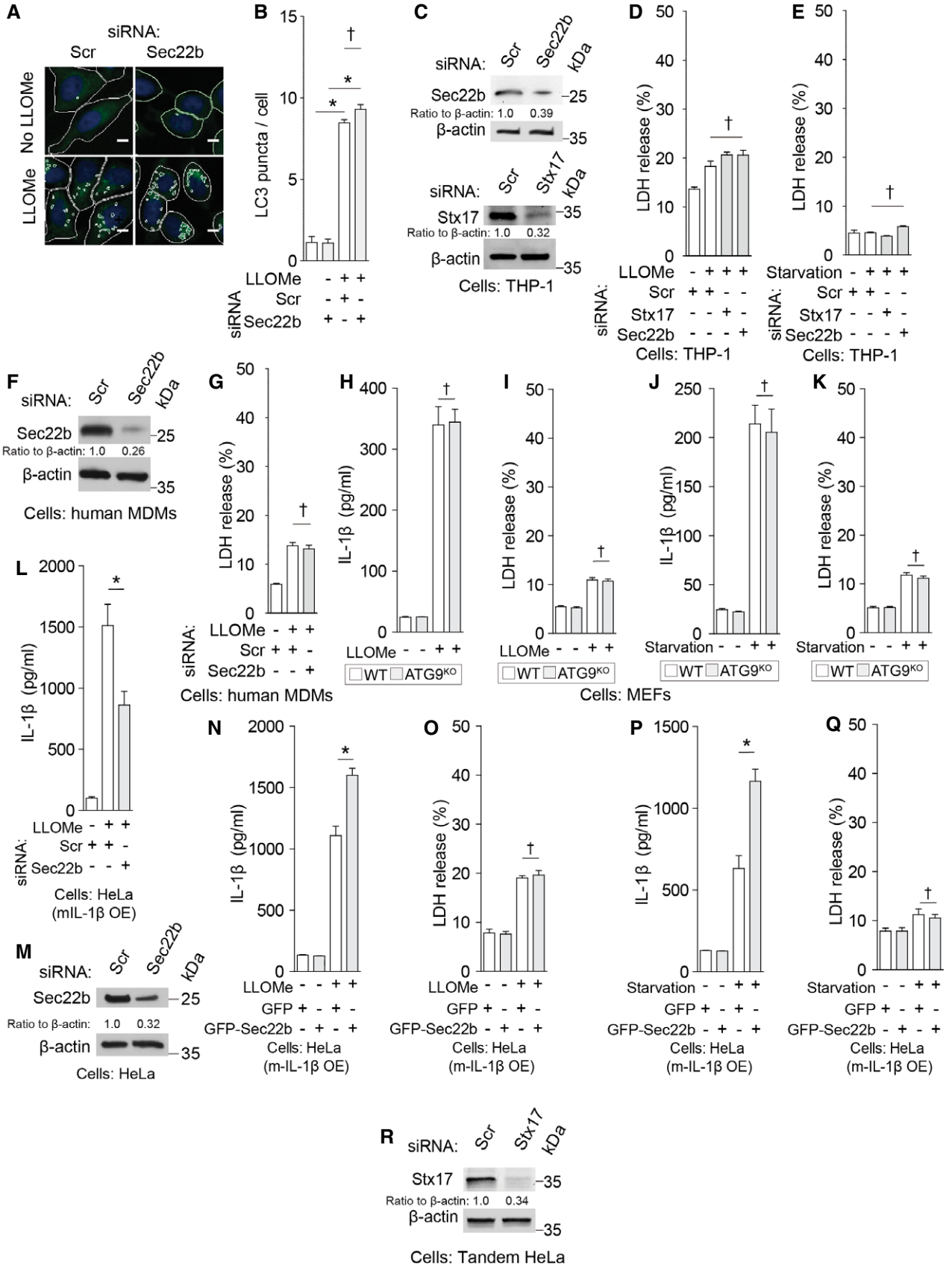


**Figure EV4.**

**Figure EV5. $Q_{bc}$-SNAREs and plasma membrane $Q_a$-SNAREs syntaxins 3 and 4 participate in IL-1β secretion.**

A Immunoblot analyses of knockdown efficacies in THP-1 cells.
B, C LDH release data for (B) Fig 5A and (C) Fig 5B.
D Immunoblot analyses of knockdown efficacies in primary human MDM cells.
E, F Levels of (E) IL-1β and (F) LDH release determined in supernatants from primary human MDM cells that were subjected to knockdowns as indicated and were sequentially treated with LPS and LLOMe.
G Immunoblot analyses of knockdown efficacies in HeLa cells.
H, I Levels of (H) IL-1β and (I) LDH release were determined in supernatants from HeLa cells reconstituted for IL-1β secretion and treated with LLOMe.
J, K Levels of (J) IL-1β and (K) LDH release were determined in supernatants from HeLa cells reconstituted for IL-1β secretion and starved in EBSS.
L Co-IP analysis between SNAP-23 with Sec22b in lysates from HeLa cells treated with NEM and DTT as indicated.
M Immunoblot analyses of knockdown efficacies in THP-1 cells.
N LDH release data from Fig 5C.
O Immunoblot analyses of knockdown efficacies in primary human MDM cells.
P, Q Levels of (P) IL-1β and (Q) LDH release determined in supernatants from primary human MDM cells that were subjected to knockdowns as indicated and were sequentially activated with LPS and treated with LLOMe.
R Conventional autophagy response to starvation measured by LC3 puncta (HC imaging and analysis) in HeLa cells knocked down as indicated.
S Knockdown efficacies in cells corresponding to panel (R).

Data information: Means ± SEM; $n \geq 5$, except for immunoblot quantifications where $n \geq 3$. $^{*}P < 0.05$, $^{\dagger}P \geq 0.05$ (*t*-test for P, Q; ANOVA for B, C, E, F, H–K, N, R).

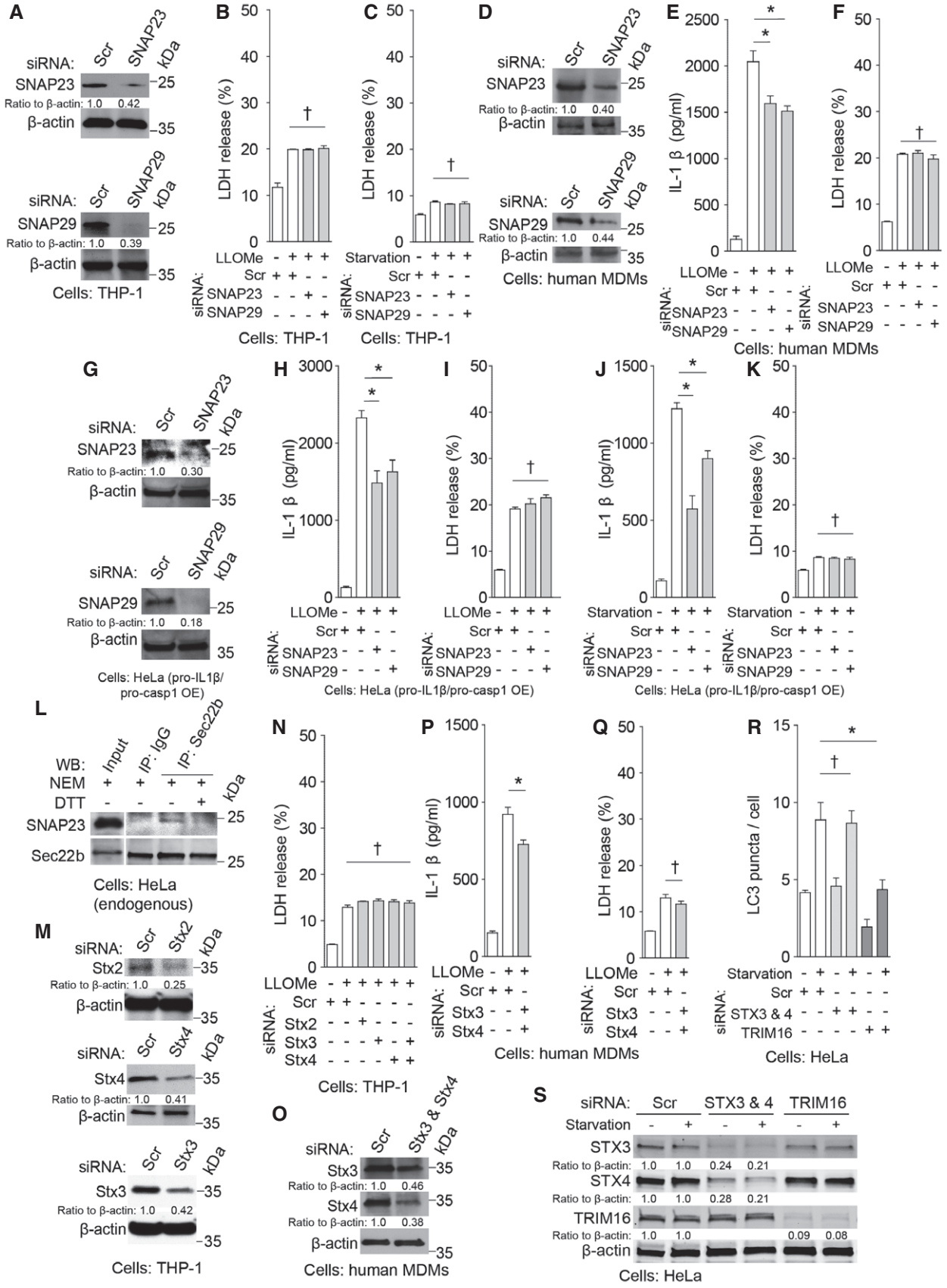


**Figure EV5.**

**Figure EV6. Secretory autophagy machinery plays a role in unconventional secretion of ferritin.**

A Immunoblot analysis of the levels of FTH1 in supernatants from THP-1 cells that were subjected to knockdown as indicated and were sequentially treated with LPS and LLOMe.

B Ferritin levels in supernatants of primary human MDM macrophages subjected to knockdowns as indicated and treated sequentially with LPS and LLOMe.

C Ferritin levels in supernatants of THP-1 cells subjected to knockdowns as indicated and infected with *M. tuberculosis* Erdman as indicated.

D, E (D) Ferritin levels and (E) LDH release in supernatants of primary human MDM cells subjected to knockdowns as indicated and infected with *M. tuberculosis* as indicated.

F, G (F) Ferritin levels and (G) LDH release in supernatants of primary human MDM cells infected with *M. tuberculosis* as indicated.

H LDH release data from Fig 6E.

I, J Immunoblot analyses of knockdown efficacies in THP-1 cells.

K, L Levels of (K) IL-1β and (L) LDH release determined in supernatants from THP-1 cells subjected to knockdowns as indicated and were sequentially treated with LPS and LLOMe.

M Co-IP analysis of interaction between FTH1 with GFP-TRIM16, GFP-galectin-8, and GFP-Sec22b.

Data information: Means ± SEM; $n \geq 5$, except for immunoblot quantifications where $n \geq 3$. *$P < 0.05$, $^{\dagger}P \geq 0.05$ (vs. LLOMe-treated or Mtb-infected scr control or indicated) (*t*-test for F–H, K, L; ANOVA for B–E).

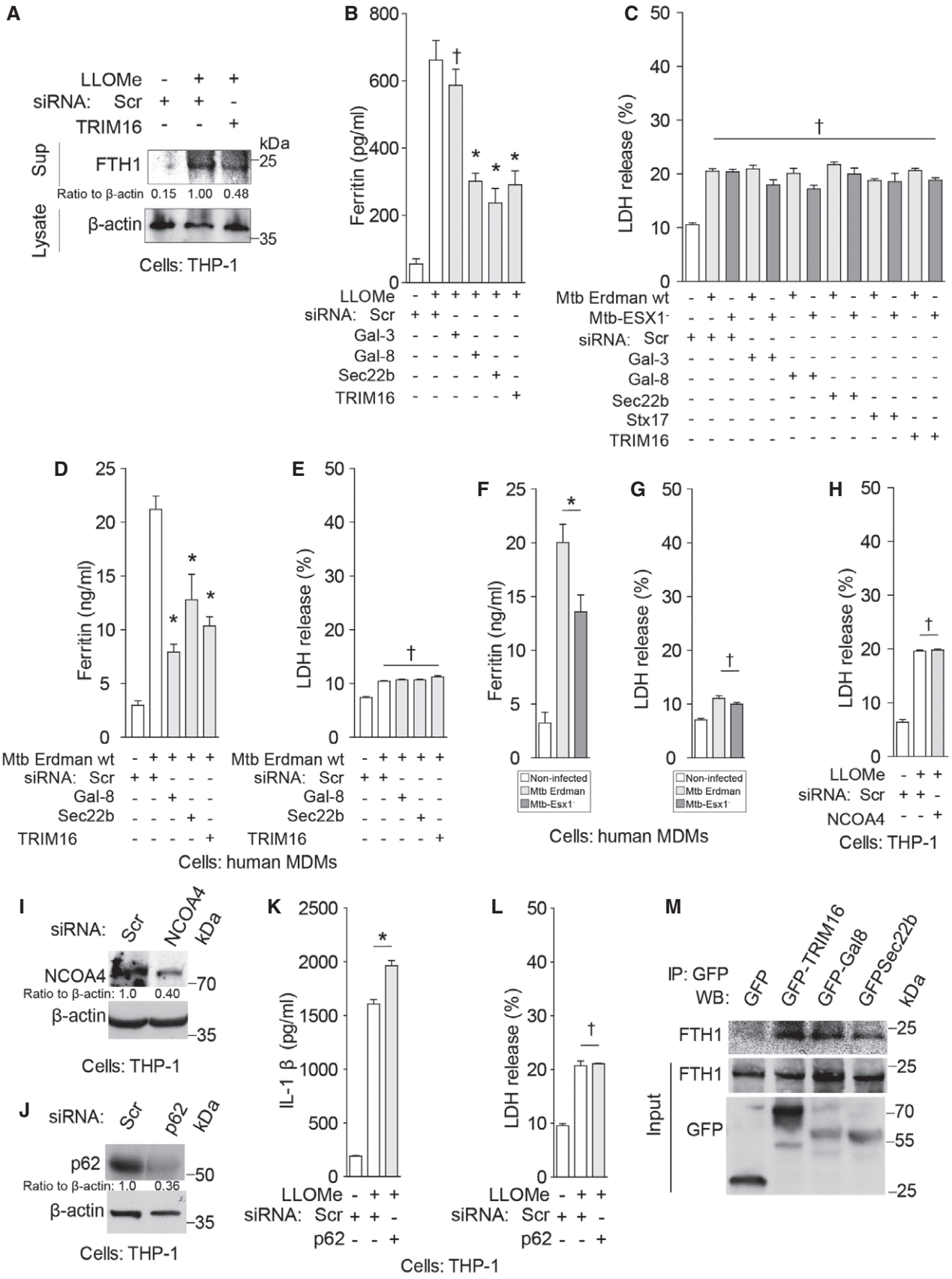


**Figure EV6.**