Supplementary Information for

Inositol triphosphate-triggered calcium release blocks lipid exchange at endoplasmic reticulum-Golgi contact sites

Mouhannad Malek, Anna M. Wawrzyniak, Peter Koch, Christian Lüchtenborg, Manuel Hessenberger, Timo Sachsenheimer, Wonyul Jang, Britta Brügger, Volker Haucke

Inventory of supplementary items

Supplementary figures

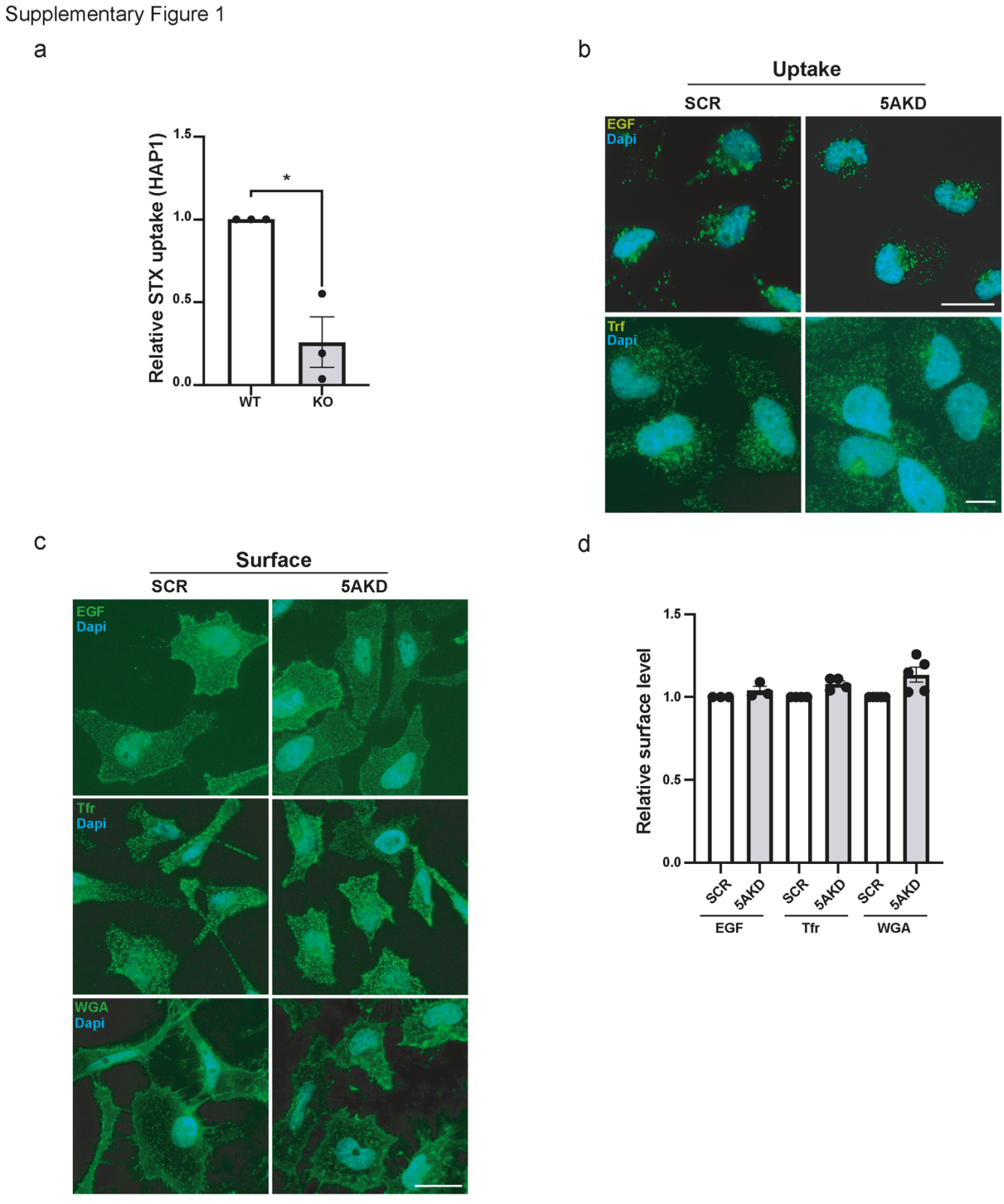
Supplementary figure 1 | INPP5A loss impairs clathrin-independent endocytosis of bacterial Shiga toxin but not CME.
Supplementary figure 2 | Cholesterol rerouting to lysosomes and lipid droplets in absence of INPP5A.
Supplementary figure 3 | | Cholesterol depletion or loss of VAP or OSBP proteins impair CIE of Shiga toxin.
Supplementary figure 4 | Levels of PI(4,5)P_2 and Golgi PI(4)P in INPP5A-depleted cells and effects of VAP or OSBP loss-of-function on SREBP-1 protein expression.
Supplementary figure 5 | INPP5A controls Shiga toxin endocytosis by regulating IP_3 levels.
Supplementary figure 6 | Phospholipase C and calcium regulate Shiga toxin endocytosis.
Supplementary figure 7 | IP_3-mediated calcium release represses lipid exchange at ER/ Golgi contact sites by inhibiting OSBP recruitment and function.

Supplementary tables

Supplementary table 1 | Antibodies
Supplementary table 2 | Oligonucleotides

Supplementary Data File 1 not contained in this PDF: Quantitative mass spectrometric analysis of total lipids or plasma membrane fractions from control and INPP5A-depleted HeLa cells

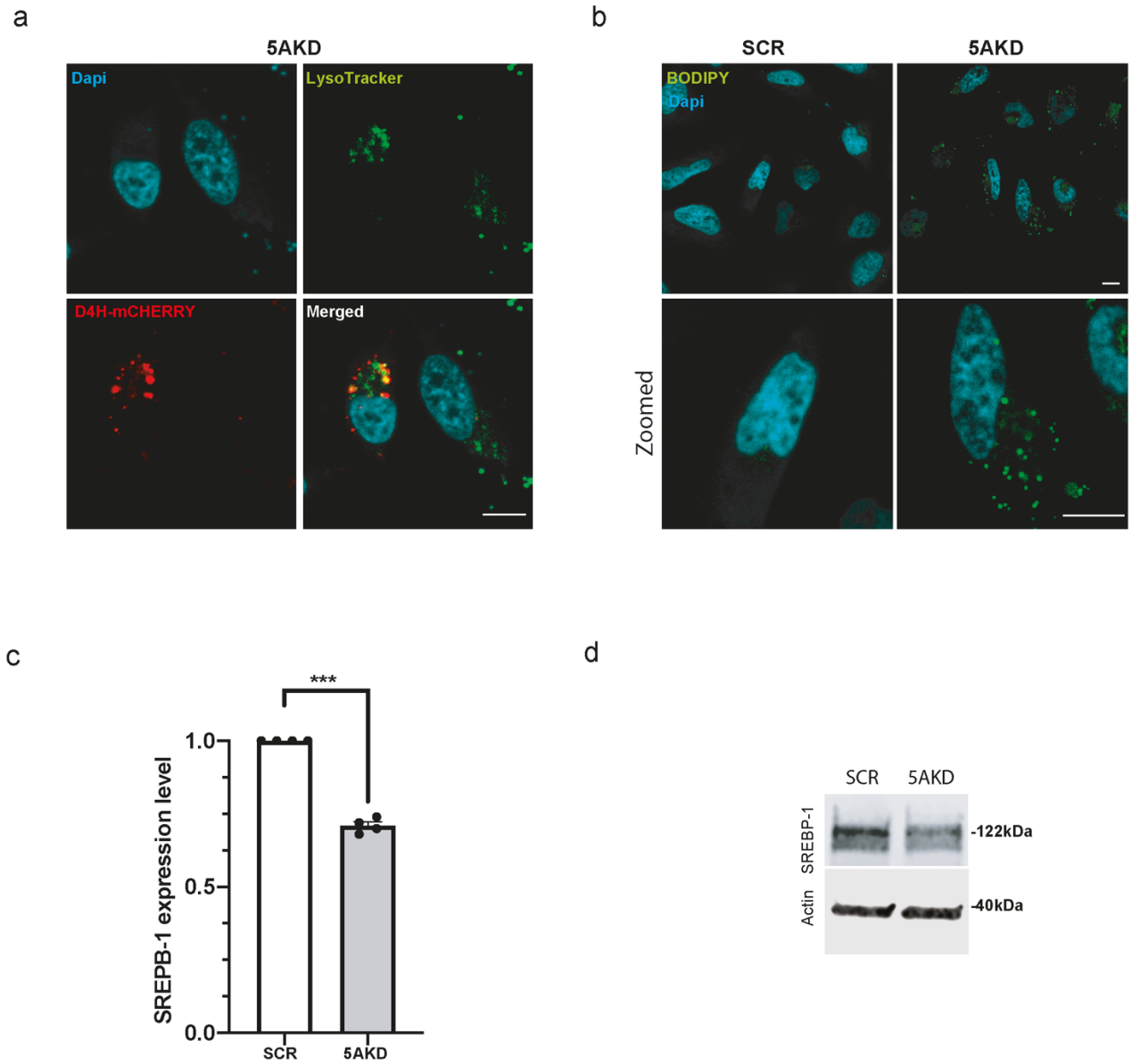
Supplementary figures



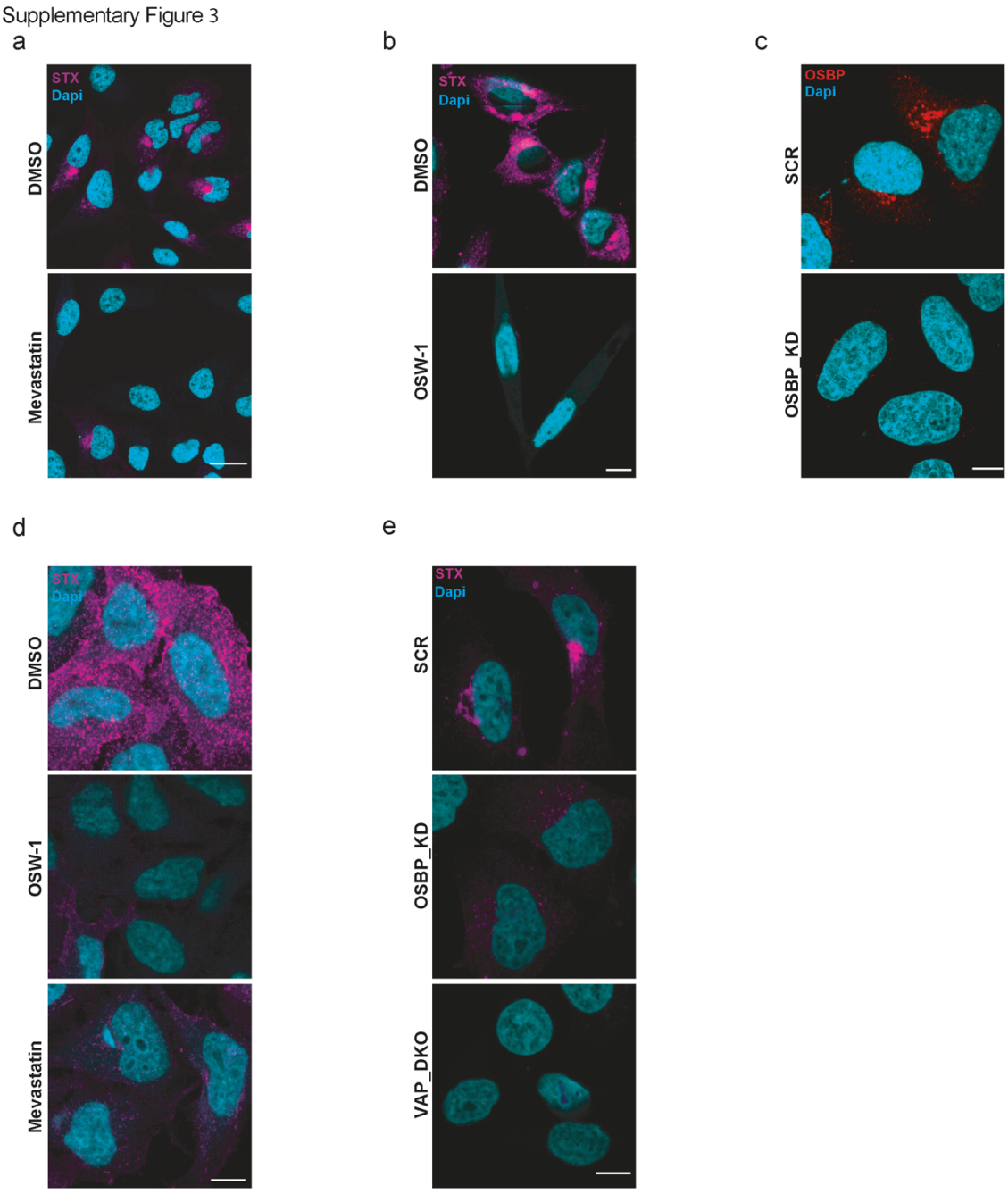
Supplementary figure 1 | INPP5A loss impairs clathrin-independent endocytosis of bacterial Shiga toxin but not CME. (a) Relative Shiga toxin (STX; 5 µg/ml) uptake in WT HAP-1 cells (set to 1) or *INPP5A* knockout (KO) cells. One sample t-test : p =0.0398 , t =4.862 , df =2. Data represent mean ± SEM from 3 independent experiments **(b)** Representative confocal images for fig1e. Scale bar, 25µm

(EGF) or 10µm (Tfr). **(c)** Representative confocal images of Alexa Fluor 647-labeled EGF, transferrin (Tfr), or wheat germ agglutinin (WGA) (all in green) bound to the surface of HeLa cells kept at 4°C and treated with scrambled (SCR) control siRNA or siRNA against INPP5A (5AKD). Blue, DAPI-stained nuclei. Scale bar, 25µm. **(d)** Quantification of representative data shown in Fig. S1c (SCR of each pair is set to 1) one sample t-test. EGF: $p = 0.2529$, $t = 1.589$, $df = 2$. Tf: $p = 0.0205$, $t = 4.496$, $df = 3$. WGA: $p = 0.0385$, $t = 3.037$, $df = 4$. Data represent mean ± SEM from 3 independent experiments for EGF, n=4 for Tfr and n=5 for WGA. Numerical source data are reported in the Source Data file.

Supplementary Figure 2

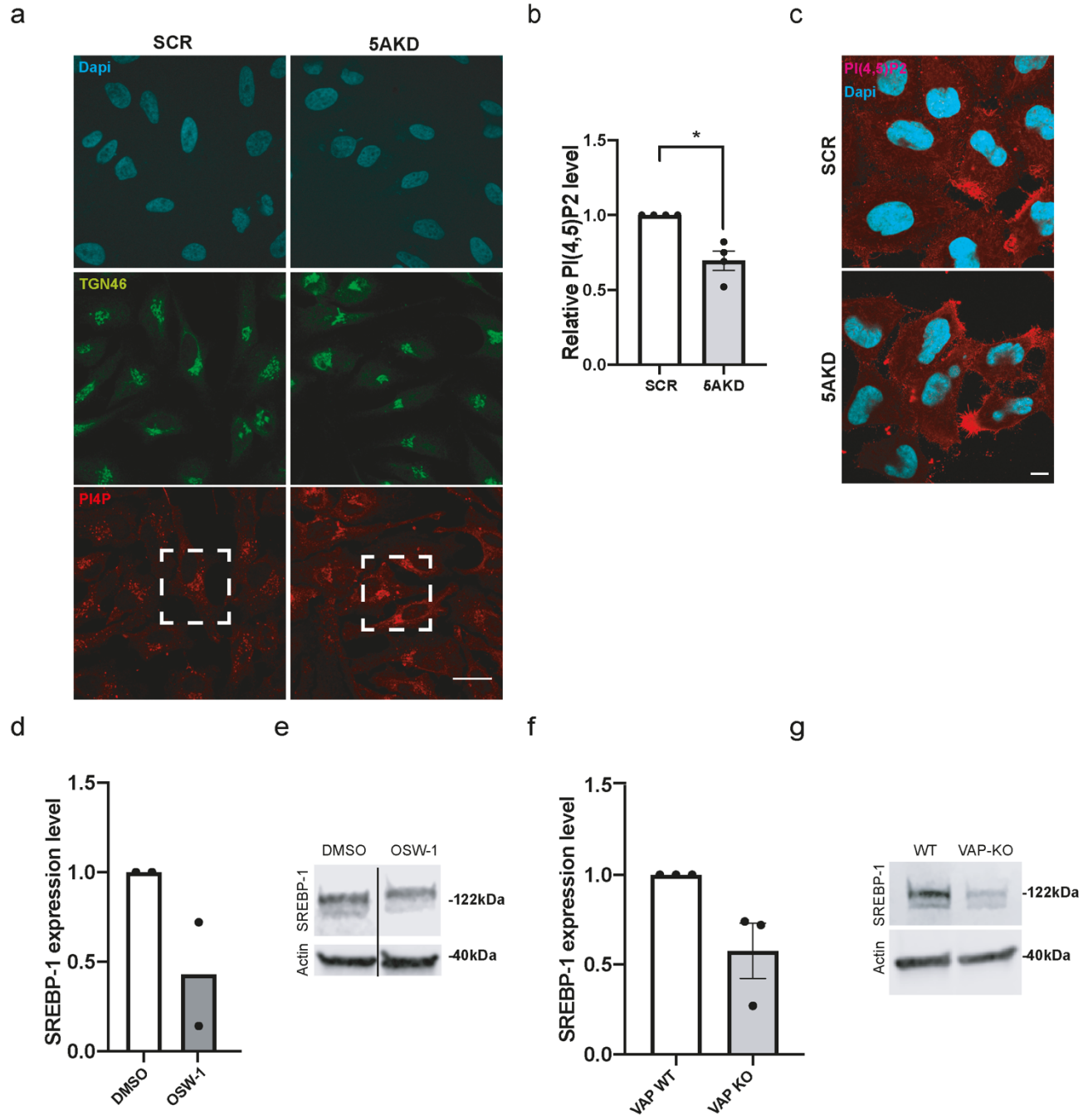


Supplementary figure 2 | In the absence of INPP5A cholesterol is directed towards lysosomes and lipid droplets. **(a)** Representative images of HeLa cells treated with INPP5A siRNA (5AKD), transfected with a plasmid encoding D4H-mCherry (red) to detect intracellular cholesterol, and incubated with Lysotracker (1:1000 dilution) for 60 minutes before fixation to detect lysosomes (green). Blue, DAPI-stained nuclei. Scale bar, 10 μm. **(b)** Top, low magnification views of HeLa cells treated with control (SCR) or INPP5A siRNA (5AKD) and incubated before fixation for 15 min with 2 μM of BODIPY 493/503 (4,4-Difluoro-1,3,5,7,8-Pentamethyl-4- Bora-3a,4a-Diaza-s-Indacene) (green). Bottom, zoomed images. Scale bar, 10 μm. **(c)** Quantification of representative data shown in S2d. One sample t test P=0.0002, t= 22.46 df =3. Data represent mean ± SEM from 4 independent experiments **(d)** Representative immunoblot analysis of HeLa cells treated with control (SCR) or INPP5A siRNA (5AKD) for 48 hours. Immunoblots were decorated with antibodies against SREBP-1 and β-actin as a loading control Numerical source data and unprocessed blots are reported in the Source Data file.



Supplementary figure 3 | Cholesterol depletion or loss of VAP or OSBP proteins impair CIE of Shiga toxin. **(a)** Representative confocal images of HeLa cells treated with control DMSO or Mevastatin (250 nM) for 24 h in presence of Serum, and incubated with labeled STX (magenta). Blue, DAPI-stained nuclei. Scale bar, 25 µm. **(b)** Representative confocal images for Fig.5g. Shiga toxin, STX (magenta) Blue, DAPI-stained nuclei. Scale Bar, 10 µm. **(c)** Representative confocal images of HeLa cells treated with control siRNA (SCR) or siRNA against OSBP (OSBP_KD) and stained for endogenous OSBP (red). Blue, DAPI-stained nuclei. Scale bar, 10 µm. **(d)** Representative confocal images for Fig.5a, Fig.5h. Shiga toxin, STX (magenta) Blue, DAPI-stained nuclei. Scale Bar, 10 µm. **(e)** Representative confocal images for Fig.5f and Fig.5i. Shiga toxin, STX (magenta) Blue, DAPI-stained nuclei. Scale Bar, 10 µm. All data are representative of data from 3 independent experiments.

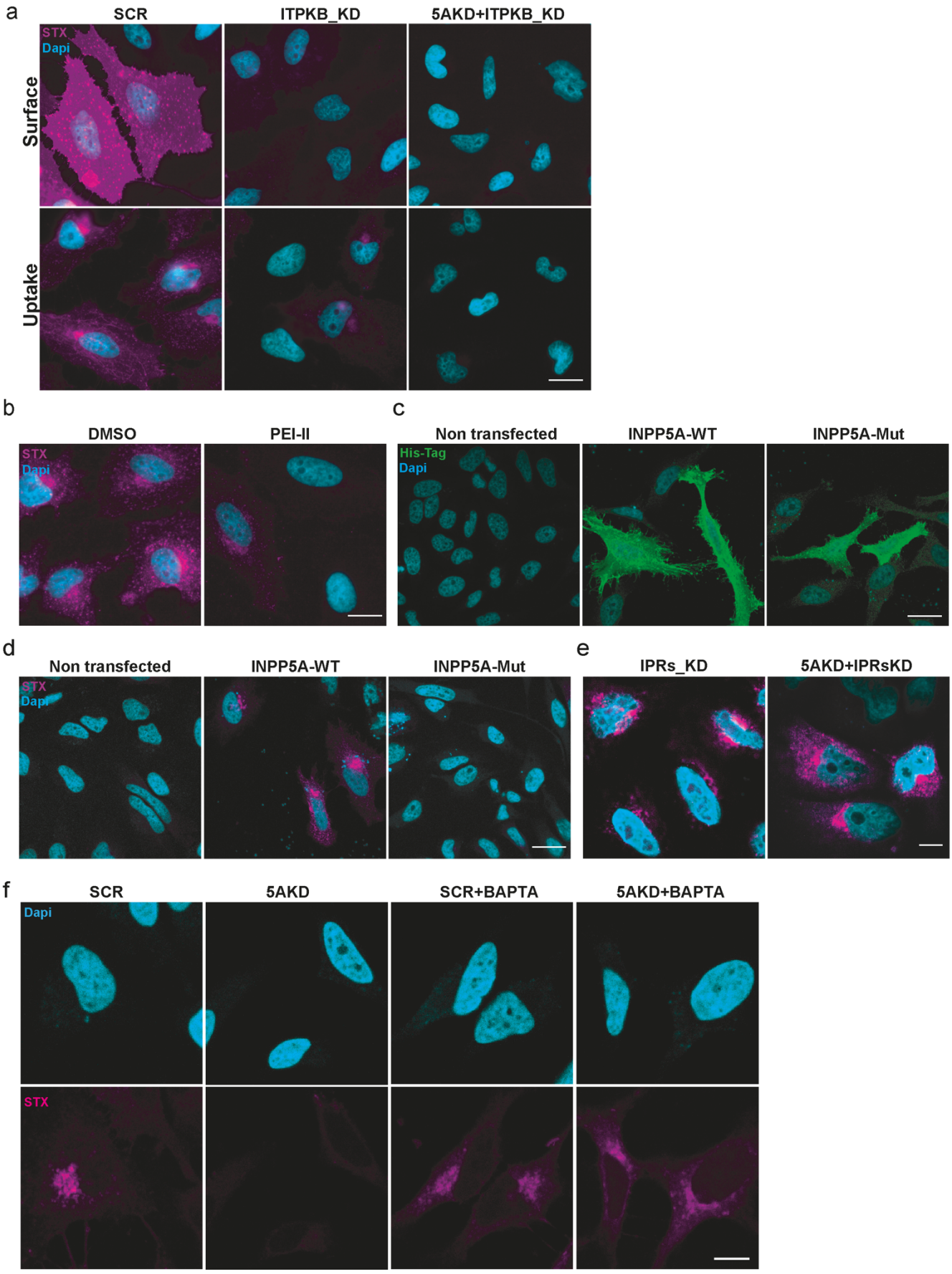
Supplementary Figure 4



Supplementary figure 4 | Levels of PI(4,5)P_2 and Golgi PI(4)P in INPP5A-depleted cells and effects of VAP or OSBP loss-of-function on SREBP-1 protein expression. **(a)** Low magnification views representative from 5 independent experiments of HeLa treated with control siRNA (SCR) or against INPP5A (5AKD) and stained with antibodies against TGN46 (green) and PI(4)P (red). White quadrants delimit the area shown in Figure 5e. Scale bar, 25 µm. **(b)** Relative phosphatidylinositol 4,5-biphosphate [PI(4,5)P_2] levels in HeLa cells treated with control (SCR, set to 1) or INPP5A siRNA (5AKD) one sample t-test. p =0.0176, t =4.758, df =3. Data represent mean ± SEM from 4 independent experiments **(c)** Representative confocal images of HeLa cells treated with control (SCR) or INPP5A siRNA (5AKD) and stained for PI(4,5)P_2 (red). Blue, DAPI-stained nuclei. Scale Bar, 10 µm. **(d,e)** Quantification (e)

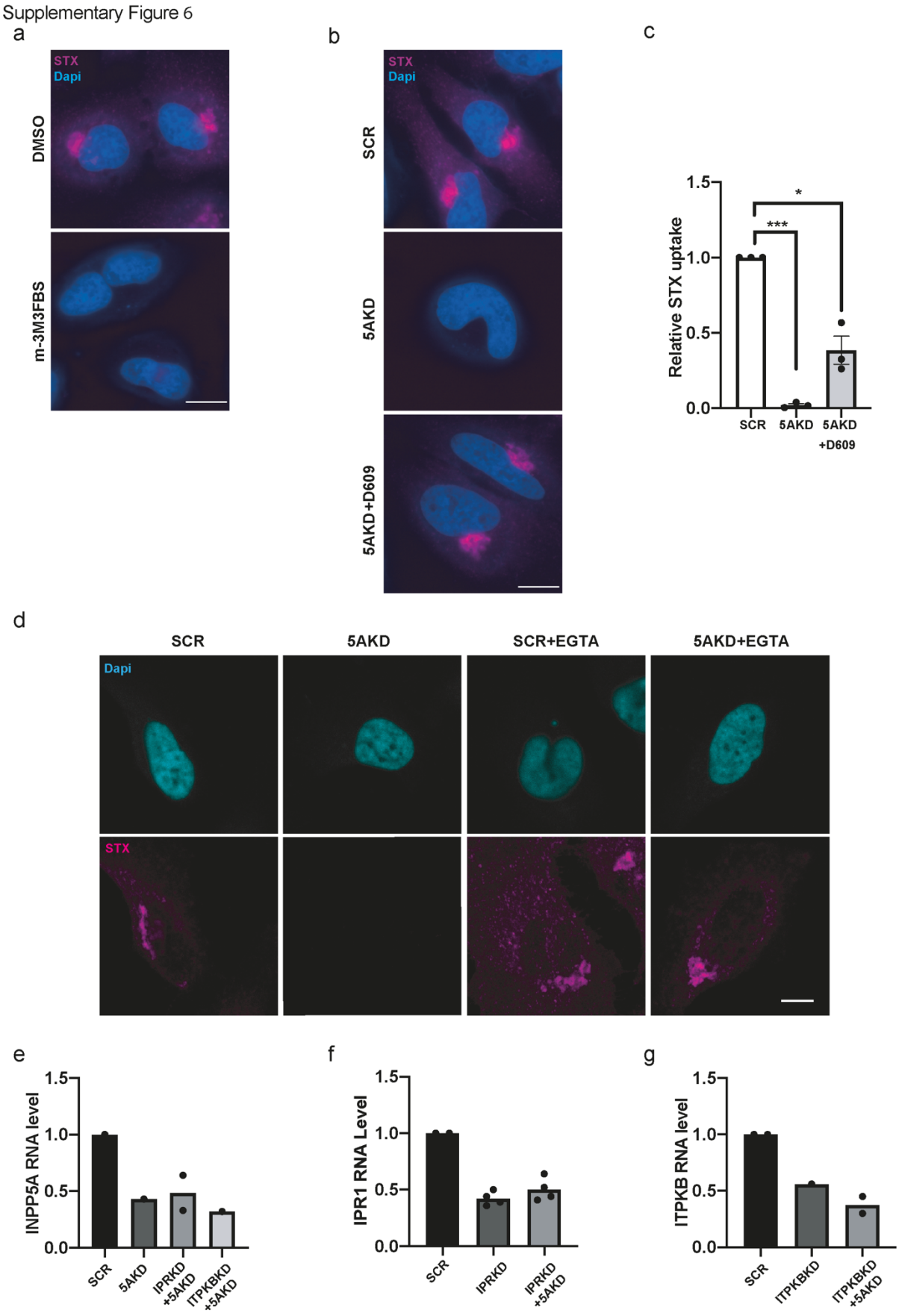
and representative immunoblot (e) analysis of HeLa cells treated with DMSO or the OSBP inhibitor OSW-1 (20 nM, 16h). Immunoblots were decorated with antibodies against SREBP-1 and β-actin as a loading control. Data represent mean ± SEM from 2 independent experiments, black vertical line indicates the fusion line of two cropped blots **(f,g)** Quantification (f) and representative immunoblot (g) analysis of wild-type (*VAP*-WT) or *VAP-A/ VAP-B* double knockout (*VAP*-DKO) HeLa cells. Immunoblots were decorated with antibodies against SREBP-1 and β-actin as a loading control. One sample t test. p=0.1101 t=2.759 df=2. Data represent mean ± SEM from 3 independent experiments. Numerical source data and unprocessed blots are reported in the Source Data file.

Supplementary Figure 5



Supplementary figure 5 | INPP5A controls Shiga toxin endocytosis by regulating IP_3 levels. (a) Representative confocal images from at least 3 independent experiments for Fig.6c. Shiga toxin (STX,

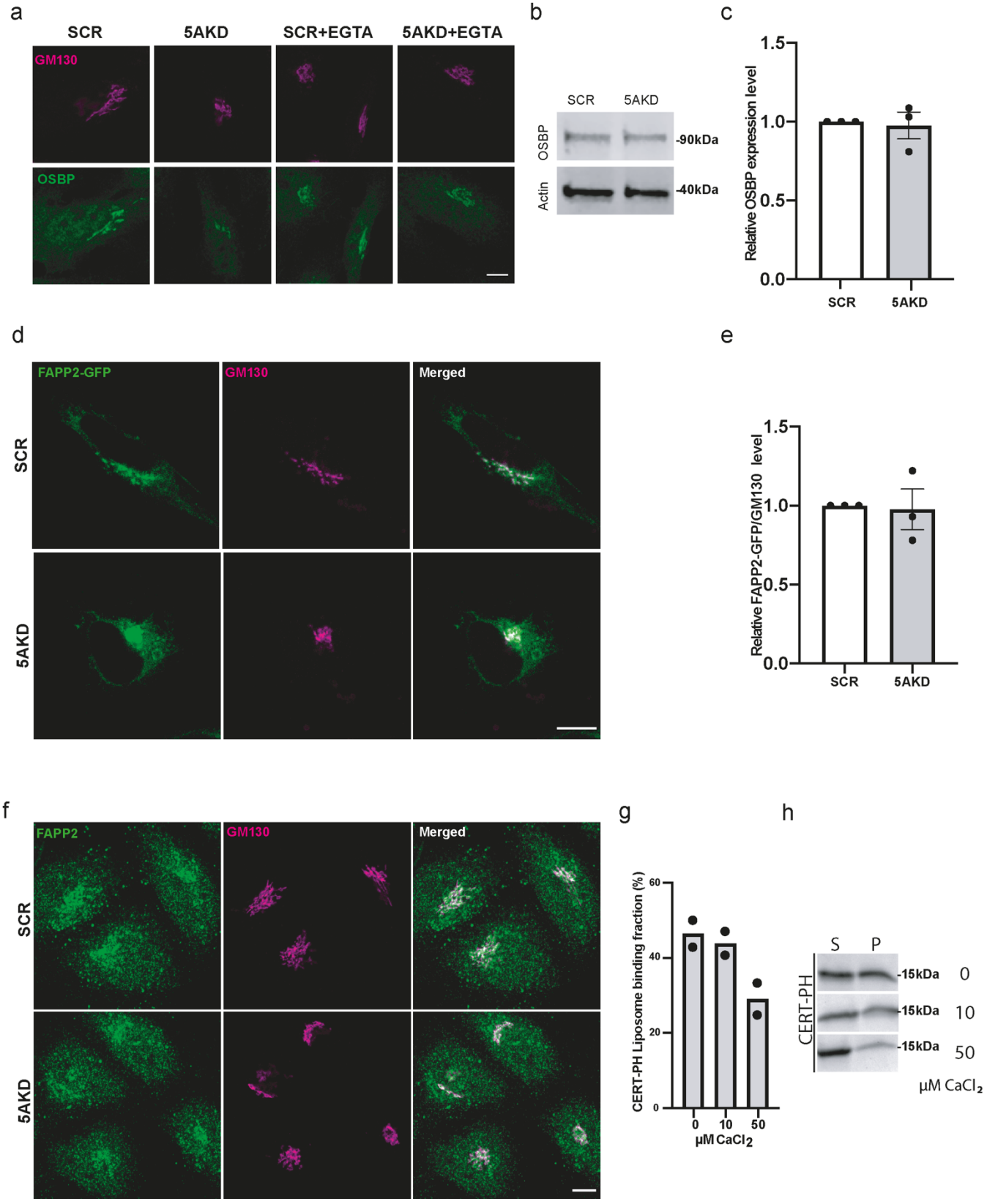
magenta); blue, DAPI-stained nuclei. Scale bar, 25 μm. **(b)** Representative confocal images from 4 independent experiments for Fig. 6d Shiga toxin (STX, magenta); blue, DAPI-stained nuclei. Scale bar, 25 μm. **(c)** Representative confocal images from at least 3 independent experiments of INPP5A-depleted HeLa cells expressing WT INPP5A-myc-6xHis (INPP5A- WT) or phosphatase-inactive (INPP5A-Mut) stained with antibodies against the 6xHis-Tag (green). Blue, DAPI-stained nuclei. Scale bar, 25 μm. **(d)** Representative confocal images from at least 3 independent experiments for Fig. 6e. STX (magenta) Blue, DAPI- stained nuclei. Scale bar, 25μm. **(e)** Representative confocal images from 6 independent experiments for Fig. 6i. STX (magenta) Blue, DAPI-stained nuclei. Scale bar, 10μm. (**f)** Representative confocal images from 3 independent experiments of Figure 6h. STX (magenta) Blue, DAPI-stained nuclei. Scale bar, 10μm. Numerical source data are reported in the Source Data file.



Supplementary figure 6 | Phospholipase C and calcium regulate Shiga toxin endocytosis. (a) Representative images for Fig.6j. Shiga toxin (STX), magenta; blue, DAPI-stained nuclei. Scale Bar,

10μm. **(b)** Representative images for Fig. S6c. Shiga toxin (STX, magenta); blue, DAPI-stained nuclei. Scale bar, 10μm. **(c)** Pharmacological inhibition of PLC activity partly rescues defective Shiga toxin endocytosis caused by loss of INPP5A. Data for SCR-siRNA transfected control cells were set to 1. One sample t-test 5AKD: p =0.0001, t =95.94, df =2. 5AKD+D609 p=0.0222 t= 6.592, df=2. Data represent mean ± SEM from three independent experiments **(d)** Defective STX endocytosis is rescued by using the cell-permeant Ca2+ chelator EGTA-AM (1 μM). Representative images where STX (magenta) Blue, DAPI-stained nuclei. Scale bar, 10 μm. **(e-f)** Efficiency of siRNA-mediated knock-downs in HeLa cells treated with scrambled control siRNA control (SCR) or specific siRNAs targeting *INPP5A* (5AKD), *IP$_3$Rs* (IPRKD), or *ITPKB* (ITPKBKD). mRNA levels were measured by real-time qPCR using specific primers for the indicated genes. (e) *INPP5A* mRNA levels. Data are representative of one (for 5AKD, ITPKKD+5AKD) or two (IPRKD + 5AKD) experiments. (f) *IP$_3$R1* mRNA levels. Data represent two (SCR) or four (IPRKD; IPRKD +5AKD) independent experiments. (g) *ITPKB* mRNA levels. Data represent one (ITPKBKD) or two (SCR; ITPKBKD +5AKD) independent experiments. Numerical source data are reported in the Source Data file.

Supplementary Figure 7



Supplementary figure 7 | IP_3-mediated calcium release represses lipid exchange at ER/ Golgi contact sites by inhibiting OSBP recruitment and function. (a) Defective OSBP localisation is rescued by using the cell-permeant Ca2+ chelator EGTA-AM (1µM). Representative images from 3 independent experiments where OSBP (Green), GM130 (Magenta) Blue, DAPI-stained nuclei. Scale

bar, 10 μm. **(b)** Representative immunoblot analysis of HeLa cells treated with control (SCR) or INPP5A siRNA (5AKD). Immunoblots were decorated with antibodies against OSBP and β-actin as a loading control. **(c)** Quantification of data shown in panel b. p=0.8015 t=0.2865 df=2 Data represent mean ± SEM from 3 independent experiments **(d)** Representative confocal images of HeLa cells treated with control (SCR) or INPP5A siRNA (5AKD) transfected with FAPP2-PH-GFP (green) for 48 hours and stained with GM130 (Magenta). Scale Bar, 10 μm. **(e)** Quantification of FAPP2-GFP colocalising with GM130 shown in Fig S7d. One sample t test p=0.8733, t=01807, df=2. Data represent mean ± SEM from 3 independent experiments **(f)** Representative confocal images of HeLa cells treated with control (SCR) or INPP5A siRNA (5AKD) stained with antibody against endogenous FAPP2 (green) and GM130 (Magenta). (Scale Bar, 10 μm). **(g)** Quantification of representative PI(4)P-liposome binding assays shown in (h). Data represent mean ± SEM from 2 independent experiments **(h)** Representative data from 3 independent experiments for Calcium effect using Liposome co-sedimentation assays to determine binding of purified recombinant CERT-PH to PI(4)P-containing liposomes. S, supernatant; P, liposomal pellet. Samples were analysed by SDS-PAGE and staining with Coomassie Blue.

Supplementary table 1 | Antibodies

Antibody	Species	Dilution		Source	Catalogue number
		ICC	IB		
Primary antibodies					
GFP	ms		1:2500	Clontech	632381
GM130	ms	1:500		BD transduction	610822
TGN46	rb	1:200		Abcam	ab50595
INPP5A	rb		1:500	ProteinTech	21723-1-AP
Ceramide	ms	1:10		Enzo	ALX-804-196
OSBP	rb	1:500		Sigma-aldrich	HPA039227
Actin	ms		1:1000	Sigma-aldrich	A5441
SREBP1	ms		5 µg/ml	Abcam	(ab3259)
FAPP2	rb	1:250		Gift from Antonella de matteis	N/A
HA-Tag	ms		1:1000	Abcam	ab130275
HIS-Tag	ms	1:200		Novagen	70796-3
PI4P	ms	1:100		Echlon	z-p004
PI(4,5)P2	ms	1:100		Echlon	Z-A045
Secondary antibodies					
α-ms IgG (H+L) AF488	gt	1:400		Thermo Fisher	A11029
α-rb IgG (H+L) AF488	gt	1:400			A11034
α-rb IgG (H+L) AF647	gt	1:400			A21244
α-r IgG (H+L) AF488	dk	1:400			A21208
α-ms IgM (H+L) AF568	gt	1:400			A21043
α-rb IRDye680RD IgG(H+L)	gt		1:10000	LI-COR Biosciences	926 -68071
α-rb IRDye800RD IgG(H+L)	gt		1:10000		926 -32211
α-msIRDye680RD IgG(H+L)	gt		1:10000		925 -68070
α-msIRDye800RDIgG(H+L)	gt		1:10000		926 -32210

Supplementary table 2 | Oligonucleotides

siRNA name	Sequence	Souce	Reference
Universal Negative Control #1	n.a	Sigma MISSION	SIC001-10NMOL
INPP5A#1	5'-GCACCGCGCUCUUGGAGUU-3'	Sigma MISSION	Customized
OSBP	n.a	Dharmacon	L-009747-00-0020
ITPKB	n.a	Dharmacon	L-006743-00-0005
ITPR1	n.a	Dharmacon	L-006207-02-0005
ITPR2	n.a	Dharmacon	L-006208-02-0005
ITPR3	n.a	Dharmacon	L-006209-02-0005
PIP5K1B	n.a	Dharmacon	8395

Primers

Name	Sequence from 5´ to 3'
ITPR1-Fw	GCGGAGGGATCGACAAATGG
ITPR1-Rev	TGGGACATAGCTTAAAGAGGCA
ITPKB-Fw	TCTCCTCATCCTACGAAGACTCA
ITPKB-Rev	GCTCACTCTAGGTTTCTGCTGG
INPP5A-Fw	ACACGAACATGGCACTAGGA
INPP5A-Rev	GGGCGTGCTCTCTAAGGTAT