Supplementary Information:

Palmitoylome profiling reveals S-palmitoylation-dependent anti-viral activity of IFITM3

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Supplementary Table 1. Proteins selectively identified with high confidence in alk-16 samples by mass spectrometry. Data was categorized as high confidence if the sum of assigned spectra was at least 10 fold greater for alk-16 samples than the sum of spectra identified in the DMSO samples. Further, in order to be considered a high confidence candidate, the protein must have been identified with spectral counts greater than 5 in at least 2 of 3 runs. Data was compared to other palmitoylome protein proteomic data sets from the El-Husseini[1], Freeman[2], and Cravatt[3] groups and proteins were shaded blue if they had not been identified in these previous experiments (X indicates identification). Ribosomal subunits not previously identified were differentiated with yellow shading as many other ribosomal subunits have been previously characterized as palmitoylated proteins. Proteins with potential myristoylation sites (N-terminal glycines) are marked with an asterisk.

Gene	Description	Access. #	DMSO-1	ALK16-1	DMSO-2	ALK16-2	DMSO-3	ALK16-3	SUM NEG	SUM POS	El-Husseini	Freeman	Cravatt
Canx	Calnexin	IPI00119618	2	36	0	461	0	275	2	772	X	X	X
Ifitm3	Interferon-Induced Transmembrane Protein 3	IPI00133243	0	74	0	55	0	76	0	205			
Mtdh	Protein LYRIC	IPI00330599	0	3	0	56	0	47	0	106	X	X	X
M6pr	Cation-dependent mannose-6-phosphate receptor	IPI00108844	0	4	0	49	0	52	0	105		X	
Hspd1	Isoform 1 of 60 kDa heat shock protein, mitochondrial	IPI00308885	3	37	0	12	5	53	8	102			
Gnai3	Guanine nucleotide-binding protein G(k) subunit alpha*	IPI00338854	0	10	0	46	0	34	0	90		X	X
Rpl4	60S ribosomal protein L4	IPI00111412	0	7	0	18	0	49	0	74			
Ckap4	Cytoskeleton-associated protein 4	IPI00223047	0	0	0	60	0	11	0	71		X	
Rpl6	60S ribosomal protein L6	IPI00313222	5	25	0	14	0	32	5	71	X		X
Snap23	Synaptosomal-associated protein	IPI00113798	0	15	0	21	0	15	0	51		X	X
-	RhoA activator C11orf59 homolog*	IPI00315187	0	10	4	23	0	18	4	51	X	X	X
Ncl	Nucleolin	IPI00317794	0	11	0	0	2	39	2	50			
Pgd	6-phosphogluconate dehydrogenase, decarboxylating	IPI00466919	4	11	0	22	0	14	4	47			
Gnai2	Guanine nucleotide-binding protein G(i), alpha-2 subunit*	IPI00228617	0	0	0	21	0	22	0	43		X	X
Atp5a1	ATP synthase subunit alpha, mitochondrial	IPI00130280	0	8	0	15	2	17	2	40	X		
Got2	Aspartate aminotransferase, mitochondrial	IPI00117312	0	11	0	11	2	16	2	38			
Vcp	Transitional endoplasmic reticulum ATPase	IPI00622235	0	17	0	4	0	16	0	37			
Rps19	40S ribosomal protein S19	IPI00113241	0	7	0	10	0	20	0	37			
Rpl5	60S ribosomal protein L5*	IPI00308706	0	7	0	12	0	18	0	37	X	X	X
Cyb5b	Cytochrome b5 type B	IPI00315794	0	10	0	15	0	11	0	36	X	X	
Rps3	40S ribosomal protein S3	IPI00134599	2	15	0	8	0	13	2	36			
CD44	CD44 antigen isoform c	IPI00223769	0	9	0	13	0	13	0	35		X	
Tfrc	Transferrin receptor protein 1	IPI00124700	0	0	0	12	0	22	0	34		X	X
Rpl7	60S ribosomal protein L7	IPI00311236	3	16	0	6	0	12	3	34			
Slc1a5	Neutral amino acid transporter, member 5	IPI00229548	0	0	0	26	0	7	0	33		X	X
Eif4a1	Eukaryotic initiation factor 4A-I	IPI00118676	0	0	0	23	0	10	0	33			
Rpl1	Ribosomal protein L1	IPI00849927	0	4	0	10	0	19	0	33			
Scamp3	Putative uncharacterized protein	IPI00653426	0	3	0	19	0	10	0	32	X	X	X
Rap2c	Ras-related protein Rap-2c	IPI00466588	0	8	0	12	0	8	0	28		X	X
Aco2	Aconitate hydratase, mitochondrial	IPI00116074	0	6	0	17	0	3	0	26			
Irgm	Isoform 1 of Immunity-related GTPase family M protein	IPI00120264	0	0	0	8	0	18	0	26			
Rpl29	60S ribosomal protein L29	IPI00222548	0	7	0	8	0	10	0	25			
Phgdh	D-3-phosphoglycerate dehydrogenase	IPI00225961	2	0	0	16	0	9	2	25	X		
Slc25a5	ADP/ATP translocase 2	IPI00127841	0	0	0	5	0	19	0	24		X	
Slc15a3	Solute carrier family 15 member 3	IPI00124097	0	3	0	0	0	21	0	24			
Rpl19	60S ribosomal protein L19	IPI00122426	0	9	0	5	0	10	0	24			
Aldh2	Aldehyde dehydrogenase, mitochondrial	IPI00111218	0	3	0	15	0	5	0	23			
Nras	GTPase NRas	IPI00775965	0	0	0	13	0	8	0	21		X	
Rps8	40S ribosomal protein S8*	IPI00466820	0	7	0	4	0	10	0	21			
Gm16408	hypothetical protein, ribosomal protein S2 pseudogene	IPI00604967	0	0	0	11	0	9	0	20			
Capg	Capping protein (Actin filament), gelsolin-like	IPI00277930	0	5	0	7	0	8	0	20			
Ywhaz	14-3-3 protein zeta/delta	IPI00116498	0	0	0	7	0	13	0	20			
Gpi	Glucose-6-phosphate isomerase	IPI00228633	0	0	0	11	2	9	2	20	X	X	
Rpl7a	60S ribosomal protein L7a	IPI00330363	0	7	0	3	0	8	0	18			
Procr	Endothelial protein C receptor	IPI00136972	0	10	0	0	0	8	0	18		X	
Rpl3	60S ribosomal protein L3	IPI00321170	0	11	0	3	0	4	0	18	X	X	X
Cap1	Adenylyl cyclase-associated protein 1	IPI00137331	0	5	0	7	0	6	0	18	X	X	
Nme2	Nucleoside diphosphate kinase B	IPI00127417	0	6	0	3	0	8	0	17	X		
Gnas	Guanine nucleotide-binding protein G(s) subunit alpha*	IPI00119853	0	6	0	8	0	2	0	16		X	X
Pfn1	Profilin-1	IPI00224740	0	0	0	5	0	10	0	15	X		
Rpl17	60S ribosomal protein L17	IPI00453768	0	6	0	0	0	9	0	15			
Rps17	40S ribosomal protein S17*	IPI00465880	0	8	0	0	0	7	0	15			X
Vdac2	Voltage-dependent anion-selective channel protein 2	IPI00122547	0	0	0	7	0	8	0	15	X		
Eef1b	Elongation factor 1-beta	IPI00320208	0	0	0	5	0	10	0	15			
Phf8	PHD finger protein 8	IPI00229377	0	0	0	5	0	9	0	14			
Phb	Prohibitin	IPI00133440	0	0	0	5	0	9	0	14		X	
Pdgfra	Alpha-type platelet-derived growth factor receptor*	IPI00844650	0	0	0	5	0	7	0	12			
Hnrnpk	Isoform 1 of Heterogeneous nuclear ribonucleoprotein K	IPI00223253	0	0	0	5	0	6	0	11			
Acat1	Acetyl-CoA acetyltransferase, mitochondrial	IPI00154054	0	6	0	5	0	0	0	11			
Atp5o	ATP synthase subunit O, mitochondrial	IPI00118986	0	0	0	5	0	5	0	10	X		X

Supplementary Tabale 2. Proteins selectively identified with medium confidence in alk-16 samples by mass spectrometry. Medium confidence proteins must have had a 5 fold or greater number of assigned spectra in the alk-16 samples as compared to DMSO samples and a spectral count of at least 5 in 1 of 3 runs. Data was compared to other lipidated protein proteomic data sets from the El-Husseini[1], Freeman[2], and Cravatt[3] groups and proteins were shaded blue if they had not been identified in these previous experiments (X indicates identification). Ribosomal subunits not previously identified were differentiated with yellow shading as many other ribosomal subunits have previously characterized as palmitoylated proteins. Proteins with potential myristoylation sites (N-terminal glycines) are marked with an asterisk. CD14, a previously characterized GPI-anchored protein is marked with two asterisks. Candidate viral proteins were shaded purple.

Gene	Description	Access. #	DMSO-1	ALK16-1	DMSO-2	ALK16-2	DMSO-3	ALK16-3	SUM NEG	SUM POS	El-Husseini	Freeman	Cravatt
Prdx1	Peroxiredoxin-1	IPI00121788	9	16	0	60	7	67	16	143	X		
Hspa8	Heat shock cognate 71 kDa protein	IPI00323357	8	22	6	60	11	57	25	139			
Rps27a	ribosomal protein S27a	IPI00470152	14	70	0	32	0	33	14	135		X	
Hadhb	Trifunctional enzyme subunit beta, mitochondrial	IPI00115607	0	0	0	72	0	0	0	72		X	X
Ppia	Peptidyl-prolyl cis-trans isomerase	IPI00554989	3	13	0	14	0	12	3	39		X	
Hspa9	heat shock protein 9	IPI00880839	4	14	0	4	0	11	4	29			
Cd14	Monocyte differentiation antigen CD14**	IPI00308990	0	2	0	0	0	25	0	27			
Ugt1a7c	UDP-glucuronosyltransferase 1-7C	IPI00417181	0	0	0	27	0	0	0	27			
Actc1	Actin, alpha cardiac muscle 1	IPI00114593	0	0	0	19	0	0	0	19			X
Rps3a	40S ribosomal protein S3a	IPI00331345	0	4	0	0	0	14	0	18		X	X
Gm6654	similar to ribosomal protein S26	IPI00261455	0	18	0	0	0	0	0	18			
Snd1	Staphylococcal nuclease domain-containing protein 1	IPI00123129	0	4	0	0	0	12	0	16			
Cd36	Platelet glycoprotein 4*	IPI00331214	0	0	0	12	0	4	0	16			
Ftl1	ferritin light chain 1	IPI00608020	2	0	0	9	0	7	2	16			
Pdia6	Protein disulfide-isomerase A6	IPI00222496	3	13	0	0	0	2	3	15			
Arhgdia	Rho GDP-dissociation inhibitor 1	IPI00322312	2	2	0	4	0	8	2	14	X		
Tmx1	Thioredoxin domain-containing protein 1	IPI00121341	0	0	0	13	0	0	0	13			
Hmox1	Heme oxygenase 1	IPI00131577	0	0	0	0	0	13	0	13			
Gm7218	ribosomal protein L21 pseudogene	IPI00473445	0	13	0	0	0	0	0	13			
Lrrc59	Leucine-rich repeat-containing protein 59	IPI00123281	0	0	0	4	0	8	0	12			
Arhgdib	Rho GDP-dissociation inhibitor 2	IPI00122568	0	0	0	4	0	8	0	12			
Rplp0	60S acidic ribosomal protein P0	IPI00314950	0	0	0	8	0	4	0	12			
Lyn	Isoform LYN B of Tyrosine-protein kinase Lyn*	IPI00230138	0	0	0	9	0	3	0	12		X	
Impdh2	Inosine-5'-monophosphate dehydrogenase 2	IPI00323971	0	0	0	9	0	3	0	12			X
Cct3	T-complex protein 1 subunit gamma	IPI00116283	0	4	0	4	0	3	0	11			
Prf1	Perforin-1	IPI00308993	0	0	0	11	0	0	0	11			
Rpl37	60S ribosomal protein L37	IPI00453924	0	8	0	3	0	0	0	11			
Rps7	40S ribosomal protein S7	IPI00136984	0	8	0	0	0	3	0	11	X		
Gna13	Guanine nucleotide-binding protein subunit alpha-13	IPI00118569	0	2	0	5	0	4	0	11		X	X
C1qb	complement component 1	IPI00132799	0	0	0	2	0	9	0	11			
Rps11	Ribosomal protein S11	IPI00117569	2	11	0	0	0	0	2	11		X	
Rps4x	40S ribosomal protein S4, X isoform	IPI00331092	0	2	0	0	0	8	0	10			
Eif5a	Eukaryotic translation initiation factor 5A-1	IPI00108125	0	0	0	4	0	6	0	10			X
Stxbp5	Isoform 2 of Syntaxin-binding protein 5	IPI00656317	0	0	0	4	0	6	0	10	X		
Cct4	T-complex protein 1 subunit delta	IPI00116277	0	0	0	6	0	4	0	10			
-	M.musculus endogenous provirus env (Fragment)	IPI00463556	0	0	0	10	0	0	0	10			
Clld6	Chronic lymphocytic leukemia deletion region 6 homolog	IPI00267667	0	0	0	4	0	6	0	10			
Fcer1g	High affinity immunoglobulin epsilon receptor gamma	IPI00119293	0	10	0	0	0	0	0	10			
Cct7	T-complex protein 1 subunit eta	IPI00331174	0	0	0	3	0	7	0	10			
Rps13	40S ribosomal protein S13*	IPI00125901	0	0	0	3	0	6	0	9			
Ywhae	14-3-3 protein epsilon	IPI00118384	0	0	0	4	0	5	0	9		X	
Rpl10	60S ribosomal protein L10*	IPI00474637	0	6	0	0	0	3	0	9			X
Cd68	Macrosialin	IPI00117031	0	0	0	9	0	0	0	9			
Pi4k2a	Phosphatidylinositol 4-kinase type 2-alpha	IPI00121277	0	0	0	9	0	0	0	9		X	X
Gdi2	Isoform 1 of Rab GDP dissociation inhibitor beta	IPI00122565	0	2	0	5	0	2	0	9			
Gm5908	hypothetical protein	IPI00474407	0	4	0	0	0	4	0	8			
Rps10	40S ribosomal protein S10	IPI00112448	0	0	0	3	0	5	0	8			
Gm3414	hypothetical protein	IPI00652753	0	0	0	8	0	0	0	8			
Gm10079	ribosomal protein S16 pseudogene	IPI00469918	0	0	0	0	0	8	0	8			
Rps25	40S ribosomal protein S15	IPI00319231	0	0	0	0	0	8	0	8			
Cct8	T-complex protein 1 subunit theta	IPI00469268	0	0	0	8	0	0	0	8			
Ebag9	Receptor-binding cancer antigen expressed on SiSo cells	IPI00154113	0	0	0	4	0	3	0	7	X		X
Msn	Moesin	IPI00110588	0	7	0	0	0	0	0	7		X	
Cd9	CD9 antigen	IPI00221921	0	0	0	7	0	0	0	7		X	
Cypt5	cysteine-rich perinuclear theca 5	IPI00742265	0	0	0	7	0	0	0	7			
Stx12	Syntaxin-12	IPI00111416	0	0	0	7	0	0	0	7	X		X
Mlec	Malectin	IPI00312018	0	0	0	3	0	4	0	7			
Mllt3	Protein AF-9	IPI00473183	0	0	0	5	0	2	0	7			

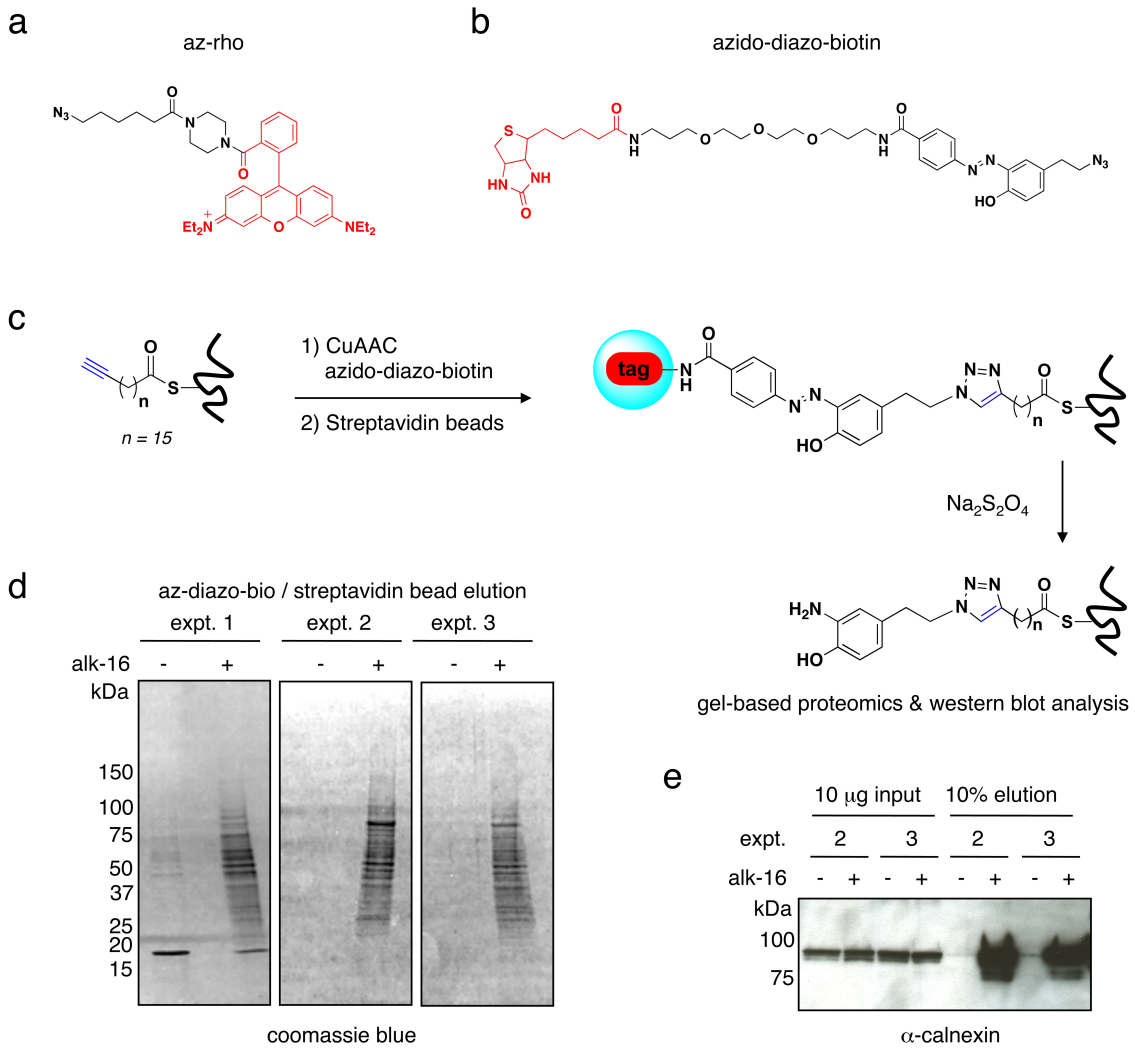
Vdac1	Voltage-dependent anion-selective channel protein 1	IPI00122549	0	0	0	0	0	7	0	7	X		
Cct2	T-complex protein 1 subunit beta	IPI00320217	0	0	0	4	0	3	0	7			X
Mela	Envelope protein	IPI00406960	0	0	0	6	0	0	0	6			
Znfx1	NFX1-type zinc finger-containing protein 1	IPI00660406	0	0	0	3	0	3	0	6			
Vamp3	Vesicle-associated membrane protein 3	IPI00132276	0	0	0	6	0	0	0	6		X	X
-	Gag-Pol polyprotein*	IPI00108206	0	0	0	0	0	6	0	6			
Tet2	Isoform 1 of Protein TET2	IPI00169923	0	0	0	6	0	0	0	6			
Zeb2	Zinc finger E-box-binding homeobox 2	IPI00420464	0	0	0	3	0	3	0	6			
Cfl1	Cofilin, non-muscle	IPI00407543	0	0	0	3	0	3	0	6			
Actr3	Actin-related protein 3	IPI00115627	0	0	0	6	0	0	0	6			
Usp32	ubiquitin specific protease 32	IPI00131330	0	6	0	0	0	0	0	6			
Rftn1	Raftlin*	IPI00469253	0	0	0	6	0	0	0	6			X
Rps9	40S ribosomal protein S9	IPI00420726	0	3	0	0	0	2	0	5		X	X
Abca14	ATP-binding cassette, sub-family A (ABC1), member 14	IPI00420146	0	0	0	5	0	0	0	5			
Rpl14	60S ribosomal protein L14	IPI00133185	0	5	0	0	0	0	0	5	X		
Bst2	Bone marrow stromal antigen 2	IPI00321222	0	0	0	5	0	0	0	5			
Psmd2	26S proteasome non-ATPase regulatory subunit 2	IPI00123494	0	0	0	0	0	5	0	5			X
Fam20b	Protein FAM20B	IPI00122494	0	0	0	5	0	0	0	5			
4930527J03Rik	Putative uncharacterized protein	IPI00675544	0	2	0	3	0	0	0	5			
Fmo9	flavin containing monooxygenase 9	IPI00223948	0	0	0	3	0	2	0	5			
Psma1	Proteasome subunit alpha type-1	IPI00283862	0	3	0	0	0	2	0	5			
Phb2	Prohibitin-2	IPI00321718	0	0	0	3	0	2	0	5			
Abcc1	Multidrug resistance-associated protein 1	IPI00129915	0	2	0	3	0	0	0	5			
Msrb2	Methionine-R-sulfoxide reductase B2, mitochondrial	IPI00109655	0	0	0	3	0	2	0	5			
H2afj	Histone H2A.J	IPI00153400	0	0	0	5	0	0	0	5			
Aplnr	Apelin receptor	IPI00124989	0	2	0	0	0	3	0	5			
Sod2	Superoxide dismutase [Mn], mitochondrial	IPI00109109	0	0	0	0	0	5	0	5			
Stat4	Signal transducer and activator of transcription 4	IPI00114970	0	0	0	2	0	3	0	5			
Itga2b	Integrin alpha-IIb	IPI00315155	0	3	0	2	0	0	0	5			
Tcp1	Isoform 1 of T-complex protein 1 subunit alpha B	IPI00459493	0	0	0	2	0	3	0	5	X	X	
Vti1b	Vesicle transport through interaction with t-SNAREs 1B	IPI00130115	0	2	0	0	0	3	0	5			
8430427H17Rik	Novel protein	IPI00453784	0	0	0	5	0	0	0	5			
Irg1	Immune-responsive gene 1 protein	IPI00111285	0	0	0	0	0	5	0	5			
Nos2	Nitric oxide synthase, inducible	IPI00125726	0	0	0	0	0	5	0	5			
Glud1	Glutamate dehydrogenase 1, mitochondrial	IPI00114209	0	5	0	0	0	0	0	5			
Ptbp1	polypyrimidine tract binding protein 1	IPI00136883	0	0	0	0	0	5	0	5			X
Hnrnpf	Heterogeneous nuclear ribonucleoprotein F	IPI00226073	0	0	0	2	0	3	0	5			
Atic	Bifunctional purine biosynthesis protein PURH	IPI00330303	0	0	0	5	0	0	0	5			
Emb	Embigin	IPI00129968	0	0	0	5	0	0	0	5			

Supplementary Table 3. Primers used for cloning and mutagenesis.

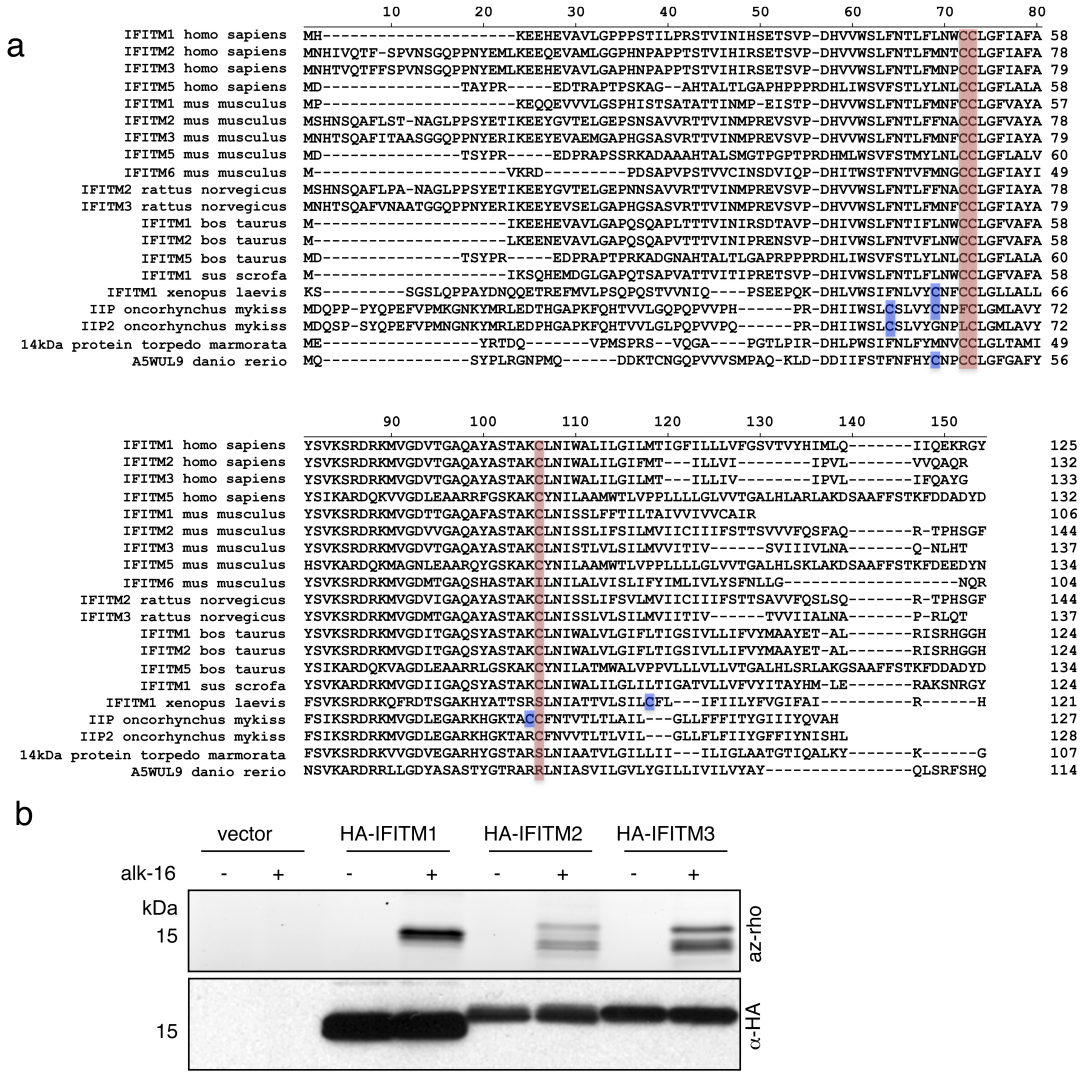
Description	Primer (5' to 3')
IFITM3 For	GAATTCCCATGAACCACACTTCTCAAGC
IFITM3 Rev	GTCGAC TTAAGTGTGAAGGTTTTGAG
CD9 For	AGATCTATGCCGGTCAAAGGAGGTAG
CD9 Rev	GAATTC TCACTAGACCATTTCTCGGCTCCTGC
IFITM3 C71A	ATACACTCTTCATGAACTTCGCATGCCTGGGCTTCATAGCCTATGCC
IFITM3 C72A	ATACACTCTTCATGAACTTCTGCGCACTGGGCTTCATAGCCTATGCC
IFITM3 C71, 72A	ATACACTCTTCATGAACTTCGCAGCACTGGGCTTCATAGCCTATGCC
IFITM3 C105A	CCTACGCCTCCACTGCTAAGGCACTGAACATCAGCACCTTGGTC
hIFITM1 For	GAATTCCCATGCACAAGGAGGAACATGA
hIFITM1 Rev	GTCGACCTAGTAACCCCGTTTTTCCTGTA
hIFITM2 For	GAATTCCCATGAACCACATTGTGCAAAC
hIFITM2 Rev	GTCGACCTATCGCTGGGCCTGGACGA
hIFITM3 For	GAATTCCCATGAATCACACTGTCCAAAC
hIFITM3 Rev	GGCC GTCGACCTATCCATAGGCCTGGAAGA

Supplementary Table 4. Primers used for quantitative PCR reactions

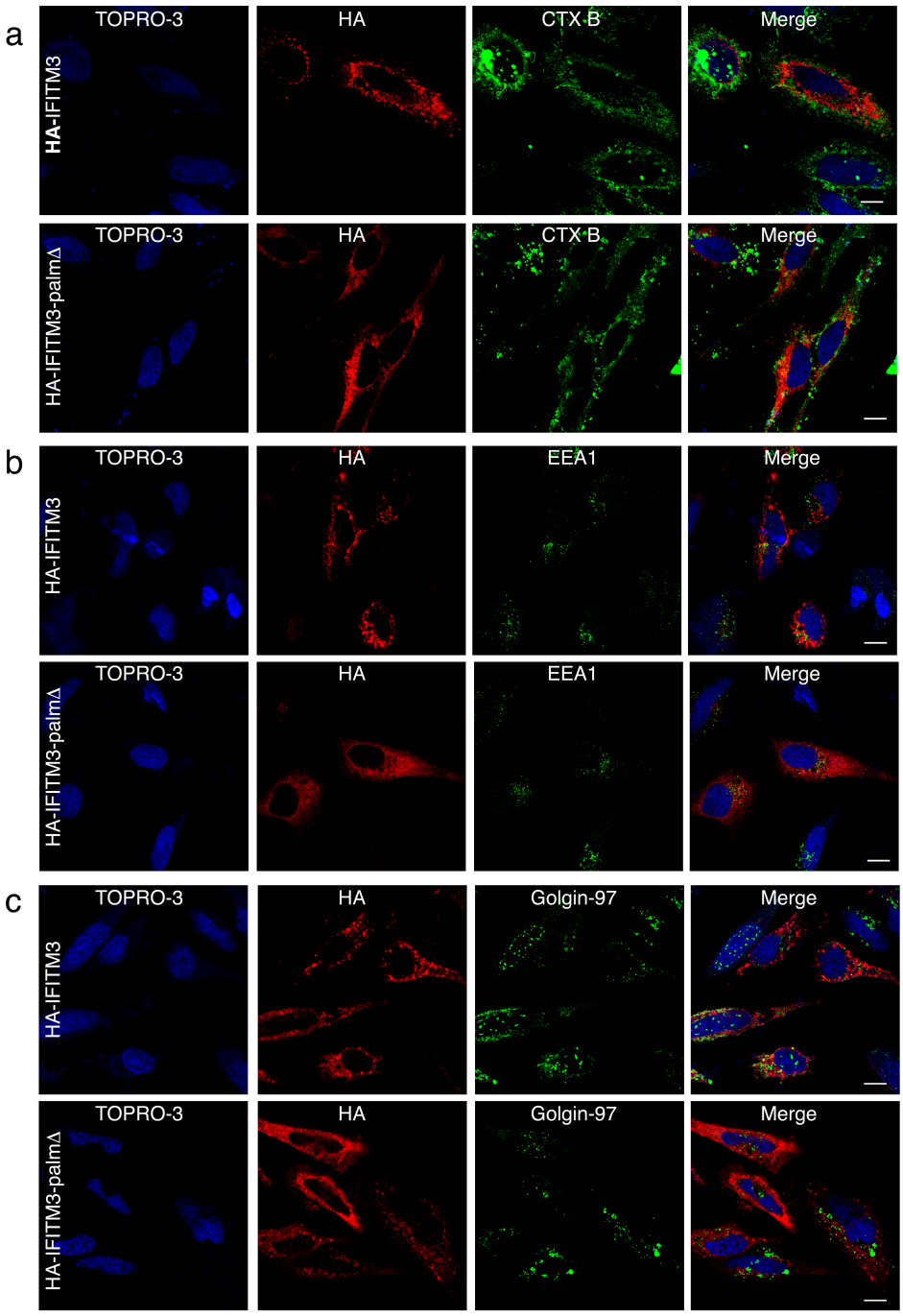
Description	Primer (5' to 3')
hβ-actin For	ACTGGAACGGTGAAGGTGAC
hβ-actin Rev	GTGGACTTGGGAGAGGACTG
hRPS11 For	GCCGAGACTATCTGCACTAC
hRPS11 Rev	ATGTCCAGCCTCAGAACTTC
hα-tubulin For	GCCTGGACCACAAGTTTGAC
hα-tubulin Rev	TGAAATTCTGGGAGCATGAC
mβ-actin For	AGGTGACAGCATTGCTTCTG
mβ-actin Rev	GCTGCCTCAACACCTCAAC
mRPS11 For	CGTGACGAAGATGAAGATGC
mRPS11 Rev	GCACATTGAATCGCACAGTC
mα-tubulin For	TGCCTTTGTGCACTGGTATG
mα-tubulin Rev	CTGGAGCAGTTTGACGACAC
mIFITM3 For	AACATGCCCAGAGAGGTGTC
mIFITM3 Rev	ACCATCTTCCGATCCCTAGAC
Influenza NP For	CAGCCTAATCAGACCAAATG
Influenza NP Rev	TACCTGCTTCTCAGTTCAAG



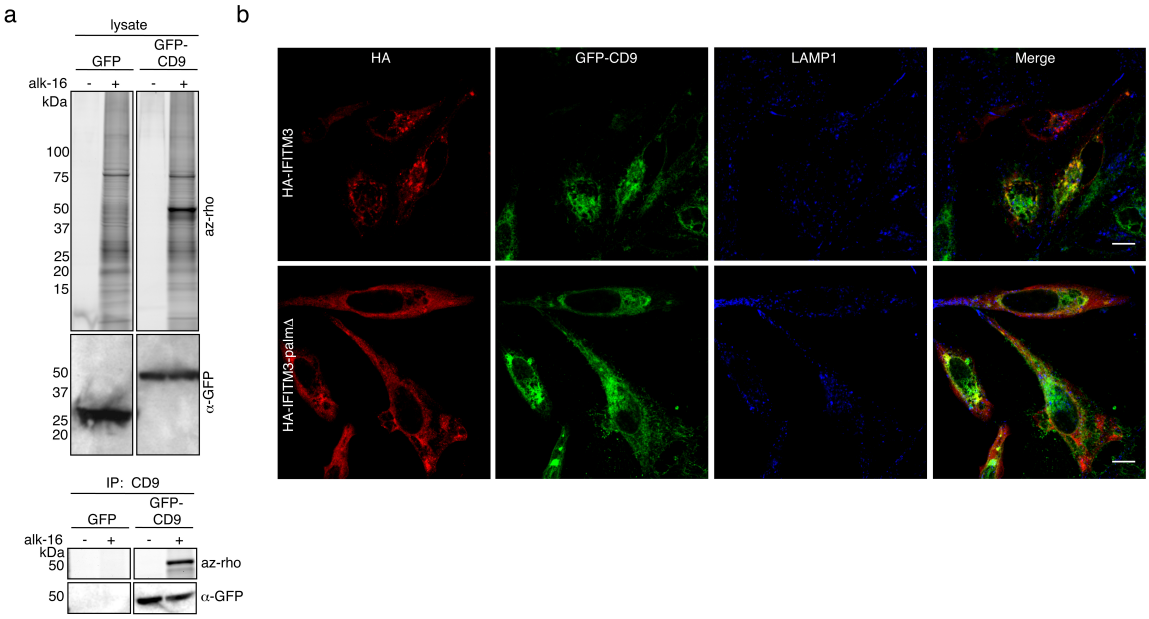
Supplementary Fig. 1. Identification of alk-16 labeled proteins. (A) Structure of az-rho. (B) Structure of azido-diazo-biotin. (C) Selective enrichment of alk-16 labeled proteins. (D and E) DC2.4 cells were incubated for 2 h with 50 μM alk-16 or DMSO as a control. Cell lysates were reacted with az-diazo-biotin by CuAAC for enrichment of alk-16 labeled proteins with streptavidin beads. (D) Proteins eluted from beads using sodium dithionite were separated by SDS-PAGE and visualized by coomassie blue staining. (E) 10 μg of lysate from alk-16 labeled DC2.4 cells and 10% of selectively enriched alk-16 labeled proteins were separated by SDS-PAGE and blotted for calnexin to confirm the selective retrieval of palmitoylated proteins.



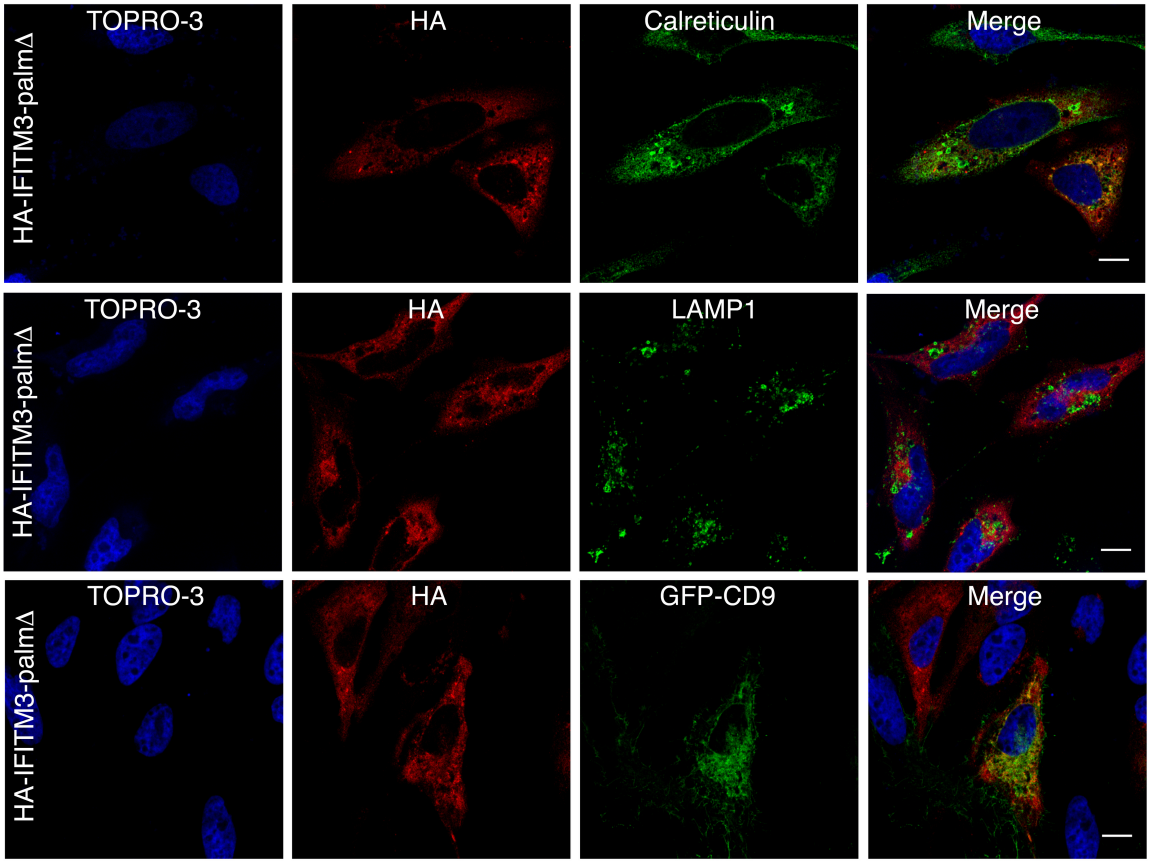
Supplementary Fig. 2. Evolutionary conservation of palmitoylated cysteine residues among IFITM family isoforms. (A) IFITM family members from various species were aligned using the Clustal V method. Amino acids corresponding to palmitoylated cysteine residues of mouse IFITM3 are shaded red. Additional membrane-proximal cysteines present in other IFITM isoforms but not in IFITM3 are shaded blue. (B)) HeLa cells grown in 6-well plates were transfected overnight with 2 ug empty vector or vector encoding the indicated human IFITM isoforms and labeled with 50 μM alk-16 for 2 h. Cell lysates were subjected to anti-HA immunoprecipitation, reacted with az-rho by CuAAC, separated by SDS-PAGE and visualized by fluorescence gel scanning. Comparable protein loading was confirmed by anti-HA western blotting.



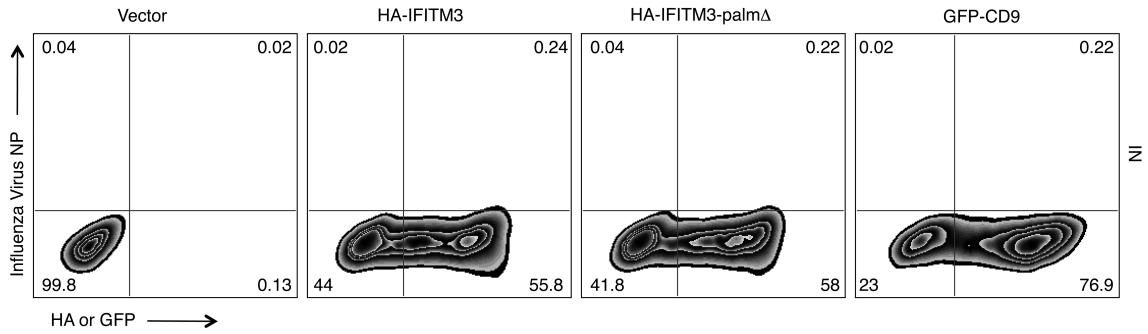
Supplementary Fig. 3. Costaining of IFITM3 and IFITM3-palmΔ with cellular markers. HeLa cells were transfected with pCMV-HA-IFITM3 and pCMV-HA-IFITM3-palmΔ and stained with anti-HA (red) and TOPRO-3 (blue). Cells were also treated with FITC-conjugated cholera toxin B (CTX B) (green) or stained with antibodies against EEA1 or golgin-97 (green). Scale bars represent 10 μm.



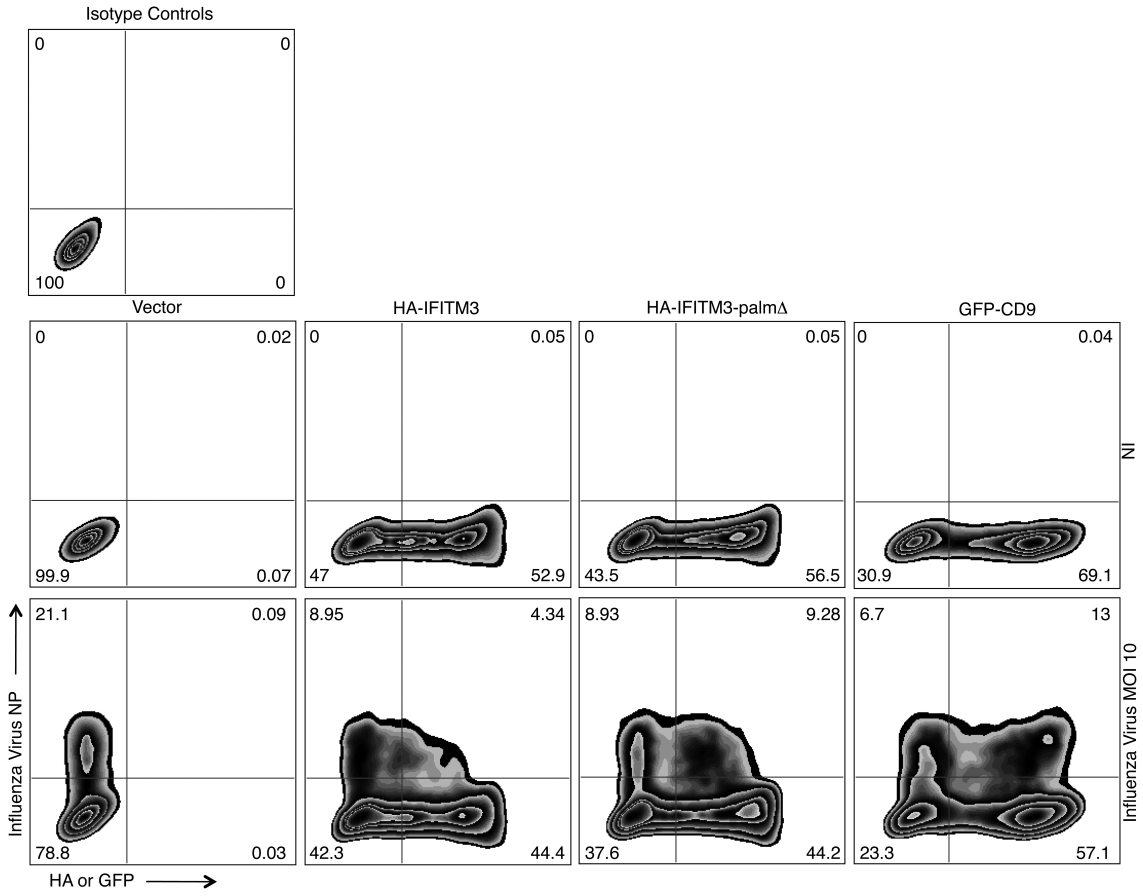
Supplementary Fig. 4. Palmitoylation of GFP-CD9. (A) HeLa cells were transfected with pEGFP-C1 or pEGFP-C1-CD9 and labeled with 50 μM alk-16 for 2 h. Cell lysates were reacted with az-rho by CuAAC followed by SDS-PAGE and visualization by fluorescence gel scanning. Anti-CD9 immunoprecipitation, reaction with az-rho by CuAAC, and SDS-PAGE allowed visualization by fluorescence gel scanning. Equal protein loading was confirmed by anti-GFP western blotting. (B) HeLa cells were transfected with pCMV-HA-IFITM3 or pCMV-HA-IFITM3-palmΔ and pEGFP-C1-CD9. Cells were stained with anti-HA (red) and anti-LAMP1 (blue), and GFP was visualized (green). Scale bars represent 10 μm.



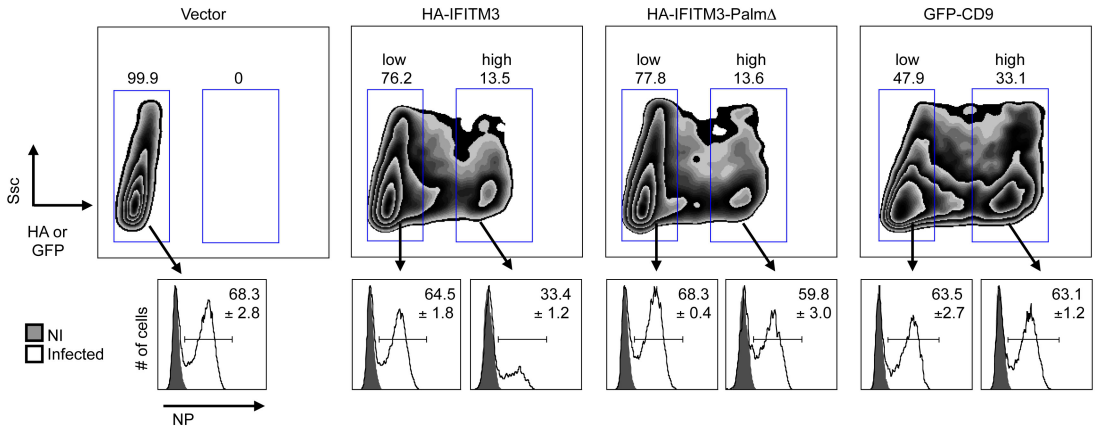
Supplementary Fig. 5. Costaining of IFITM3-PalmΔ with cellular markers. HeLa cells were transfected with pCMV-HA-IFITM3-palmΔ and stained with anti-HA (red) and TOPRO-3 (blue). Cells were also stained with antibodies against cellular markers, calreticulin and LAMP1 (green), or cotransfected with GFP-CD9 (green). Scale bars represent 10 μm.



Supplementary Fig. 6. Non-infected (NI) controls corresponding to Fig. 4b.



Supplementary Fig. 7. Anti-viral activity of IFITM3 is dependent on palmitoylation. HeLa cells were transfected with pCMV-HA, pCMV-HA-IFITM3, pCMV-HA-IFITM3-palmΔ, or pEGFP-C1-CD9 and infected with influenza virus at an MOI of 10 for 6 h. Virus NP and IFITM3 protein levels were examined by flow cytometry using anti-NP and anti-HA staining respectively. NI; non-infected. Note: the HA-tag epitope is derived from an H3 influenza virus and is not present in the PR8 strain of H1N1 influenza virus.



Supplementary Fig. 8. Palmitoylation-dependent anti-viral activity of IFITM3. Cells grown in 12-well plates were transfected with 1 ug pCMV-HA, pCMV-HA-IFITM3, pCMV-HA-IFITM3-palmΔ, or pEGFP-C1-CD9 and infected with influenza virus at an MOI of 1 for 6 h. Non-transfected and transfected cells expressing the proteins of interest were gated on (labeled low and high respectively) and analyzed for the percentage of these cells that were infected. Histograms show the percentage of cells staining positive for influenza NP antigen. Gates were set by comparing to non-infected cells transfected with the same plasmids. Error represents standard deviation, n=4.

Supplementary References

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