SUPPLEMENTAL METHODS

Cell Culture

The SV40 transformed human lung fibroblast cell line, IMR90SV, and human embryonic kidney 293 cells expressing the SV40 large T antigen (HEK293T) cells were cultured in RPMI1640 (WAKO, Japan; Cat. #: 189-02025) and DMEM (WAKO, 043-30085), respectively supplemented with 10% FBS (Biowest, Nuaillé, France; Cat. #: S1530-500), but without antibiotics. Human HeLa cervical carcinoma cells, HepG2 hepatoma cells and the THP1 monocytic cell line were grown in DMEM supplemented with 10% FBS, but without antibiotics. The cell-types used in this study are listed in **Supplemental Table 1**. All cells were grown in a humidified incubator in an atmosphere containing 5% CO_2. As stipulated in the results, in some short-term experiments, cells were assessed using medium without FBS. Under these conditions there was no change in cellular viability as judged by cellular morphology and Trypan blue staining.[1]

For collection of EVs, cells were cultured in 0.5% FBS that had been depleted of bovine EVs with or without FAC (0-20 µg/mL). The bovine EV-depleted FBS was prepared as described previously.[2] In brief, EVs were depleted from FBS by centrifugation at 100,000 x *g*/20 h/4 °C. The collected supernatant was kept at -80 °C until use.

Chemical compounds used for iron-loading, iron-chelation and inhibition of autophagy are listed in **Supplemental Table 2**.

Lentiviral Production

Lentivirus-based shRNA was used for gene knockdown of human IRP1, IRP2 and NCOA4. The lentiviral vector was co-transfected with the packaging vectors, psPAX2, pMD2 VSV-G in HEK293T cells using polyethylenimine HCl MAX 4000 (PEI), as described previously.[3]

pLKO-puro-scramble was used as control. After a 48 h/37 °C incubation, culture supernatants were collected and virus concentrated 10x overnight with a Lenti-X concentrator according to the manufacturer's instructions. Cells were transduced with virus using polybrene (2 µg/mL). To prepare stable cells, cells were selected with puromycin (0.2–1 µg/mL) 48 h after transduction. Those cells were maintained with puromycin (0.2–1 µg/mL) after selection. Expression of the target protein was verified by immunoblotting. The chemical compounds used for lentivirus production are listed in **Supplemental Table 2**.

Gene Knockdown

Lentiviral shRNA pLKO.1 plasmids were obtained as bacterial stocks from Sigma-Aldrich: human IRP1 #1; human IRP1 #2, human IRP2 #1, human IRP2 #2 and human NCOA4. For silencing of the *IRP1, IRP2* or *NCOA4* gene, lentiviral pLKO.1-IRP1, pLKO.1-IRP2, or pLKO.1-NCOA4 shRNA was used to transduce HEK293T cells, as described previously.[4] Scrambled shRNA was a gift from David Sabatini (Massachusetts Institute of Technology, MA). The shRNAs used in this study are listed in **Supplemental Table 3**.

Immunofluorescence Staining and Light Microscopy

Cells were plated on coverslips transfected with the indicated plasmids. Cells were fixed with 4% paraformaldehyde for 20 min/4 °C, permeabilized for 3 min in 0.1% Triton X-100 and then blocked with 2% BSA in PBS. Cells were stained with specific antibodies listed in **Supplemental Table 4**, namely mouse anti-CD63, rabbit anti-FtH, rabbit anti-CD81 and rabbit anti-NCOA4. Highly cross-absorbed H+L secondary antibodies conjugated to Alexa 488, Alexa 568, or Alexa 647 were used at 1:2000. Glass coverslips were mounted with ProLong™ Diamond Antifade Mountant (Thermo Fisher Scientific; Cat. #: P36961).

Images were obtained using Olympus SpinSR10 confocal microscope and 100X 1.4NA oil immersion objective. Images were analyzed using the image processing package, Fiji (https://fiji.sc/), with data being presented as maximum intensity projections.

General Cloning and Plasmids

DNA constructs were amplified in *E. coli* DH5α or Stbl3 and purified using mini prep columns (Econospin, Aji-biopharma, Osaka, Japan). DNA sequence verification of all plasmids was performed using an ABI 3100 automated DNA sequencer (Thermo Fisher Scientific).

Lysing and Immunoblotting

Cells were lysed in lysis buffer (20 mM Tris pH 7.4, 0.5% NP-40, 60 mM NaCl, 1 mM DTT) and centrifuged at 10,000 x *g*/10 min/4 ºC. Supernatant was collected and protein concentration was measured by the BCA assay (Nacalai Tesque, Kyoto, Japan; Cat. #: 06385-00). Samples were analyzed by immunoblotting with the specific antibodies listed in **Supplemental Table 4**, namely: anti-mouse human CD63, rabbit anti-human PDI, rabbit anti-human IRP1, rabbit anti-human IRP2, rabbit anti-human calnexin, rabbit anti-human CD81, rabbit anti-human ferritin heavy chain, rabbit anti-human ferritin light chain, rabbit anti-LC3B, mouse anti-human β-actin, mouse anti-human α-tubulin, mouse anti-human LAMP2 and anti-human NCOA4.

Collection of EVs from Cellular Supernatants

IMR90SV cells were incubated with 0, 5, 20 µg/mL FAC for 48 h/37 °C supplemented with 0.5% FBS depleted of bovine EVs. EVs were collected from cellular supernatants by a well characterized protocol.[2] Briefly, medium was collected and centrifuged at 500 x *g*/10 min/4 ºC, the supernatant carefully decanted and then centrifuged again at 2000 x *g*/30 min/4 ºC. The supernatant was transferred to new tube and mixed with 2 x PEG buffer (16% polyethylene glycol 6000, 1.0 M NaCl) and then incubated for 16 h/4 ºC. The solution was then centrifuged at 2900 x *g/* 70 min/4 ºC. The collected pellet was resuspended in PBS and then centrifuged at 100,000 x *g/*70 min/4 ºC. The cell pellet was resuspended in RIPA buffer (10 mM Tris pH 7.4, 150 mM NaCl, 5 mM EDTA, 1% Triton X-100, 1% sodium deoxycholate) and the protein concentration measured by the BCA protein assay.

Electron microscopy

The collected EVs from cellular supernatants described above were resuspended in PBS and added dropwise onto a grid supported by Excel Support Film (Nisshin EM Tokyo, Cu200). EVs were imaged using TEM (JEM-1400Plus; JEOL, Tokyo, Japan) after 1% uranyl acetate staining. Thirty transmission electron micrograph fields over 3 experiments at a magnification of 20,000x were randomly chosen and the EVs counted by a blinded investigator (up to 500-1000 vesicles were counted/experiment). EVs number was analyzed by Fiji software.

RNA Pulldown

The IRE region of *CD63* mRNA (NM_001257389.2 on NCBI) was analyzed by SIREs Web Server 2.0 (http://ccbg.imppc.org/sires/).[5] The predicted IRE of *CD63* 5'-UTR RNA (5'-GAGGGGGCUUGCACAGAGUUGGAGCCAGAGG-3') was synthesized by Eurofins Scientific (Luxembourg). The RNA 3'-end was biotinylated using a Pierce RNA 3'-End Biotinylation kit according to the manufacturer (Thermo Fisher Scientific, Cat. #: 20160). Briefly, 50 pmol RNA was heated 5 min/85 ºC and cooled on ice immediately. The RNA and biotinylated cytidine (bis)phosphate were mixed with T4 RNA ligase overnight at 16 ºC. Total protein was extracted from IMR90SV cells in pulldown buffer (20 mM Tris pH 7.5, 150 mM KCl, 0.5% NP40, 5 mM EDTA, 0.5 mM DTT and proteinase inhibitor cocktail (Roche, Basel, Switzerland, Cat. #: 11873580001).

The non-IRE-containing RNA (5'-CCUGGUUUUUAAGGAGUGUCGCCAGAGUGCCGCGAAUGAAAAA-3') included in the kit (Thermo Fisher Scientific, Cat. #: 20160) was used as negative control according to the manufacturer's instructions. Biotinylated RNA was mixed with precleared total protein extraction from IMR90SV cells, recombinant GST-IRP1 or GST-IRP2 for 1 h/4 ºC and then magnetic streptavidin R280 beads (Veritas, Tokyo, Japan; Cat. #: 11205D) were added to mixture and

incubated for additional 45 min/4 ºC. Beads were washed with pulldown buffer and analyzed by immunoblotting with anti-IRP1 (Cell Signaling Technology, 1:4000; Cat. #: 20272S) and anti-IRP2 (Cell Signaling Technology, 1:2000; Cat. #: 37135S) antibodies.

Purification of Recombinant IRPs

To generate recombinant IRP1 and IRP2 proteins, the full-length of human IRP1 or IRP2 was cloned into pGEX5X-1 and transformed in *E. coli* BL21 DE3. Bacterial cells were grown at 37 °C in Lucia Broth medium and induced at $A_{600\ nm}$ = 0.6-0.7 by the addition of 0.5 mM isopropyl-1-thio-β-D-galactopyranoside (Wako) and harvested after 4 h/18 °C. The cell pellets were resuspended in ice-cold pulldown buffer and disrupted by sonication. Lysates were centrifuged at 15,000 x *g*/15 min/4 °C. Clarified lysates were incubated with glutathione Sepharose 4B beads (Cytiva, Marlborough, MA) for 2 h/4 °C. The beads were then washed three times with 5 column volumes of pulldown buffer and eluted in 15 mM reduced glutathione-containing 50 mM Tris (pH 8).

Electrophoretic Mobility Shift Assay (EMSA)

The IRE RNA included in the RNA 3' biotinylation kit (Thermo Fisher Scientific, Cat. #: 20160) described above was used as positive control. The specific unlabeled tRNA from yeast (Thermo Fisher Scientific, Cat. #: AM7119) competitors were added in excess (200-fold) in pulldown buffer.

The incubated reactions included 62.5 nM biotinylated RNA and 0.5 µg purified GST-IRP1 or GST-IRP2 with unlabeled RNA competitors. Before adding the biotinylated RNA, the reaction components were pre-incubated for 15 min/room temperature (RT) and the binding reactions were incubated for 30 min/RT before adding the loading buffer. The binding reactions for RNAs and GST-IRP1 or GST-IRP2 were electrophoresed on native 6% TBE/acrylamide gels. The gels were transferred to a nylon membrane using in 0.5 x TBE, at 90 mA for 40 min. After UV crosslinking of biotinylated RNA to the nylon membrane, the biotinylated RNA was detected with the streptavidin-HRP (Dako, Santa Clara, CA, Cat. #: P0397).

Loading Cells with Zinc

Cells were incubated with 40 µM $ZnSO_4$ for 6 h/37 °C in -FBS media and then washed with PBS. Then the fluorescent zinc indicator, FluoZin-3 AM, was incubated with cells according to the manufacturer's instructions. Briefly, a 2 mM stock solution of FluoZin-3 AM (Thermo Fisher Scientific; Cat. #: F24195) in DMSO was diluted to a final concentration of 5 µM in FluoroBrite DMEM Media (Thermo Fisher Scientific; Cat. #: A1896701). Cells were incubated with FluoZin-3 AM for 1 h/37 °C with 5% CO_2, followed by a 30 min wash in FluoroBrite DMEM Media at 37 °C. Imaging was performed immediately after washing. To apply Pluronic F-127 non-ionic surfactant during the staining procedure, the stock solution of the zinc indicator was mixed with an equal

volume of Pluronic F-127 20% (w/v) solution (Thermo Fisher Scientific; Cat. #: P3000MP) and then the mixture was diluted in phosphate-free Hank's balanced salt solution, making the final Pluronic concentration 0.025%.[6,7]

RNA Isolation, cDNA Synthesis and Reverse Transcription Quantitative Real-Time PCR (RT-qPCR)

Total RNA was isolated from cells by using an RNeasy Mini Kit System (Qiagen, Hilden, Germany**;** Cat. # 74104) according to the manufacturer's protocol. cDNAs were synthesized by using a SuperScript™ III First-Strand Synthesis System (Thermo fisher, Cat. #: 18080051) with 1 µg of total RNA. Real-time PCR was performed by using the Platinum™ SYBR™ Green qPCR SuperMix-UDG (Thermo Fisher, Cat #: 11733046) with a CFX96 thermocycler (Bio-Rad, Hercules, CA). The relative mRNA levels of *CD63*, *CD81*, *FtH* and *FtL* were calculated using the $2^{-\Delta\Delta Cq}$ method[8-10] and normalized to *GAPDH*. Primers are listed in **Supplemental Table 5**.

Native-PAGE, Prussian Blue Staining and Immunoblotting Analysis

To determine whether ferritin in cells and EVs store iron after incubation with FAC, total cell extracts or EVs were resolved by 7.5% native PAGE and then stained with a mixture (1:1; v/v) of 2% $K_4Fe(CN)_6$ and 2% 11.6 M HCl prepared immediately before use.[11,12] Cellular expression of FtH-

and FtL- chain was identified using the same native PAGE procedure followed by immunoblotting analysis with anti-FtL and -FtH antibodies listed in **Supplemental Table 4**.

Statistical Analysis

Statistical analyses were conducted using GraphPad, Prism 8 (GraphPad Software, San Diego, CA). Results are presented as mean ± standard error of the mean (SEM) of 3 independent experiments and were compared using Student's *t*-test. Results were considered significant when $p < 0.05$.

Supplemental Table 1. List of cell-types used in this study

Reagent type (species) or resource	Designation	Source or reference	Identifiers
Cell line (Homo sapiens)	HEK293T	Riken Bank	RCB2202
Cell line (Homo sapiens)	IMR90SV	Riken Bank	RCB1024
Cell line (Homo sapiens)	HeLa	Riken Bank	RCB0007
Cell line (Homo sapiens)	HepG2	Riken Bank	RCB1886
Cell line (Homo sapiens)	THP1	Riken Bank	RCB1189

Supplemental Table 2. List of chemical compounds used in this study

Reagent type or resource	Designation	Source or reference	Identifiers	Additional information
Chemical compound	Bafilomycin A1	Medchem	HY -100558	
Chemical compound	Ferric ammonium citrate	Sigma-Aldrich	F5879	
Chemical compound	Deferasirox	Novartis Pharma	147868	
Chemical compound	polyethylenimine HCl MAX 4000	Polysciences, Inc	24885-2	Reed SE *et al*. 2006
Chemical compound	Lenti-X concentrator	Takara-Bio	631231	
Chemical compound	Pluronic F-127	Thermo Fisher	P3000MP	
Chemical compound	FluoZin™ -3、AM	Thermo Fisher	F24195	

Supplemental Table 3. List of shRNAs used in this study

Reagent type (species) or resource	Designation	Source or reference	Identifiers
Genetic reagent (shRNA) / (Homo sapiens)	human IRP1 #1	Sigma-Aldrich	TRCN0000333171
Genetic reagent (shRNA) / (Homo sapiens)	human IRP1 #2	Sigma-Aldrich	TRCN0000333172
Genetic reagent (shRNA) / (Homo sapiens)	human IRP2 #1	Sigma-Aldrich	TRCN0000029191
Genetic reagent (shRNA) / (Homo sapiens)	human IRP2 #2	Sigma-Aldrich	TRCN0000335543
Genetic reagent (shRNA) / (Homo sapiens)	human NCOA4	Sigma-Aldrich	TRCN0000236186
Genetic reagent (shRNA)	Scrambled shRNA	Addgene	RRID:Addgene_1864

Supplemental Table 4. List of antibodies used in this study

Reagent type or resource	Designation	Source or reference	Identifiers	Additional information
Antibody	mouse anti-human CD63	Thermo Fisher Scientific	10628D	IB;1:1000, IF;1:500
Antibody	rabbit anti-human PDI	Cell Signaling Technology	3501S	IB;1:1000
Antibody	rabbit anti-human IRP1	Cell Signaling Technology	20272S	IB;1:4000
Antibody	rabbit anti-human IRP2	Cell Signaling Technology	37135S	IB;1:2000
Antibody	rabbit anti-human calnexin	Cell Signaling Technology	2679S	IB;1:2000
Antibody	rabbit anti-human CD81	Cell Signaling Technology	56039S	IB;1:1000, IF;1:200
Antibody	rabbit anti-human FtH	Santa Cruz	sc25617	IB;1:1000, IF;1:200
Antibody	rabbit anti-human FtL	Abcam	69090	IB;1:2000
Antibody	rabbit anti-LC3B	MBL	PM036	IB;1:1000
Antibody	mouse anti-human β-actin	Wako	017-24556	IB;1:5000

Antibody	mouse anti-human α-tubulin	Sigma	049K4764	IB;1:5000
Antibody	mouse anti-human Lamp2	Santa Cruz	sc18822	IB;1:2000
Antibody	rabbit anti-human NCOA4	Thermo Fisher Scientific	PA5-96398	IB;1:1000, IF;1:200
Antibody	mouse anti-human HERC2	BD Transduction Laboratories	612366	IB;1:2000
Antibody	Highly cross-absorbed H+L secondary antibodies conjugated to Alexa 488	Life Technologies	A11029	IF;1:2000
Antibody	Highly cross-absorbed H+L secondary antibodies conjugated to Alexa 488	Life Technologies	A11008	IF;1:2000
Antibody	Highly cross-absorbed H+L secondary antibodies conjugated to Alexa 568	Life Technologies	A11031	IF;1:2000
Antibody	Highly cross-absorbed H+L secondary antibodies conjugated to Alexa 568	Life Technologies	A21069	IF;1:2000
Antibody	Highly cross-absorbed H+L secondary antibodies conjugated to Alexa 647	Life Technologies	A21244	IF;1:2000

IB; immunoblotting, IF; immunofluorescence

Supplemental Table 5. List of primers for RT-qPCR in this study

	Forward (5'-3')	Reverse (5'-3')	Additional information
CD63	ATGCAGGCAGATTTTAAGTGCT	GTTCTTCGACATGGAAGGGATTT	Kudo K *et al*. 2018
CD81	GGGAGTGGAGGGCTGCACCAAGTGC	GATGCCACAGCACAGCACCATGCTC	ZHANG Y *et al*. 2020
FtH	ACTGATGAAGCTGCAGAACC	GTCACCCAATTCTTTGATGG	Huang BW *et al*. 2013
FtL	CAGCCTGGTCAATTTGTACCT	CGGTCGAAATAGAAGCCCAGAG	Huang BW *et al*. 2013
GAPDH	CCAAAATCAGATGGGGCAATGCTGG	TGATGGCATGGACTGTGGTCATTCA	ZHANG Y *et al*. 2020

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SUPPLEMENTAL FIGURE LEGENDS

Supplemental Figure 1: Expression of CD63 is increased by cellular iron-loading and decreased by iron depletion in (A-D) HeLa and (E-H) HepG2 cells. (A) Confocal immunofluorescence microscopy analysis of HeLa cells that were incubated in the absence or presence of 10 µg/mL FAC or 5 µM DFX for 16-h/37°C (-FBS). Cells were then fixed and stained with anti-CD63 (red) or CD81 (green) antibodies. Scale bar = 10 µm. Photographs are typical from 3 experiments. **(B)** Quantitation of CD63 and CD81 as intensity/cell of the data in **(A)** was performed using Fiji software. Results are from the analysis of >60 cells/condition over 3 experiments. **(C)** Immunoblotting analysis of HeLa cells that were incubated in the absence or presence of 10 µg/mL FAC or 5 µM DFX for 16-h/37°C (-FBS). Cells were lysed in lysis buffer and 20 µg of protein/sample analyzed with anti-CD63, anti-CD81, anti-FtH and anti-β-actin antibodies. **(D)** Quantitation of CD63, CD81 and FtH intensity from the immunoblotting analysis in **(C)**. **(E)** Confocal immunofluorescence microscopy analysis of HepG2 cells that were incubated in the absence and presence of 10 µg/mL FAC or 5 µM DFX for 16-h/37°C (-FBS). Cells were then fixed and stained with anti-CD63 (red) or CD81 (green) antibodies. Scale bar = 10 µm. Photographs are typical from 3 experiments. **(F)** Quantitation of CD63 and CD81 as intensity/cell of the data in **(E)** was performed using Fiji software. Results are from the analysis of >60 cells/condition over 3 experiments. **(G)** Immunoblotting analysis of HepG2 cells that were incubated in the absence or presence of 10 µg/mL FAC or 5 µM DFX for 16-h/37°C (-FCS).

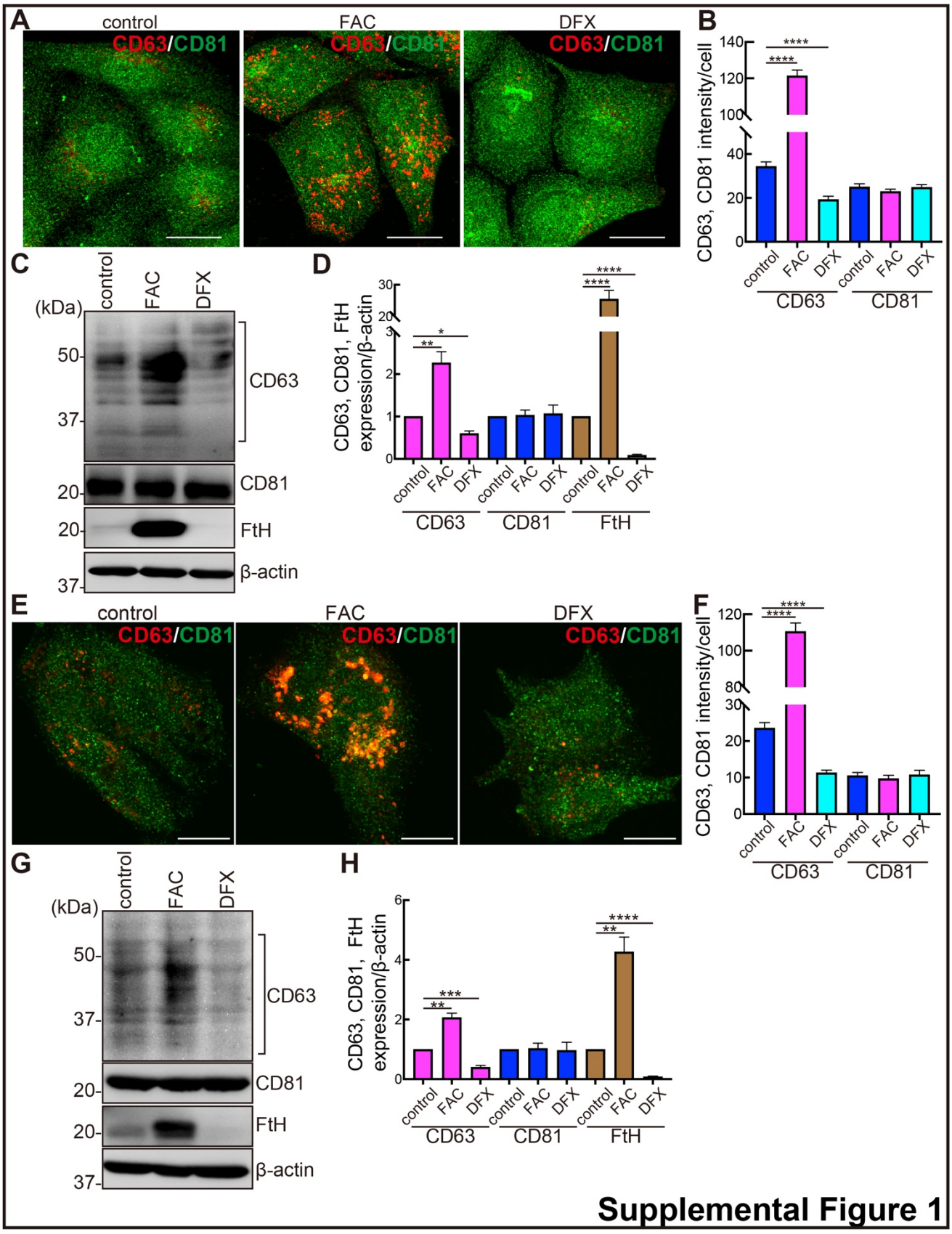
Cells were lysed in lysis buffer and 20 µg of protein/sample analyzed with anti-CD63, anti-CD81, anti-FtH and anti-β-actin antibodies. **(H)** Quantitation of CD63, CD81, and FtH intensity from the immunoblotting analysis in **(G)**. Results are mean ± SEM (3 experiments). Analysis of significance determined in (**B, D, F** and **H**) was performed using Student's *t*-test; *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$; ****, $p<0.0001$.

Supplemental Figure 2: CD63 protein expression is induced by iron, but not by Zn(II)-loading of cells. (A) IMR90SV cells were incubated with 40 µM $ZnSO_4$ for 6-h/37 °C in -FBS media. After washing cells with PBS, cells were incubated with 5 µM FluoZin-3 AM and 0.025% Pluronic F-127 for 1-h/37 °C, followed by a 30 min wash in FluoroBrite DMEM Media at 37 °C. **(B)** Quantitation of FluoZin-3 AM intensity in **(A**). **(C)** IMR90SV cells were incubated with 40 µM $ZnSO_4$ or 10 µg/mL FAC for 6-h/37 °C in -FBS media. The relative mRNA levels of *CD63*, *CD81*, *FtH* and *FtL* were calculated using the $2^{-\Delta\Delta Cq}$ method and normalized to *GAPDH*. **(D)** IMR90SV cells were incubated with 0, 5, 20 and 40 µM $ZnSO_4$ or 10 µg/mL FAC for 24-h/37 °C in -FBS media. Cells were lysed in lysis buffer and 20 µg of protein/sample analyzed with anti-CD63, anti-CD81, anti-FtH, anti-FtL and anti-β-actin antibodies. **(E)** Quantitation of CD63, CD81, FtH and FtL intensity from the immunoblotting analysis in **(D)**. Results are mean ± SEM (3 experiments). Analysis of significance

determined in (**B, C** and **E**) was performed using Student's *t*-test; **, $p<0.01$; ***, $p<0.001$; ****, $p<0.0001$.

Supplemental Figure 3: Expression of CD63 in cells and EV's is increased by cellular iron-loading of THP1 cells. (A) Immunoblotting analysis of THP1 cells incubated in the presence of 5 or 20 µg/mL FAC for 24-h/37°C (-FBS). Cells or EVs were prepared in lysis buffer, the protein concentration was measured by the BCA assay, and 20 µg of protein/sample analyzed using anti-CD63, anti-CD81, anti-FtH, and anti-FtL antibodies. **(B)** Quantitation of CD63, CD81 and FtH intensity from the immunoblotting analysis in **(A)**. **(C)** THP1 cells were incubated in the presence of 5 or 20 µg/mL FAC for 48-h/37°C (0.5% FBS). Isolated EVs from cell culture supernatants were collected by the procedures described in the *Materials and Methods* section. Twenty µg of EV protein/sample were then analyzed by immunoblotting with anti-CD63, anti-FtH, anti-FtL and anti-CD81 antibodies. **(D)** Quantitation of CD63, CD81, FtH and FtL were normalized by Ponceau S staining in **(C)**. **(E)** Quantification of FtH expression as a ratio between EVs/cells from data in **(A)** and **(C)**. Results are mean ± SEM (3 experiments). Significance was determined using the Student's *t*-test; *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$; ****, $p<0.0001$.

Supplemental Figure 4: Confocal immunofluorescence microscopy demonstrates that NCOA4 forms larger fluorescent foci upon incubation with iron. (A) IMR90SV cells were incubated with 10 µg/ml FAC (-FBS) for 2-24 h/37°C. Cells were fixed with 4% PFA and stained with anti-NCOA4 antibodies and DAPI. Scale bar (upper panel- **(i)**) = 10 µm; Scale bar (lower panel- **(ii)**) = 2 µm. Photographs are typical from 3 experiments. Quantitation of the number **(B)** and size **(C)** of NCOA4 in **(A)**. For analysis, >20 cells were examined *per* condition over 3 experiments. Significance was determined using Student's *t*-test; *, $p<0.05$; ***, $p<0.001$; ****, $p<0.0001$. **(D)** IMR90SV cells were incubated with 20 µg/ml FAC (-FBS) for the indicated time. Cells were lysed in lysis buffer and 20 µg of protein/sample analyzed by immunoblotting with anti-HERC2, anti-IRP2, anti-NCOA4, anti-FtL, anti-FtH and anti-β-actin antibodies. Results are mean ± SEM (3 experiments). **(E)** Quantitation of HERC2, IRP2, NCOA4, FtL and FtH protein levels from the immunoblotting analysis in **(D)**. Significance was determined by Student's *t*-test; *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$; ****, $p<0.0001$.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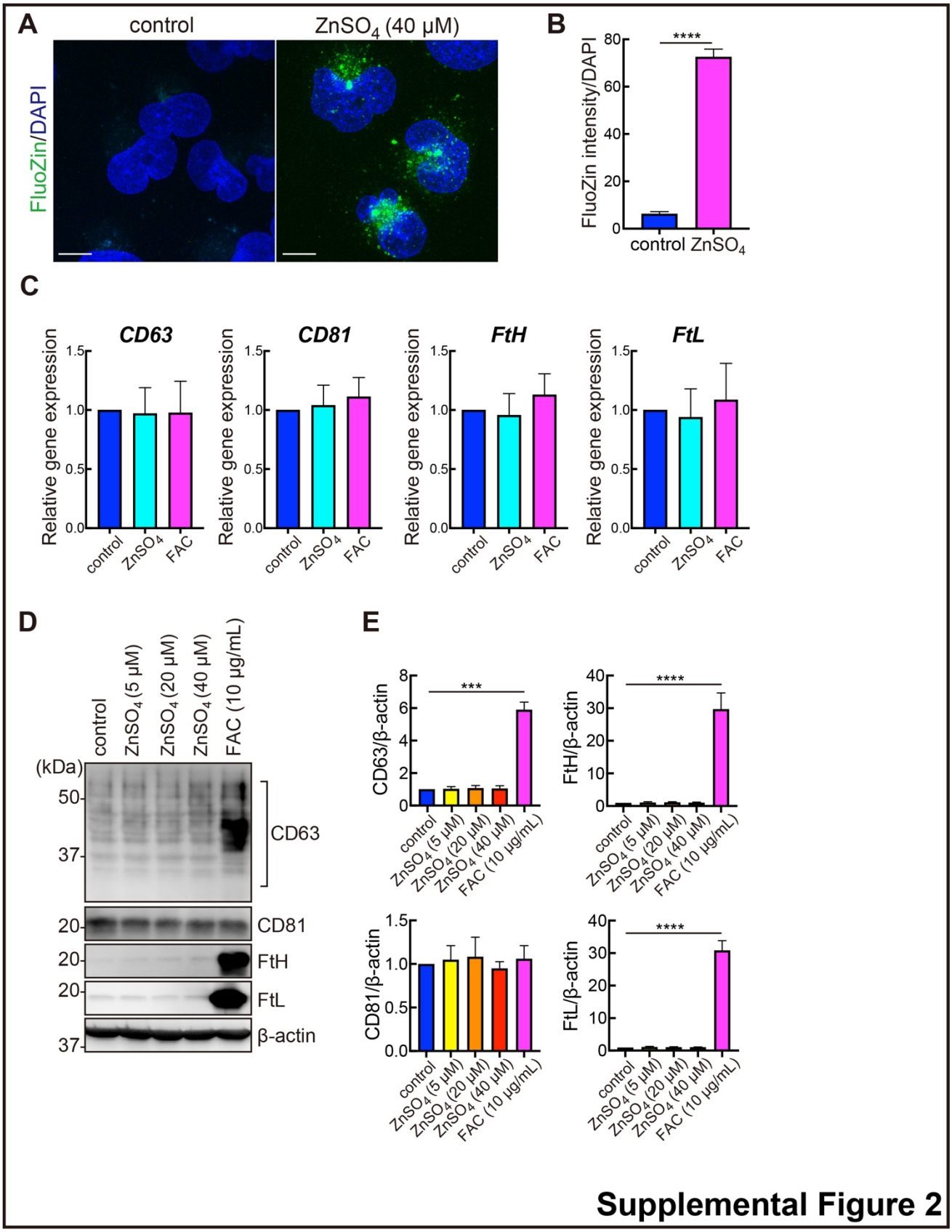
Supplemental Figure 1



Supplemental Figure 2

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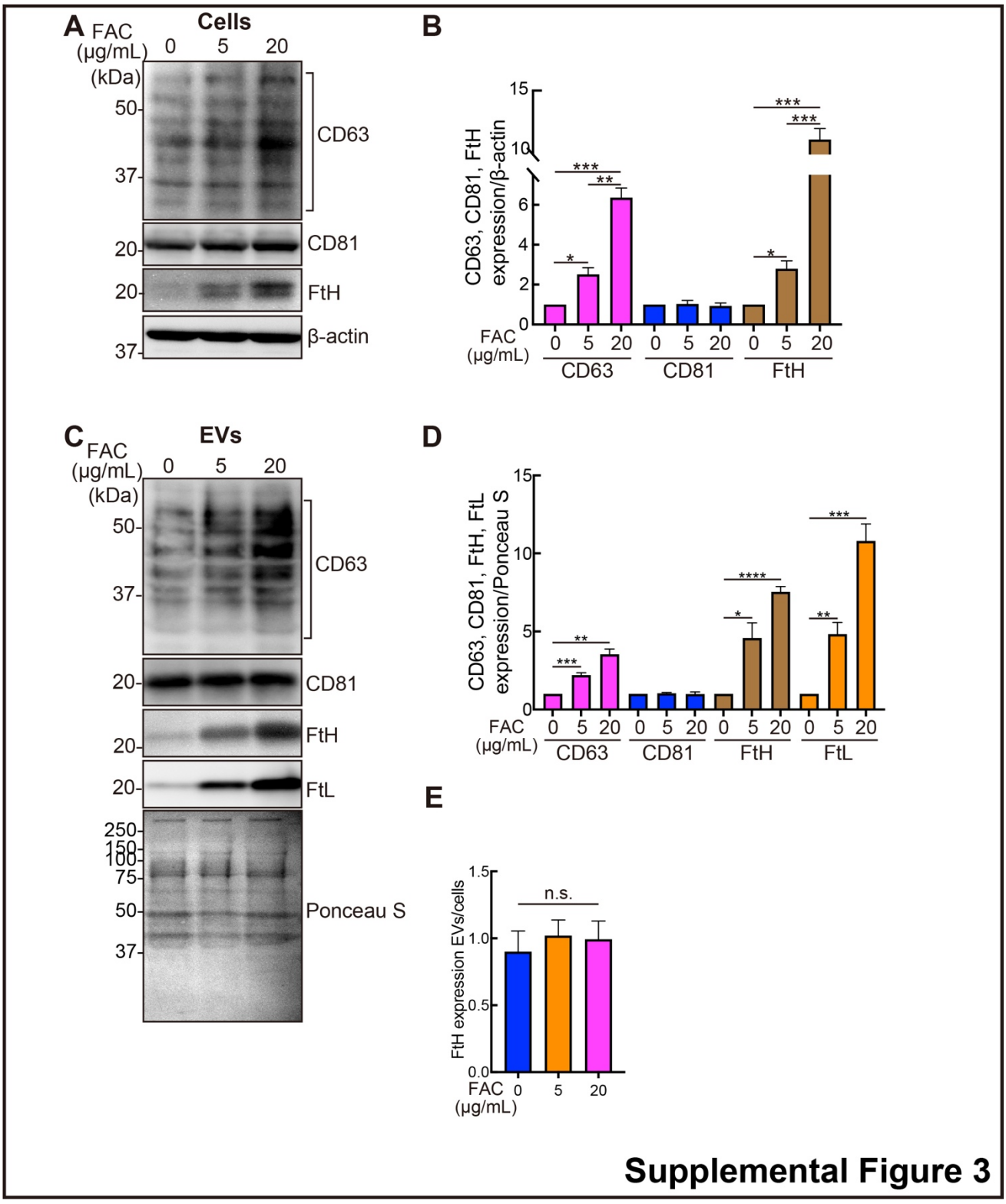
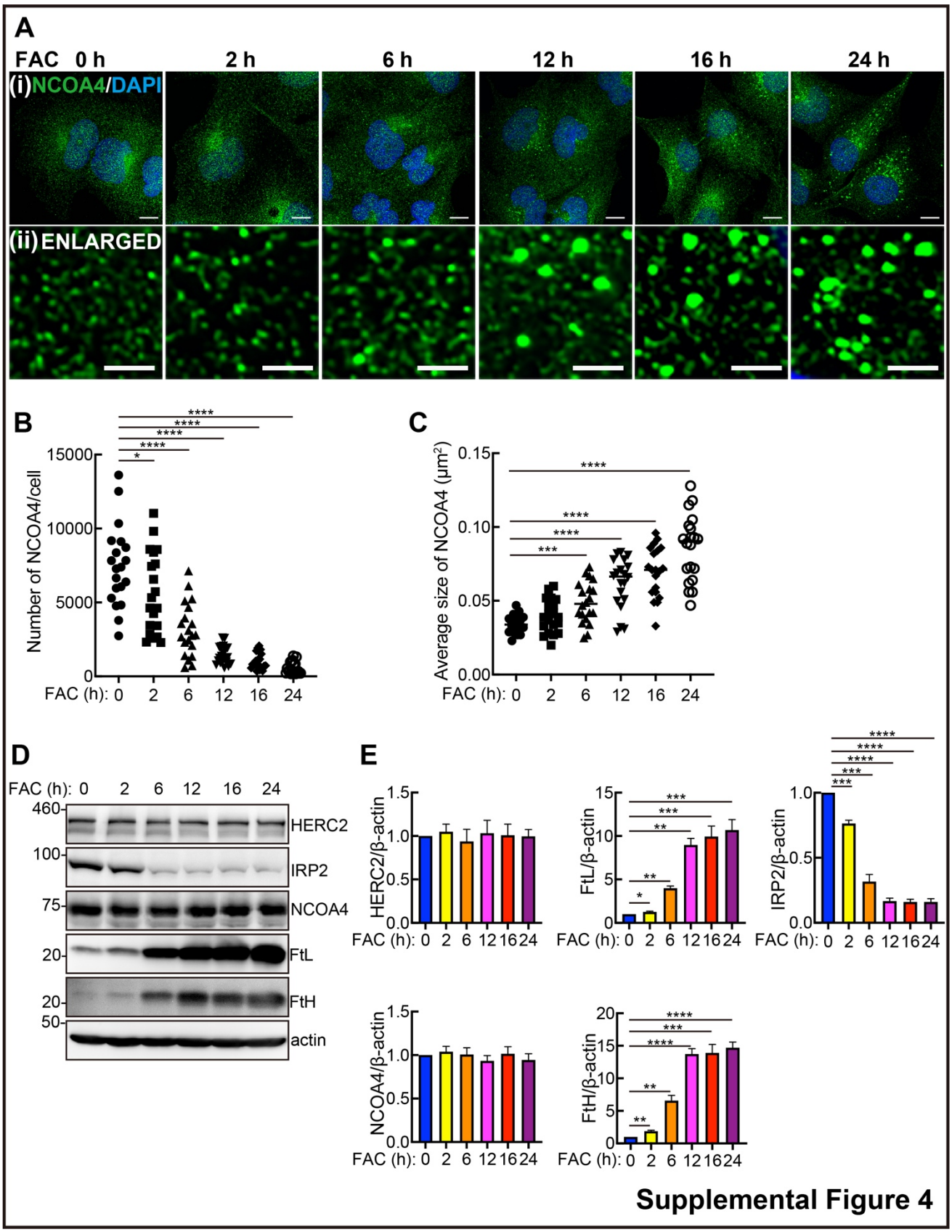


Supplemental Figure 3



Supplemental Figure 4