Supplementary information:

Endocytosis of Extracellular Vesicles and Release of Their Cargo from Endosomes

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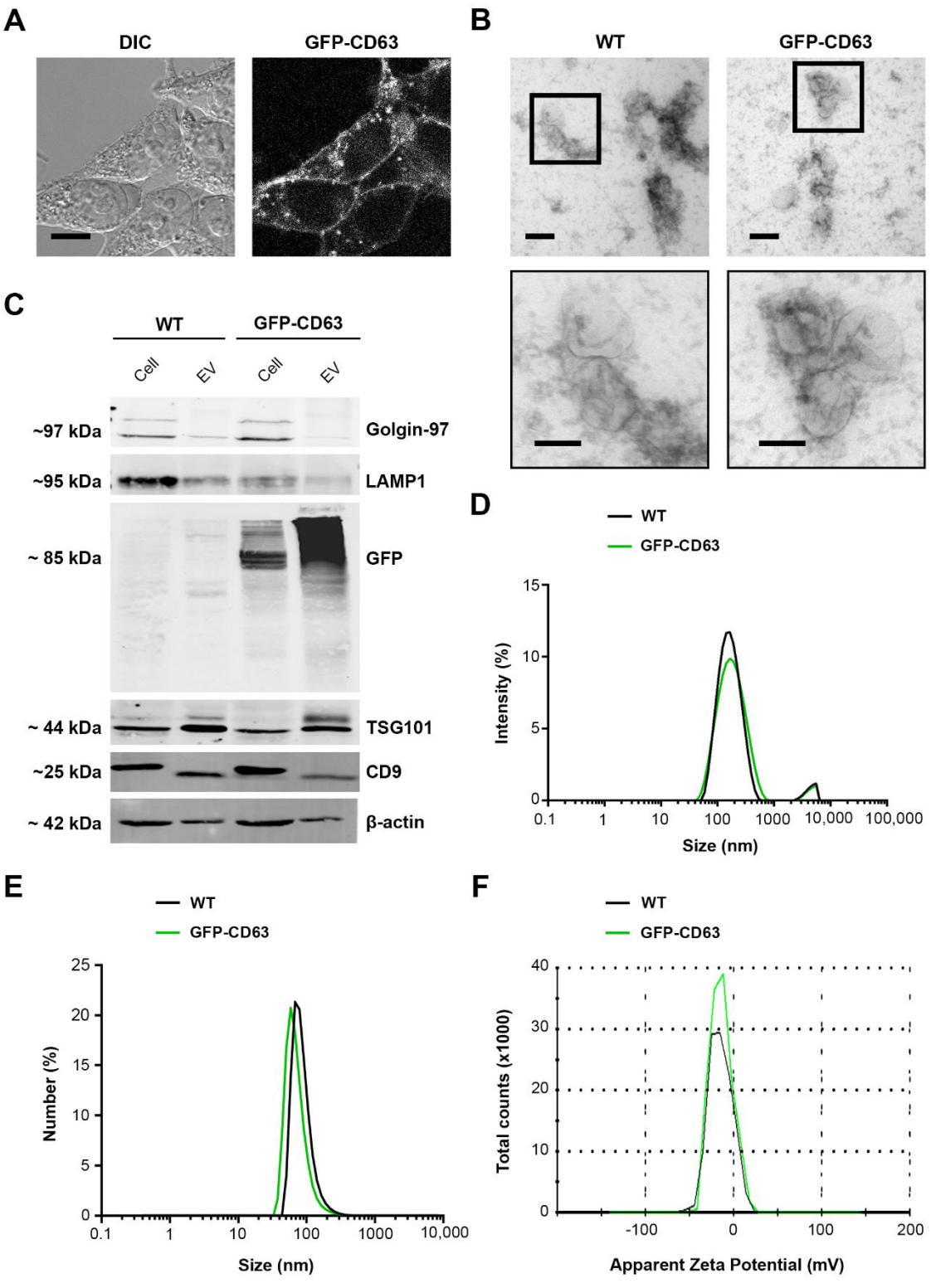
Contents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DIC
GFP-CD63
B
WT
GFP-CD63
C
WT
GFP-CD63
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EV
Cell
EV
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LAMP1
~ 85 kDa
GFP
~ 44 kDa
TSG101
~25 kDa
CD9
~ 42 kDa
β-actin
D
WT
GFP-CD63
Intensity (%)
Size (nm)
E
WT
GFP-CD63
Number (%)
Size (nm)
F
WT
GFP-CD63
Total counts (x1000)
Apparent Zeta Potential (mV)

Figure S1. Biophysical characterization of WT EVs and GFP-CD63 EVs isolated from HEK293T cells and GFP-CD63 HEK293T cells, respectively. (A) HEK293T cells expressing GFP-CD63. Note the punctate staining pattern, resembling endosomal localization. (Scale bars, 10 μm.) (B) Top: TEM of purified WT and GFP-CD63 EVs derived from HEK293T cells. Bottom: higher magnification of boxed areas. (Scale bars, top 2 μm, bottom 0.1 μm.) (C) Western blot analysis of lysates of WT and GFP-CD63 HEK293T producer cells and isolated EVs (30 μg protein per lane). GFP is only present in GFP-CD63 cells and EVs. EV marker TSG101 is enriched in both WT and GFP-CD63 EVs relative to cells. EV markers CD9 and LAMP1 are present in both EV types. EV-negative marker golgin-97 is depleted from both EV types relative to cells. β-actin: loading control for cell lysates. *EV*: EV lysate; *Cell:* whole cell lysate. (D) Size distribution by intensity of purified WT and GFP-CD63 EVs derived from HEK293T determined by Malvern zeta sizer. WT and GFP-CD63 EVs have an average diameter of 159.7 ± 3.4 nm and 163 ± 0.9 nm and polydispersity index of 0.264 ± 0.021 and 0.276 ± 0.027, respectively. (E) Size distribution by number of purified WT and GFP-CD63 EVs derived from HEK293T determined by Malvern zeta sizer. (F) Zeta potential (surface charge) profiles of purified WT and GFP-CD63 EVs determined by Malvern zeta sizer. GFP-CD63 overexpression does not alter size and surface charge of EVs.

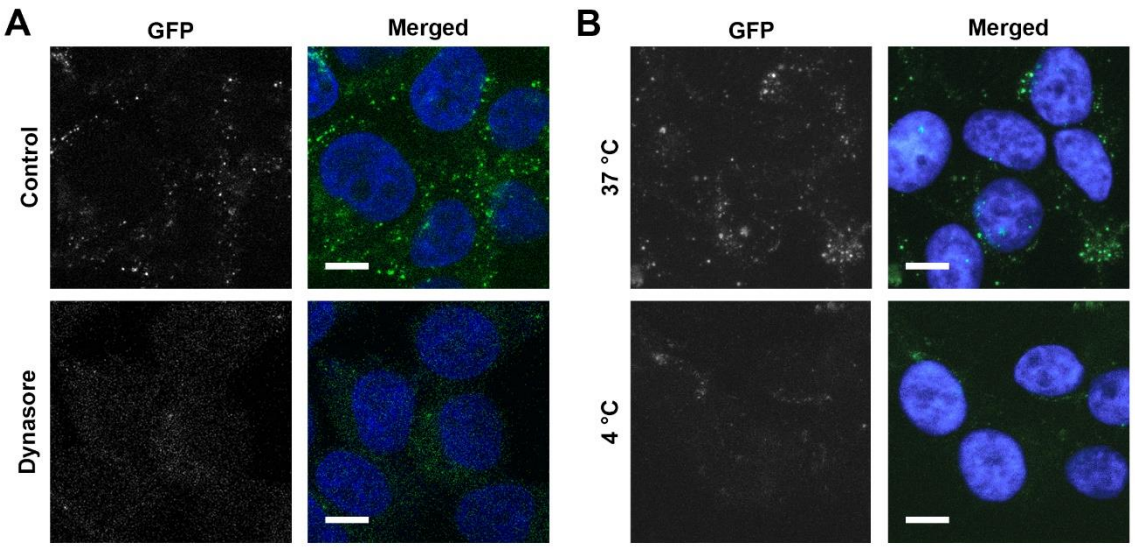
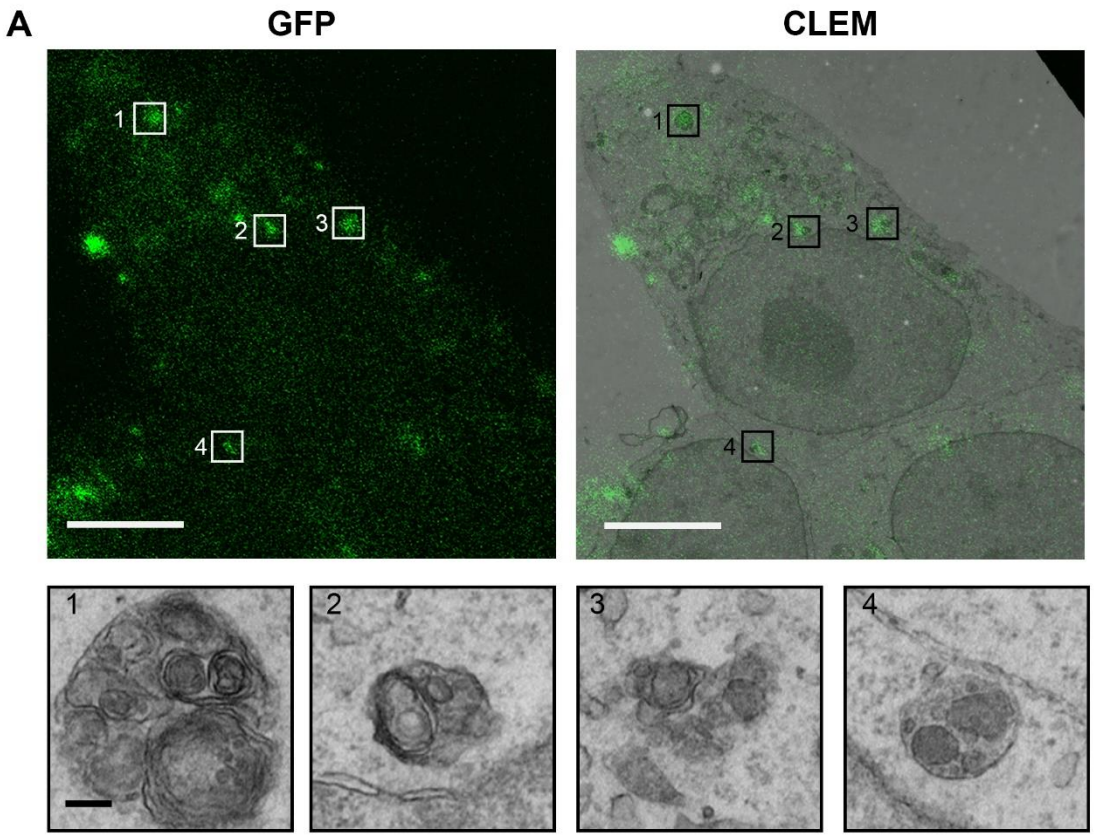


Figure S2. GFP-CD63 EVs are taken up *via* endocytosis. (A) HEK293T cells were incubated with GFP-CD63 EVs in the absence and presence of dynasore (dynamin inhibitor) for 2 hours. Note the absence of EVs in dynasore-treated cells, indicating a role for dynamin-dependent endocytosis in EV uptake. (B) HEK293T cells were incubated with GFP-CD63 EVs at 37 °C and 4°C for 1 hour. Note the absence of EV uptake at 4 °C, indicating a role for active transport in EV uptake. GFP: EV; Blue: nucleus. (Scale bars, 10 µm.)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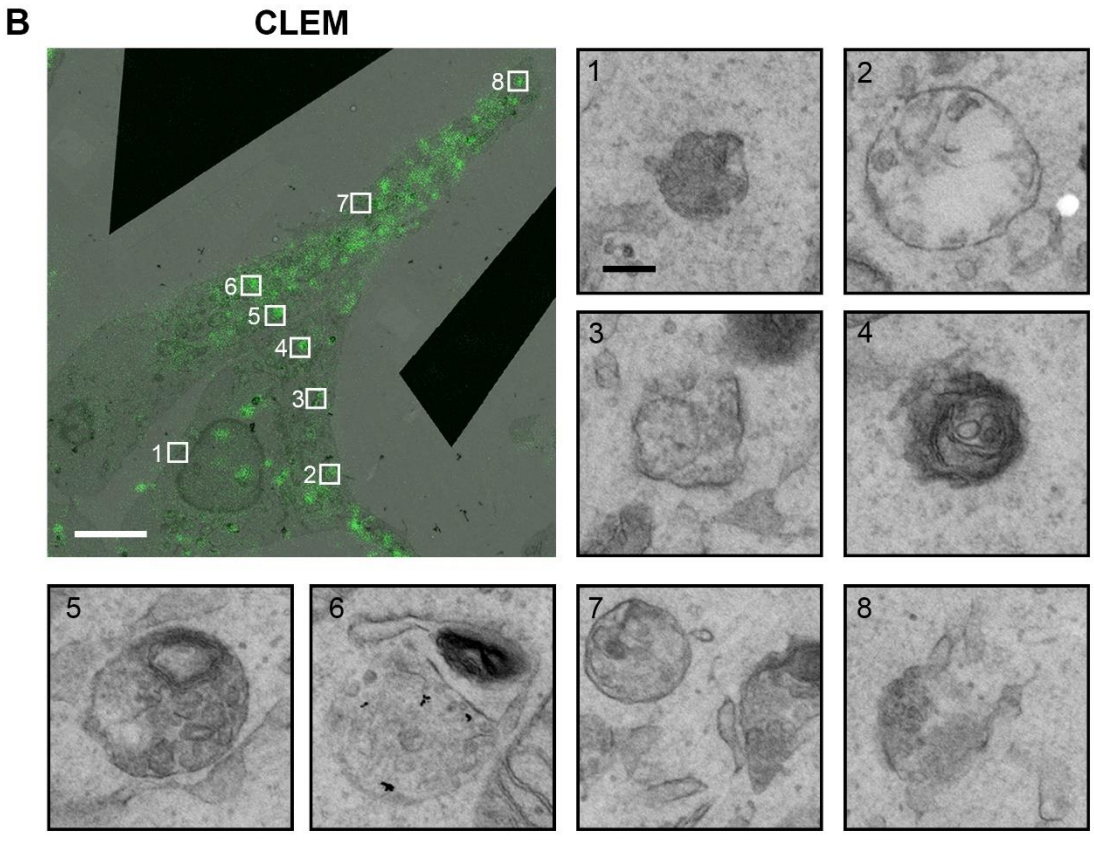
B
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2
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6
7
8

Figure S3. EVs localize in membrane-bound compartments in HEK293T acceptor cells. The underlying structures of GFP-positive spots, representing internalized GFP-CD63 EVs in HEK293T cells following (A) 4 hours and (B) 12 hours of incubation, are membrane-bound compartments, as revealed by CLEM. (Scale bars, CLEM 5 µm, EM 0.2 µm.) Full data set is available *via* www.nanotomy.org.

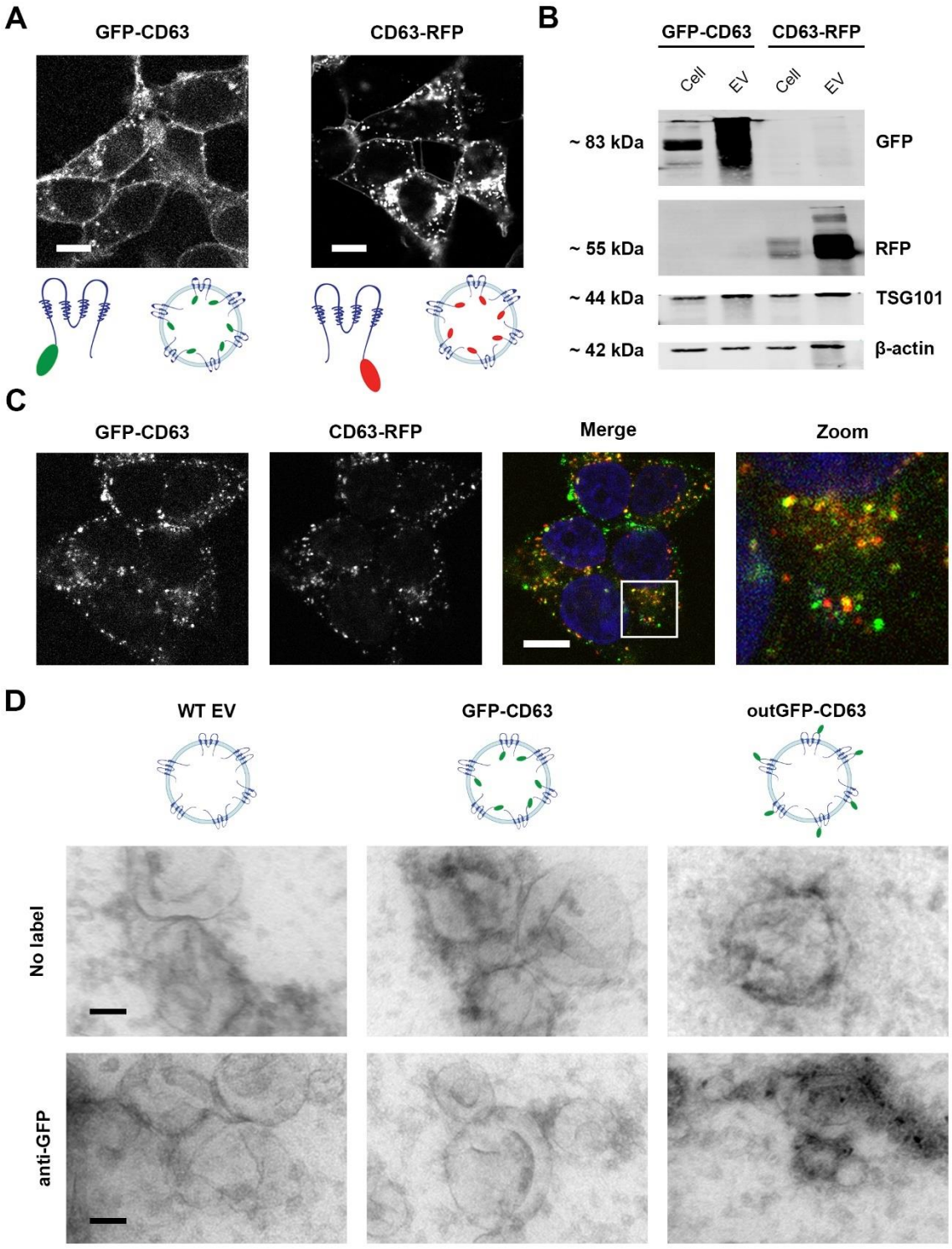

Figure S4. GFP-CD63 and CD63-RFP EVs show similar EV characteristics and intracellular localization in recipient cells. (A) Top: GFP-CD63 and CD63-RFP HEK293T cells. Note the similar fluorescence localization pattern in both producer cell lines. Bottom: Cartoon of GFP-CD63 and

CD63-RFP fusion proteins and EVs displaying the respective fluorescent protein inside the EV. (Scale bars, 10 µm.) (B) Western blot analysis of lysates of GFP-CD63 and CD63-RFP HEK293T producer cells and derived EVs (30 µg protein per lane). Note that GFP and RFP are exclusively present in corresponding cell lines and derived EVs, *i.e.* GFP-CD63 and CD63-RFP cells and EVs, respectively. EV marker TSG101 is enriched in EVs compared to producer cells. β-actin: loading control for cell lysates. (C) Cells were incubated for 12 hours with equal amounts of GFP-CD63 and CD63-RFP EVs simultaneously and imaged following fixation. Note partial colocalization (yellow dots, Pearson's Colocalization Coefficient = 0.37), showing the presence of both EV types in the same endocytic compartments. This confirmed that GFP-CD63 and CD63-RFP EVs show similar EV characteristics and intracellular localization in recipient cells, and were fit to be used interchangeably. Green, GFP-CD63 EV; red, CD63-RFP EV; blue, nucleus. (Scale bars, 10 µm.) (D) GFP fused to the N-terminus of CD63 is located at the inside of GFP-CD63 EVs. Wildtype EVs, GFP-CD63 EVs (GFP at N-terminus of CD63), and outGFP-CD63 EVs (GFP in the loop of CD63) were labeled, without prior permeabilization, with anti-GFP antibody and secondary antibody conjugated to gold, and investigated by TEM. Only outGFP-CF63 EVs show gold-labeling. (Scale bars, 50nm.)

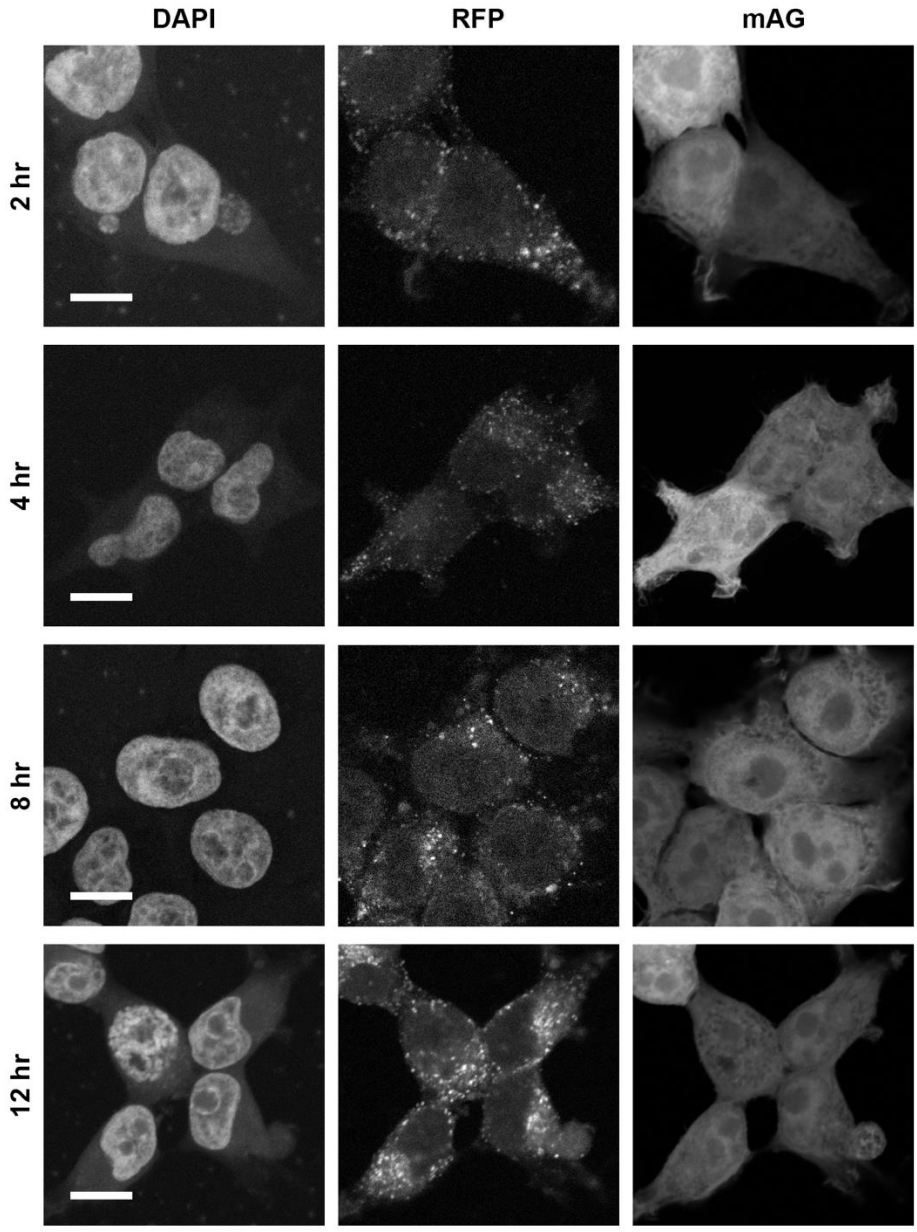

Figure S5. CD63-RFP EVs do not induce endosomal permeabilization. Fluorescence images of mAG-gal3 expressing HEK293T cells incubated with 20 µg/ml CD63-RFP EVs (EV) for 2, 4, 8 and 12 hours. Cells were fixed before imaging. DAPI: Nucleus; RFP: EVs; mAG: Gal3. (Scale bars, 10 µm.)

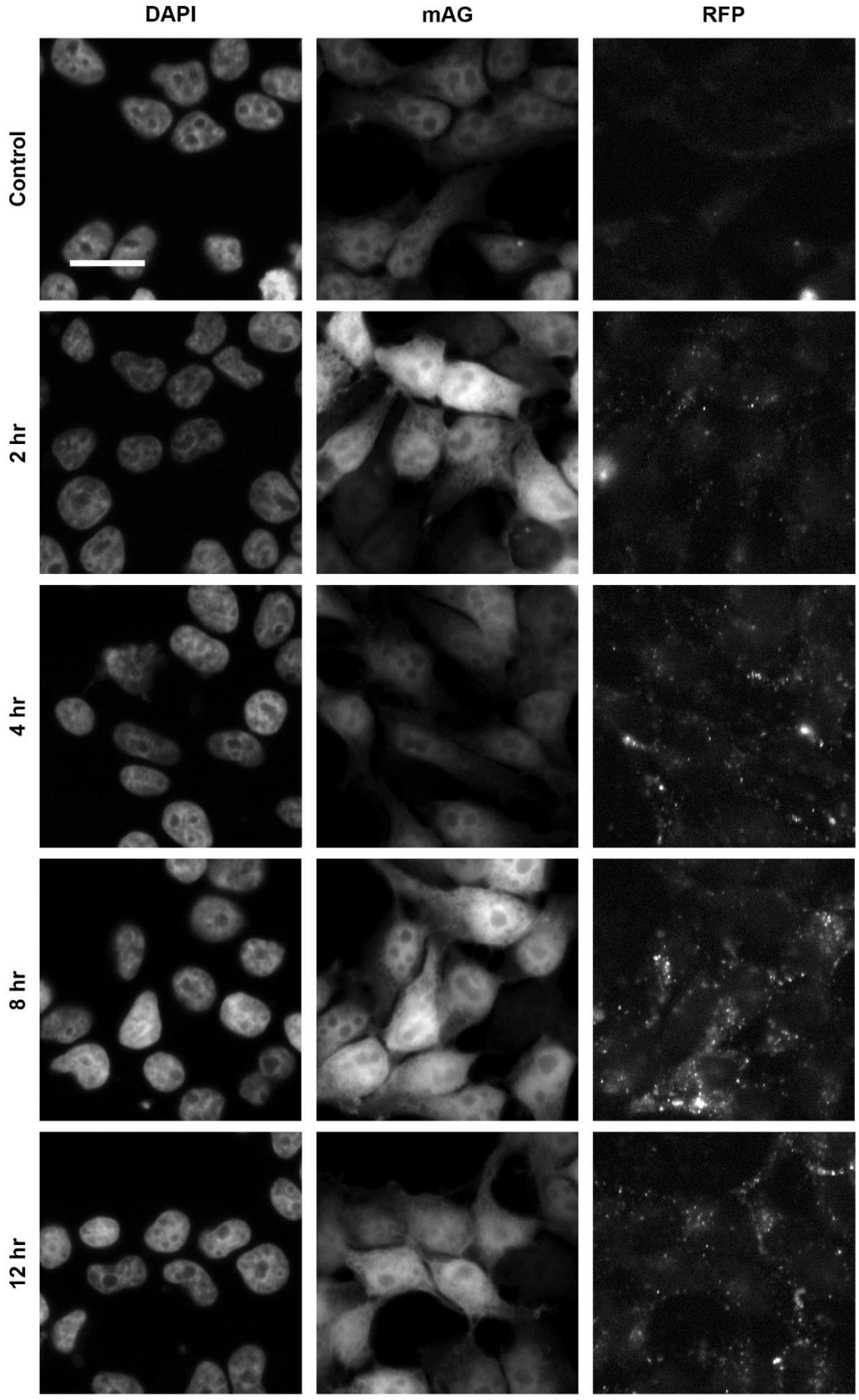
DAPI
mAG
RFP
Control
2 hr
4 hr
8 hr
12 hr

Figure S6. Increased EV concentration does not induce endosomal permeabilization. Fluorescent images of mAG-Gal3 expressing HEK293T cells incubated with 100 µg/ml CD63-RFP EVs for 2, 4, 8 and 12 hours. Cells were fixed before imaging. Note the absence of mAG-gal3 punctae at all investigated time points, similar to in control (untreated) cells. DAPI: Nucleus; mAG: Gal3; RFP: EVs. (Scale bars, 20 µm.)

Movie 1. EVs do not induce endosomal permeabilization. Of note, chemical fixatives may induce protein extraction or relocalization in cells[1]. To substantiate the absence of mAG-Gal3 punctae formation upon incubation of CD63-RFP EVs with mAG-gal3 HEK293T cells, the experiment was performed on live cells. mAG-gal3 HEK293T cells were incubated with 20 µg/ml CD63-RFP EVs for 4 hr and followed by live cell imaging. Following uptake of EVs by mAG-gal3 HEK293T cells, as revealed by the appearance of red fluorescent punctae in the cell cytosol, mAG-gal3 punctae formation was not observed. Still images in Figure S7.

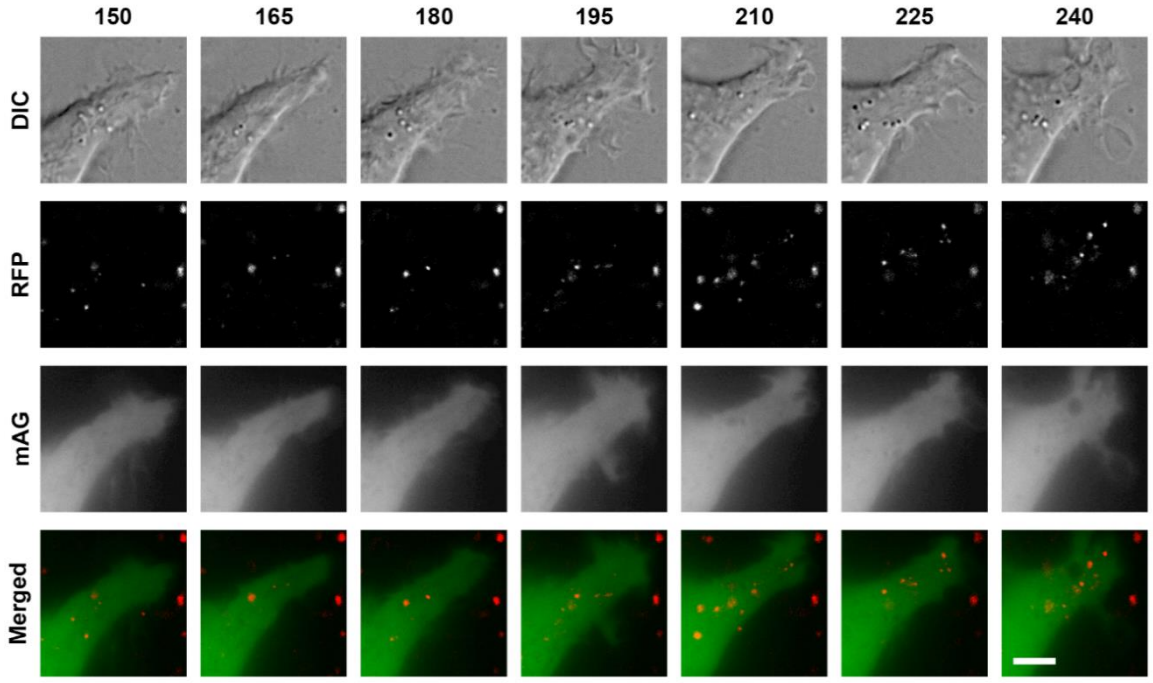

Figure S7. EVs do not induce endosomal permeabilization. Still images of Movie 1. Live-cell imaging of mAG-gal3 HEK293T cells incubated with 20 µg/ml CD63-RFP EVs. Time (min). RFP: EV; mAG: Gal3. (scale bar, 5 µm.)

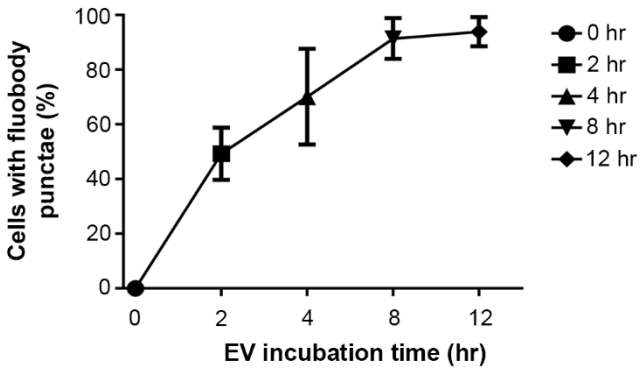

Figure S8. Quantification of fraction of mCherry-fluobody-expressing cells that show fluobody punctae after 2, 4, 8, and 12 hours incubation with 20 µg/ml CD63-GFP EVs. Note that fluobody punctae are seen in 94% ± 4.4 of the cells at the 12-hour time point (>24 cells/condition, error bars represent SD, n=3).

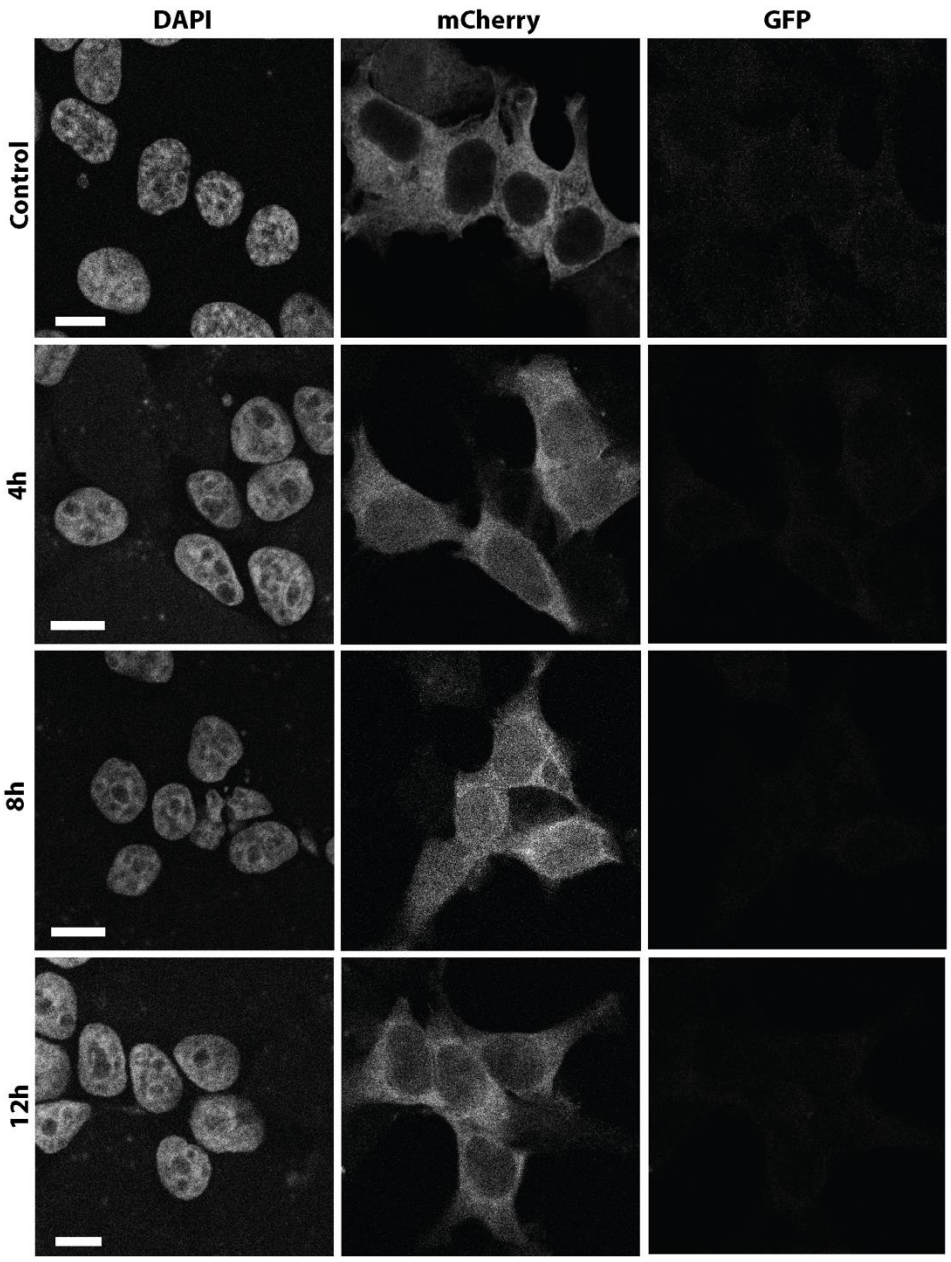

Figure S9. WT EVs do not induce fluobody punctae in anti-GFP fluobody (mCherry) HEK293T cells. Fluorescence images of HEK293T cells expressing anti-GFP fluobody (mCherry) incubated with HEK293T WT EVs for 4, 8 and 12 hours. Cells were fixed before imaging. Note the absence of fluobody punctae at all investigated time points, similar to in control (untreated) cells. DAPI: nucleus; mCherry: fluobody; GFP: absence of GFP signal in WT EVs. (Scale bars, 10 µm.)

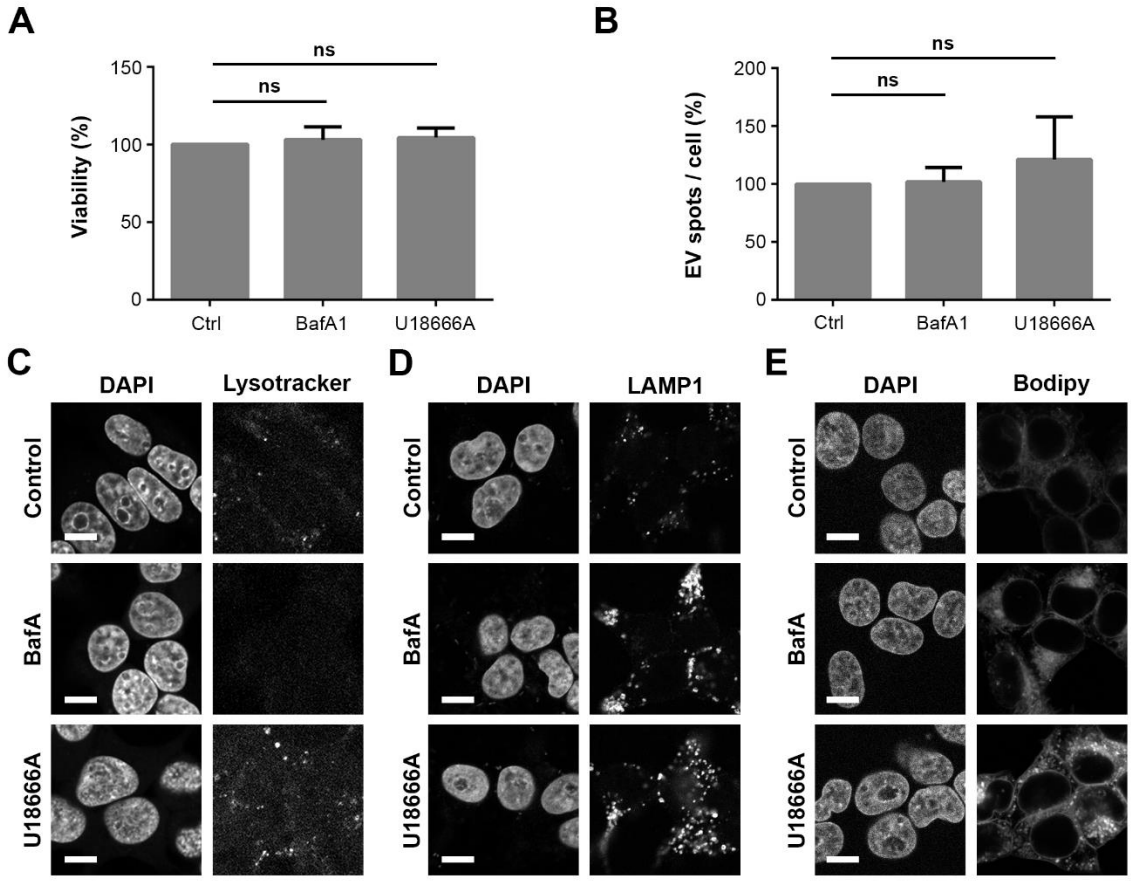

Figure S10. Effects of BafA1 and U18666A metabolic inhibitors on HEK293T cells (A) MTT cell viability assay of HEK293T cells with and without metabolic inhibitors BafA1 and U18666A (error bars represent SD, n=3; ns, not significant, two-tailed Student's *t*-test). Cell viability is not affected upon inhibitor treatments. (B) GFP-CD63 EV internalization (t = 12 h) in HEK293T cells in absence and presence of metabolic inhibitors. Number of EV-positive spots in control cells is set at 100%. (3-6 imaging fields and >8 cells/experiment, error bars represent SD, n=3; ns, not significant, two-tailed Student's *t*-test). Internalization of EVs is not affected upon inhibitor treatments. (C) Lysotracker staining of cells with and without inhibitor treatments. Note the decrease in lysotracker fluorescence in BafA1 treated cells, indicating neutralization of endosomal pH. (Scale bars, 10 µm.) (D) Late endosomal marker LAMP1 staining of cells with and without inhibitor

treatments. U18666A and BafA1 treatment led to enlarged LAMP1-positive structures. (Scale bars, 10 µm.) (E) Incubation of cells with Bodipy-cholesterol showed an endosomal staining pattern with increased intensity, indicating cholesterol accumulation in endosomes, in U18666A-treated cells compared to control and BafA1-treated cells. (Scale bars, 10 µm.)

Table S1. EV isolation method by means of sequential centrifugation.

Step	Time (min)	Centrifugal force (g)	Description	Collect	Centrifugation, rotor
1	10	300	Cell removal	Supernatant	Beckman Coulter, Allegra A-15R
2	10	2,000	Remove cellular debris	Supernatant	Beckman Coulter, Allegra A-15R
3	30	10,000	Remove apoptotic vesicles and microvesicles	Supernatant	Sorvall Discovery 90SE, Beckman SW32i
4	70	100,000	Collect EVs	Pellet	Sorvall Discovery 90SE, Beckman SW32i
5	70	100,000	Wash EVs with 5 ml PBS	Pellet	Sorvall Discovery 90SE, Beckman SW32i

Table S2. List of antibodies used in the study

Technique	Target	Host	Brand	Dilution
Western blot, primary	Golgin-97	Mouse	Invitrogen, A21270	1:1000
	LAMP1	Mouse	DSHB, AB-2296838	1:1000
	GFP	Goat	Rockland, 600-101-275	1:1000
	RFP/mCherry	Rabbit	Abcam, ab167453	1:1000
	TSG101	Mouse	Genetex, GTX70255	1:1000
	CD9	Rabbit	Abcam, ab92726	1:1000
	β-actin	Rabbit	Abcam, ab8227	1:2000
Western blot, secondary	Mouse IgG (H+L)	Goat	Li-COR Bioscience, LI 926-68070	1:5000
	Rabbit IgG (H+L)	Goat	Li-COR Bioscience, LI 926-32211	1:5000
	Goat IgG (H+L)	Donkey	Li-COR Bioscience, Li926-68074	1:5000
FM, primary	LAMP1	Mouse	DSHB, AB-2296838	1:200
FM, secondary	Mouse IgG (H+L), AlexaFluor 633	Goat	Invitrogen, A-21050	1:500
EM, primary	GFP	Goat	Rockland, 600-101-275	1:100
EM, secondary	Rabbit, biotinylated	Goat	DAKO, E0432	1:200
	Streptavidin, QD655		Life Technologies, Q10121MP	1:1000
	Rabbit IgG, 10 nm gold	Goat	BBI solutions, EM.GAR10	1:50

Supplementary data: Nucleotide and corresponding amino acid sequence of cDNAs used in this study

List of marker proteins used in this study, including abbreviations, and references.

Name	**Abbreviation**	**Reference**
Monomeric Red Fluorescent protein	mRFP	[2, 3]
Emerald Green Fluorescent protein	emGFP	[4, 5]
Monomeric Azami-Green	mAG	[6, 7]
mCherry	mCh	[8]
Ascorbate peroxidase 2	APEX2	[5, 9]
Human Galectin-3	Gal-3	[7]
Tetraspanin CD63	CD63	[5]
Nanobody anti-GFP	NB	[10]

Sequence GFP-CD63
APEX2-EmGFP-CD63

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```
cagcagaacacccccatcggcgacggccccgtgctgctgcccgacaaccactacctgagcacc
 Q  Q  N  T  P  I  G  D  G  P  V  L  L  P  D  N  H  Y  L  S  T

cagtccgccctgagcaaagaccccaacgagaagcgcgatcacatggtcctgctggagttcgtg
 Q  S  A  L  S  K  D  P  N  E  K  R  D  H  M  V  L  L  E  F  V

accgccgccgggatcactctcggcatggacgagctgtacaagaactacacagattgggagaaa
 T  A  A  G  I  T  L  G  M  D  E  L  Y  K  N  Y  T  D  W  E  K

atcccttccatgtcgaagaaccgagtccccgactcctgctgcattaatgttactgtgggctgt
 I  P  S  M  S  K  N  R  V  P  D  S  C  C  I  N  V  T  V  G  C

gggattaatttcaacgagaaggcgatccataaggagggctgtgtggagaagattgggggctgg
 G  I  N  F  N  E  K  A  I  H  K  E  G  C  V  E  K  I  G  G  W

ctgaggaaaaatgtgctggtggtagctgcagcagcccttggaattgcttttgtcgaggttttg
 L  R  K  N  V  L  V  V  A  A  A  A  L  G  I  A  F  V  E  V  L

ggaattgtctttgcctgctgcctcgtgaagagtatcagaagtggctacgaggtgatgtag
 G  I  V  F  A  C  C  L  V  K  S  I  R  S  G  Y  E  V  M  *
```

Sequence CD63-mRFP

CD63-mRFP

```
atggcggtggaaggaggaatgaagtgtgtcaagtttttgctctacgttctcctgctggccttc
 M  A  V  E  G  G  M  K  C  V  K  F  L  L  Y  V  L  L  L  A  F

tgcgcctgtgcagtgggattgatcgccattggtgtagcggttcaggttgtcttgaagcaggcc
 C  A  C  A  V  G  L  I  A  I  G  V  A  V  Q  V  V  L  K  Q  A

attacccatgagactactgctggctcgctgttgcctgtggtcatcattgcagtgggtgccttc
 I  T  H  E  T  T  A  G  S  L  L  P  V  V  I  I  A  V  G  A  F

ctcttcctggtggcctttgtgggctgctgtggggcctgcaaggagaactactgtctcatgatt
 L  F  L  V  A  F  V  G  C  C  G  A  C  K  E  N  Y  C  L  M  I

acatttgccatcttcctgtctcttatcatgcttgtggaggtggctgtggccattgctggctat
 T  F  A  I  F  L  S  L  I  M  L  V  E  V  A  V  A  I  A  G  Y

gtgtttagagaccaggtgaagtcagagtttaataaaagcttccagcagcagatgcagaattac
 V  F  R  D  Q  V  K  S  E  F  N  K  S  F  Q  Q  Q  M  Q  N  Y

cttaaagacaacaaaacagccactattttggacaaattgcagaaagaaaataactgctgtgga
 L  K  D  N  K  T  A  T  I  L  D  K  L  Q  K  E  N  N  C  C  G

gcttctaactacacagactgggaaaacatccccggcatggccaaggacagagtccccgattct
 A  S  N  Y  T  D  W  E  N  I  P  G  M  A  K  D  R  V  P  D  S

tgctgcatcaacataactgtgggctgtgggaatgatttcaaggaatccactatccatacccag
 C  C  I  N  I  T  V  G  C  G  N  D  F  K  E  S  T  I  H  T  Q

ggctgcgtggagactatagcaatatggctaaggaagaacatactgctggtggctgcagcggcc
 G  C  V  E  T  I  A  I  W  L  R  K  N  I  L  L  V  A  A  A  A

ctgggcattgcttttgtggaggtcttgggaattatcttctcctgctgtctggtgaagagtatt
 L  G  I  A  F  V  E  V  L  G  I  I  F  S  C  C  L  V  K  S  I

cgaagtggctatgaagtaatggggatccaccggccggtcgccaccatggcctcctccgaggac
 R  S  G  Y  E  V  M  G  I  H  R  P  V  A  T  M  A  S  S  E  D

gtcatcaaggagttcatgcgcttcaaggtgcgcatggagggctccgtgaacggccacgagttc
 V  I  K  E  F  M  R  F  K  V  R  M  E  G  S  V  N  G  H  E  F

gagatcgagggcgagggcgagggccgcccctacgagggcacccagaccgccaagctgaaggtg
 E  I  E  G  E  G  E  G  R  P  Y  E  G  T  Q  T  A  K  L  K  V

accaagggcggccccctgcccttcgcctgggacatcctgtcccctcagttccagtacggctcc
 T  K  G  G  P  L  P  F  A  W  D  I  L  S  P  Q  F  Q  Y  G  S

aaggcctacgtgaagcaccccgccgacatccccgactacttgaagctgtccttccccgagggc
 K  A  Y  V  K  H  P  A  D  I  P  D  Y  L  K  L  S  F  P  E  G
```

```
ttcaagtgggagcgcgtgatgaacttcgaggacggcggcgtggtgaccgtgacccaggactcc
 F  K  W  E  R  V  M  N  F  E  D  G  G  V  V  T  V  T  Q  D  S

tccctgcaggacggcgagttcatctacaaggtgaagctgcgcggcaccaacttcccctccgac
 S  L  Q  D  G  E  F  I  Y  K  V  K  L  R  G  T  N  F  P  S  D

ggccccgtaatgcagaagaagaccatgggctgggaggcctccaccgagcggatgtaccccgag
 G  P  V  M  Q  K  K  T  M  G  W  E  A  S  T  E  R  M  Y  P  E

gacggcgccctgaagggcgagatcaagatgaggctgaagctgaaggacggcggccactacgac
 D  G  A  L  K  G  E  I  K  M  R  L  K  L  K  D  G  G  H  Y  D

gccgaggtcaagaccacctacatggccaagaagcccgtgcagctgcccggcgcctacaagacc
 A  E  V  K  T  T  Y  M  A  K  K  P  V  Q  L  P  G  A  Y  K  T

gacatcaagctggacatcacctcccacaacgaggactacaccatcgtggaacagtacgagcgc
 D  I  K  L  D  I  T  S  H  N  E  D  Y  T  I  V  E  Q  Y  E  R

gccgagggccgccactccaccggcgccctgtacaagtaa
 A  E  G  R  H  S  T  G  A  L  Y  K  *
```

Sequence mAG-Galectin-3
mAG-Gal3

```
atggtgagcgtgatcaagcccgagatgaagatcaagctgtgcatgaggggcaccgtgaacggc
 M  V  S  V  I  K  P  E  M  K  I  K  L  C  M  R  G  T  V  N  G

cacaacttcgtgatcgagggcgagggcaagggcaacccctacgagggcacccagatcctggac
 H  N  F  V  I  E  G  E  G  K  G  N  P  Y  E  G  T  Q  I  L  D

ctgaacgtgaccgagggcgccccccctgcccttcgcctacgacatcctgaccaccgtgttccag
 L  N  V  T  E  G  A  P  L  P  F  A  Y  D  I  L  T  T  V  F  Q

tacggcaacagggccttcaccaagtaccccgccgacatccaggactacttcaagcagaccttc
 Y  G  N  R  A  F  T  K  Y  P  A  D  I  Q  D  Y  F  K  Q  T  F

cccgagggctaccactgggagaggagcatgacctacgaggaccagggcatctgcaccgccacc
 P  E  G  Y  H  W  E  R  S  M  T  Y  E  D  Q  G  I  C  T  A  T

agcaacatcagcatgaggggcgactgcttcttctacgacatcaggttcgacggcaccaacttc
 S  N  I  S  M  R  G  D  C  F  F  Y  D  I  R  F  D  G  T  N  F

ccccccaacggccccgtgatgcagaagaagaccctgaagtgggagcccagcaccgagaagatg
 P  P  N  G  P  V  M  Q  K  K  T  L  K  W  E  P  S  T  E  K  M

tacgtggaggacggcgtgctgaagggcgacgtgaacatgaggctgctgctggagggcggcggc
 Y  V  E  D  G  V  L  K  G  D  V  N  M  R  L  L  L  E  G  G  G

cactacaggtgcgacttcaagaccacctacaaggccaagaaggaggtgaggctgcccgacgcc
 H  Y  R  C  D  F  K  T  T  Y  K  A  K  K  E  V  R  L  P  D  A

cacaagatcgaccacaggatcgagatcctgaagcacgacaaggactacaacaaggtgaagctg
 H  K  I  D  H  R  I  E  I  L  K  H  D  K  D  Y  N  K  V  K  L

tacgagaacgccgtggccaggtactccatgctgcccagccaggccaagggaggaggaggatct
 Y  E  N  A  V  A  R  Y  S  M  L  P  S  Q  A  K  G  G  G  G  S

gcagacaatttttcgctccatgatgcgttatctgggtctggaaacccaaaccctcaaggatgg
 A  D  N  F  S  L  H  D  A  L  S  G  S  G  N  P  N  P  Q  G  W

cctggcgcatgggggaaccagcctgctggggcaggggctacccaggggcttcctatcctggg
 P  G  A  W  G  N  Q  P  A  G  A  G  G  Y  P  G  A  S  Y  P  G

gcctaccccgggcaggcacccccaggggcttatcctggacaggcacctccaggcgcctaccct
 A  Y  P  G  Q  A  P  P  G  A  Y  P  G  Q  A  P  P  G  A  Y  P

ggagcacctggagcttatcccggagcacctgcacctggagtctacccagggccacccagcggc
 G  A  P  G  A  Y  P  G  A  P  A  P  G  V  Y  P  G  P  P  S  G

cctggggcctacccatcttctggacagccaagtgccaccggagcctaccctgccactggcccc
 P  G  A  Y  P  S  S  G  Q  P  S  A  T  G  A  Y  P  A  T  G  P
```

```
tatggcgcccctgctgggccactgattgtgccttataacctgcctttgcctgggggagtggtg
 Y  G  A  P  A  G  P  L  I  V  P  Y  N  L  P  L  P  G  G  V  V

cctcgcatgctgataacaattctgggcacggtgaagcccaatgcaaacagaattgctttagat
 P  R  M  L  I  T  I  L  G  T  V  K  P  N  A  N  R  I  A  L  D

ttccaaagagggaatgatgttgccttccactttaacccacgcttcaatgagaacaacaggaga
 F  Q  R  G  N  D  V  A  F  H  F  N  P  R  F  N  E  N  N  R  R

gtcattgtttgcaatacaaagctggataataactggggaagggaagaaagacagtcggttttc
 V  I  V  C  N  T  K  L  D  N  N  W  G  R  E  E  R  Q  S  V  F

ccatttgaaagtgggaaaccattcaaaatacaagtactggttgaacctgaccacttcaaggtt
 P  F  E  S  G  K  P  F  K  I  Q  V  L  V  E  P  D  H  F  K  V

gcagtgaatgatgctcacttgttgcagtacaatcatcgggttaaaaaactcaatgaaatcagc
 A  V  N  D  A  H  L  L  Q  Y  N  H  R  V  K  K  L  N  E  I  S

aaactgggaatttctggtgacatagacctcaccagtgcttcatataccatgatataa
 K  L  G  I  S  G  D  I  D  L  T  S  A  S  Y  T  M  I  *
```

Sequence Fluobody
mCh-APEX2-Nanonody

atggtgagcaagggcgaggaggataacatggccatcatcaaggagttcatgcgcttcaaggtg
M V S K G E E D N M A I I K E F M R F K V

cacatggagggctccgtgaacggccacgagttcgagatcgagggcgagggcgagggccgcccc
H M E G S V N G H E F E I E G E G E G R P

tacgagggcacccagaccgccaagctgaaggtgaccaagggtggcccccctgcccttcgcctgg
Y E G T Q T A K L K V T K G G P L P F A W

gacatcctgtcccctcagttcatgtacggctccaaggcctacgtgaagcaccccgccgacatc
D I L S P Q F M Y G S K A Y V K H P A D I

cccgactacttgaagctgtccttccccgagggcttcaagtgggagcgcgtgatgaacttcgag
P D Y L K L S F P E G F K W E R V M N F E

gacggcggcgtggtgaccgtgacccaggactcctccctgcaggacggcgagttcatctacaag
D G G V V T V T Q D S S L Q D G E F I Y K

gtgaagctgcgcggcaccaacttcccctccgacggccccgtaatgcagaagaagaccatgggc
V K L R G T N F P S D G P V M Q K K T M G

tgggaggcctcctccgagcggatgtaccccgaggacggcgccctgaagggcgagatcaagcag
W E A S S E R M Y P E D G A L K G E I K Q

aggctgaagctgaaggacggcggccactacgacgctgaggtcaagaccacctacaaggccaag
R L K L K D G G H Y D A E V K T T Y K A K

aagcccgtgcagctgcccggcgcctacaacgtcaacatcaagttggacatcacctcccacaac
K P V Q L P G A Y N V N I K L D I T S H N

gaggactacaccatcgtggaacagtacgaacgcgccgagggccgccactccaccggcggcatg
E D Y T I V E Q Y E R A E G R H S T G G M

gacgagctgtacaagggatccggaaagtcttacccaactgtgagtgctgattaccaggacgcc
D E L Y K G S G K S Y P T V S A D Y Q D A

gttgagaaggcgaagaagaagctcagaggcttcatcgctgagaagagatgcgctcctctaatg
V E K A K K K L R G F I A E K R C A P L M

ctccgtttggcattccactctgctggaacctttgacaagggcacgaagaccggtggacccttc
L R L A F H S A G T F D K G T K T G G P F

ggaaccatcaagcaccctgccgaactggctcacagcgctaacaacggtcttgacatcgctgtt
G T I K H P A E L A H S A N N G L D I A V

aggcttttggagccactcaaggcggagttccctattttgagctacgccgatttctaccagttg
R L L E P L K A E F P I L S Y A D F Y Q L

```
gctggcgttgttgccgttgaggtcacgggtggacctaaggttccattccaccctggaagagag
 A  G  V  V  A  V  E  V  T  G  G  P  K  V  P  F  H  P  G  R  E

gacaagcctgagccaccaccagagggtcgcttgcccgatcccactaagggttctgaccatttg
 D  K  P  E  P  P  P  E  G  R  L  P  D  P  T  K  G  S  D  H  L

agagatgtgtttggcaaagctatggggcttactgaccaagatatcgttgctctatctgggggt
 R  D  V  F  G  K  A  M  G  L  T  D  Q  D  I  V  A  L  S  G  G

cacactattggagctgcacacaaggagcgttctggatttgagggtccctggacctctaatcct
 H  T  I  G  A  A  H  K  E  R  S  G  F  E  G  P  W  T  S  N  P

cttattttcgacaactcatacttcacggagttgttgagtggtgagaaggaaggtctccttcag
 L  I  F  D  N  S  Y  F  T  E  L  L  S  G  E  K  E  G  L  L  Q

ctaccttctgacaaggctcttttgtctgaccctgtattccgccctctcgttgacaaatatgca
 L  P  S  D  K  A  L  L  S  D  P  V  F  R  P  L  V  D  K  Y  A

gcggacgaagatgccttctttgctgattacgctgaggctcaccaaaagctttccgagcttggg
 A  D  E  D  A  F  F  A  D  Y  A  E  A  H  Q  K  L  S  E  L  G

tttgctgatgccgcggccgctatggatcaagtccaactggtggagtctggtggcgctttggtg
 F  A  D  A  A  A  A  M  D  Q  V  Q  L  V  E  S  G  G  A  L  V

cagccaggtggctctctgcgtttgtcctgtgccgcttctggcttcccagtgaaccgctattcc
 Q  P  G  G  S  L  R  L  S  C  A  A  S  G  F  P  V  N  R  Y  S

atgcgctggtatcgccaggctccaggcaaagagcgtgagtgggtagccggtatgtccagcgcg
 M  R  W  Y  R  Q  A  P  G  K  E  R  E  W  V  A  G  M  S  S  A

ggtgatcgtagctcctatgaagactccgtgaagggccgtttcaccatcagccgtgacgatgcc
 G  D  R  S  S  Y  E  D  S  V  K  G  R  F  T  I  S  R  D  D  A

cgtaacacggtgtatctgcaaatgaacagcttgaaacctgaagatacggccgtgtattactgt
 R  N  T  V  Y  L  Q  M  N  S  L  K  P  E  D  T  A  V  Y  Y  C

aatgtgaacgtgggcttcgagtattggggccaaggcacccaggtcaccgtctccagctaa
 N  V  N  V  G  F  E  Y  W  G  Q  G  T  Q  V  T  V  S  S  *
```

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