Supporting Information for

Bifunctional Fatty Acid Chemical Reporter for Analyzing S-palmitoylated Membrane Protein-Protein Interactions in Mammalian Cells

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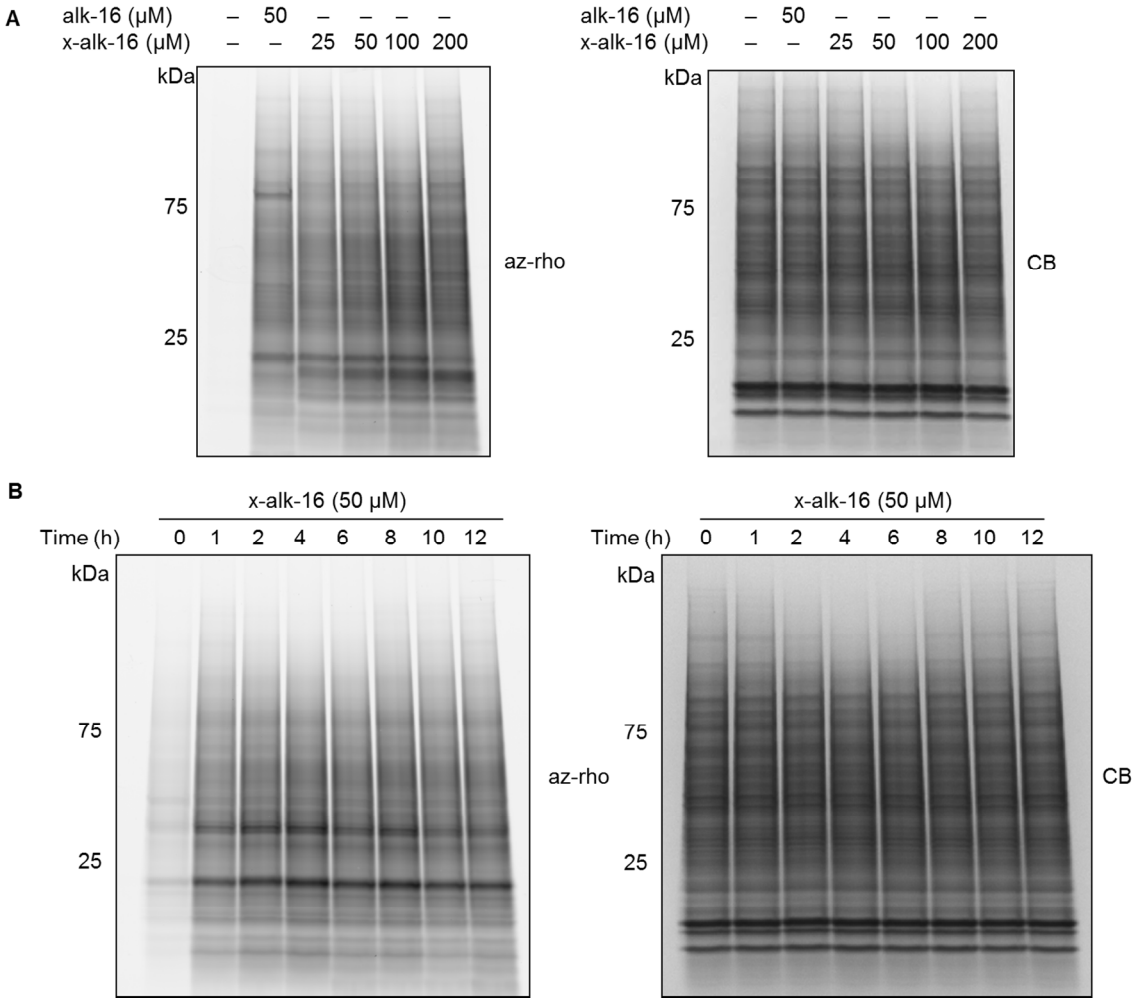


Figure S1. Dose- and time-dependent labeling of fatty-acylated proteins with x-alk-16 in HEK293T cells. (A) HEK293T cells were incubated with DMSO, alk-16, and x-alk-16 at indicated concentrations for 2 h and lysed, followed by reaction with azido-rhodamine (az-rho) and in-gel fluorescence analysis. (B) HEK293T cells were incubated with 50 μM of x-alk-16 for indicated time and lysed, followed by reaction with azido-rhodamine (az-rho) and in-gel fluorescence analysis. Equal protein loading was demonstrated by Coomassie blue staining (CB) of the gels.

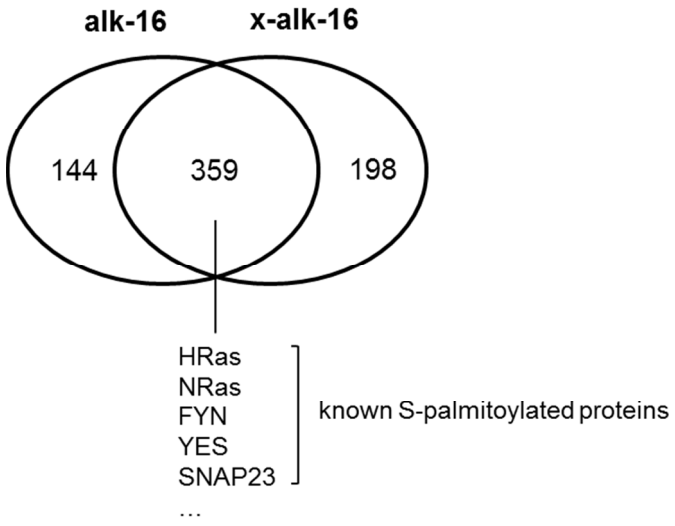


Figure S2. Mass spectrometry identification of alk-16 and x-alk-16 labeled proteins. HEK293T cells were metabolically labeled with DMSO, alk-16 (50 μM), or x-alk-16 (50 μM) for 2 h and lysed. The lysates were clicked with azido-biotin, enriched with streptavidin beads, and then digested with trypsin for protein identification and quantification with mass spectrometry. Presented are numbers of proteins identified in labeled samples with more than 20-fold intensity increases over DMSO control and at least two unique peptides (also see Table S1).

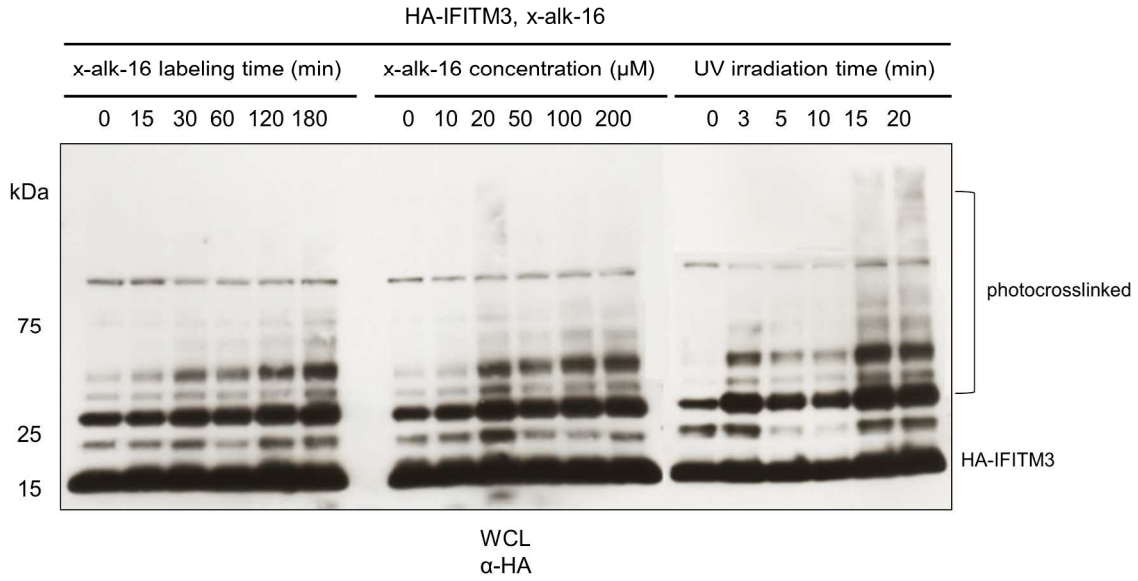


Figure S3. Optimization of x-alk-16 photocrosslinking conditions. HEK293T cells were transfected with HA-IFITM3 before x-alk-16 labeling and UV irradiation. Unless indicated, cells were labeled with 50 μM of x-alk-16 for 2 h, and then UV irradiated at 365 nm for 5 min. Whole cell lysates (WCL) were analyzed by anti-HA western blot.

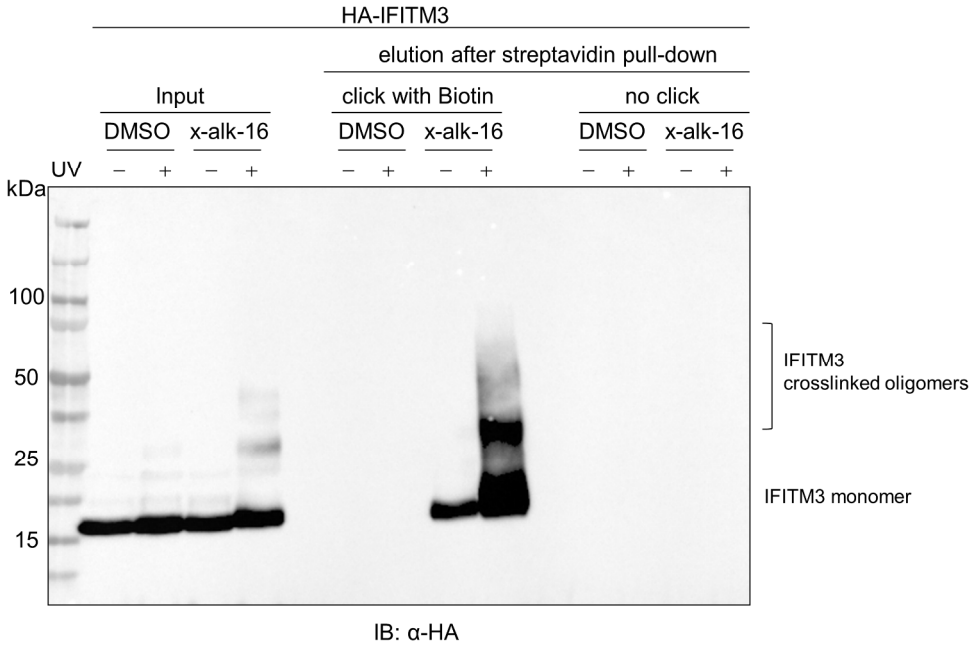


Figure S4. Analysis of IFITM3 photocrosslinking complexes after pull-down with the alkyne handle. HEK293T cells were transfected with HA-IFITM3, labeled with DMSO or x-alk-16, UV irradiated or not, and then lysed. The lysates were clicked with azido-biotin, enriched with streptavidin beads, and then eluted with 4% SDS. The eluates were analyzed by anti-HA western blotting. As revealed here, the alkynyl group is present on the photocrosslinking complexes, further validating the photocrosslinking complex formation.

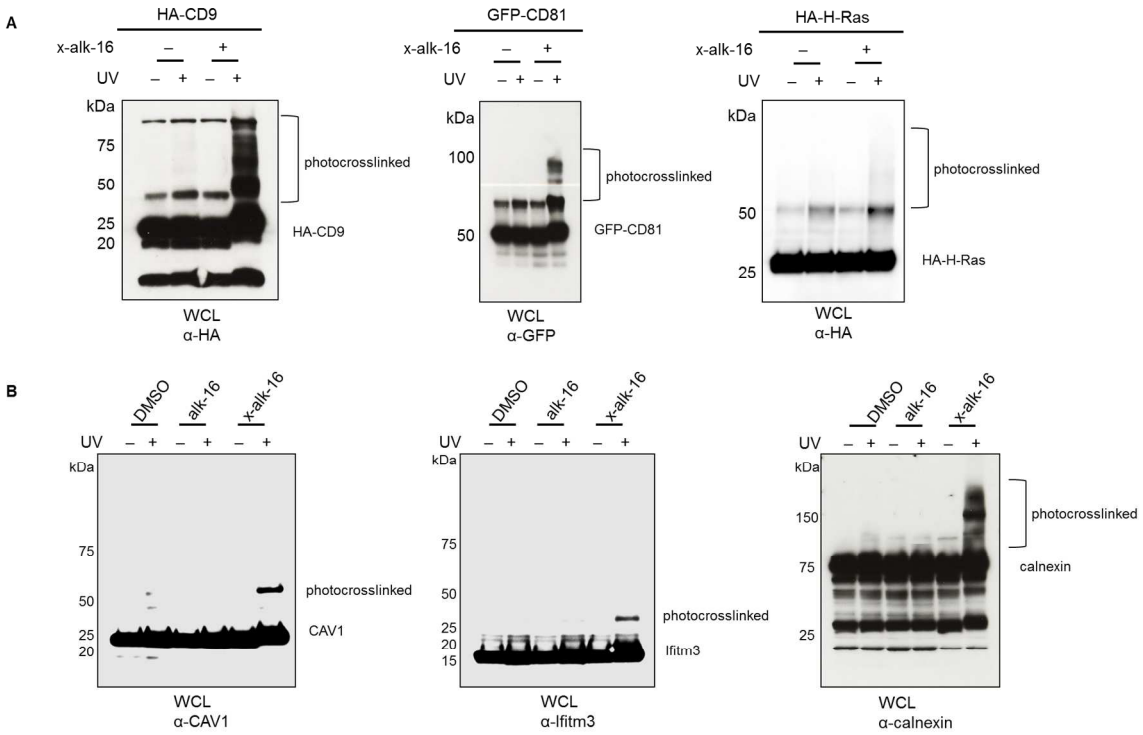


Figure S5. Photocrosslinking of S-palmitoylated proteins induced by x-alk-16. (A) HEK293T cells were transfected with HA- or GFP-tagged CD9, CD81, or H-Ras, labeled with DMSO or x-alk-16, and then irradiated with UV or not. Photocrosslinking complexes were detected by western blotting using indicated antibodies in whole cell lysates (WCL). (B) MEFs were labeled with DMSO, alk-16, or x-alk-16, and then irradiated with UV or not. Photocrosslinking complexes of endogenous CAV1, Ifitm3, and calnexin were detected by western blotting using appropriate antibodies in whole cell lysates (WCL). For Ifitm3 detection, MEFs were treated with 500 ng ml^{-1} IFN-α2 (eBioscience) for 8 h before labeling.

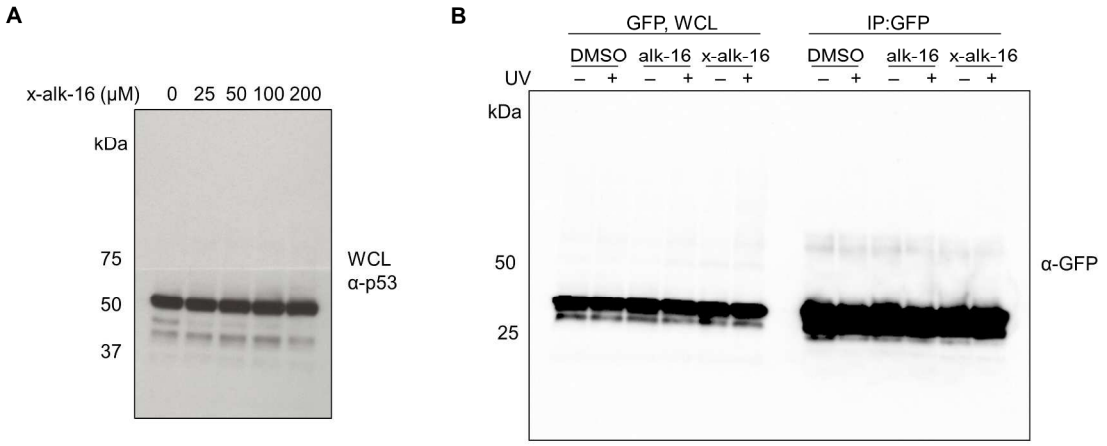


Figure S6. No photocrosslinking complex was observed for non-palmitoylated proteins with x-alk-16**.** (A) HEK293T cells were labeled with DMSO or x-alk-16 at indicated concentrations for 2 h, and irradiated with UV for 10 min. Endogenous p53 protein levels were blotted using an anti-p53 antibody in whole cell lysates (WCL). (B) HEK293T cells were transfected with pEGFP-C1 plasmid (Clontech), labeled with DMSO, alk-16, or x-alk-16, and then irradiated with UV or not. GFP was detected by western blotting using an anti-GFP antibody in whole cell lysates (WCL), or after immunoprecipitation (IP) with an anti-GFP antibody.

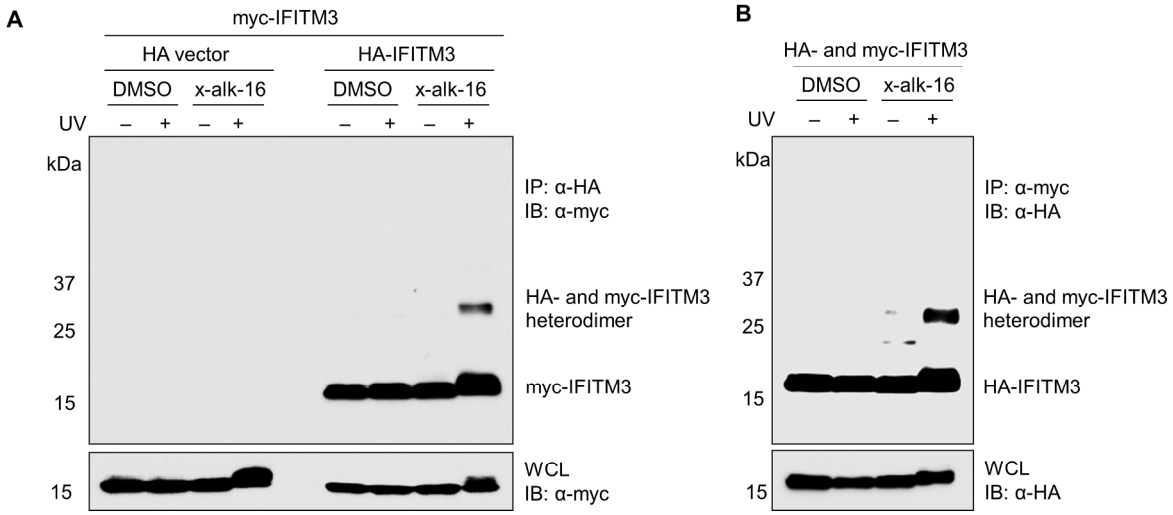


Figure S7. Validation of x-alk-16-induced photocrosslinking of IFITM3 dimerization by reciprocal immunoprecipitation. HEK293T cells were co-transfected with HA- and myc-tagged IFITM3, labeled with DMSO or x-alk-16, and then irradiated with UV or not. Cells were lysed and lysates were immunoprecipitated (A) with anti-HA beads and blotted with an anti-myc antibody, or (B) with anti-myc beads and blotted with an anti-HA antibody. Co-transfection of cells with HA vector and myc-IFITM3 in (A) confirmed that myc-IFITM3 co-immunoprecipitates with HA-IFITM3.

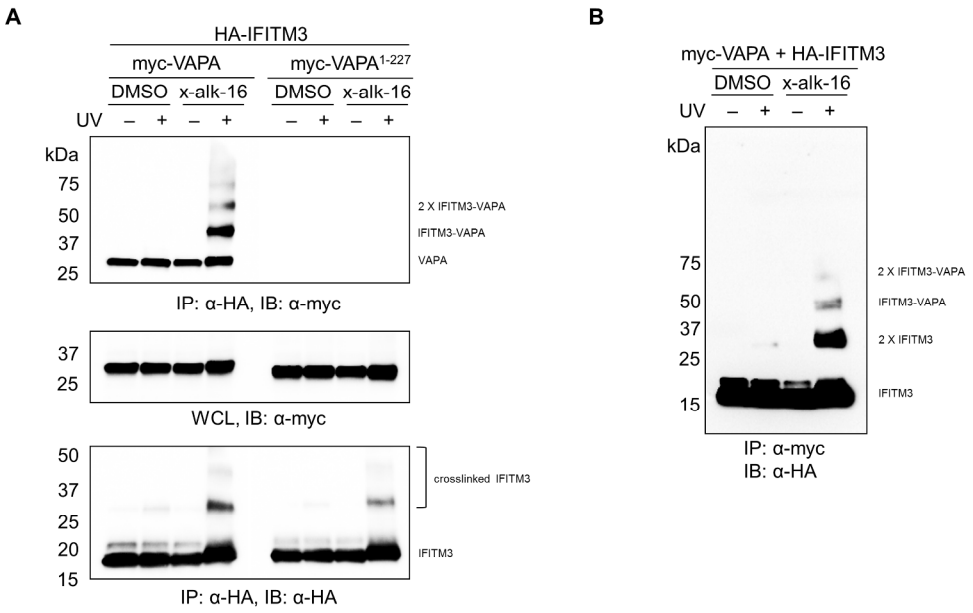


Figure S8. Validation of x-alk-16-induced photocrosslinking of IFITM3-VAPA complexes by immunoprecipitation and western blotting. HEK293T cells were co-transfected with HA-IFITM3 and myc-VAPA or myc-VAPA$^{1-227}$, a truncation mutant lacking the IFITM3 interaction domain, labeled with DMSO or x-alk-16, and then irradiated with UV or not. Cells were lysed and lysates were immunoprecipitated (A) with anti-HA beads and blotted with an anti-myc antibody, or (B) with anti-myc beads and blotted with an anti-HA antibody.

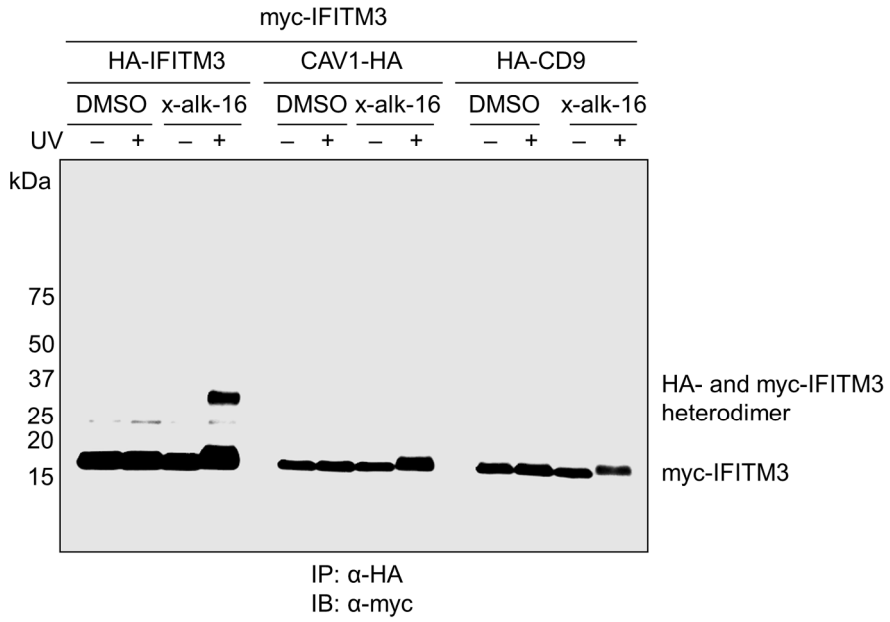


Figure S9. Specificity of x-alk-16-induced IFITM3 photocrosslinking. HEK293T cells were co-transfected with myc-IFITM3 and HA-tagged proteins, labeled with DMSO or x-

alk-16, and then irradiated with UV or not. Cells were lysed and subjected to immunoprecipitation with anti-HA beads followed with western blotting with an anti-myc antibody. Although HA-tagged IFITM3, CAV1, and CD9 all co-immunoprecipitated with myc-IFITM3, only photocrosslinking complexes between HA-IFITM3 and myc-IFITM3 were captured by x-alk-16, confirming the photocrosslinking specificity.

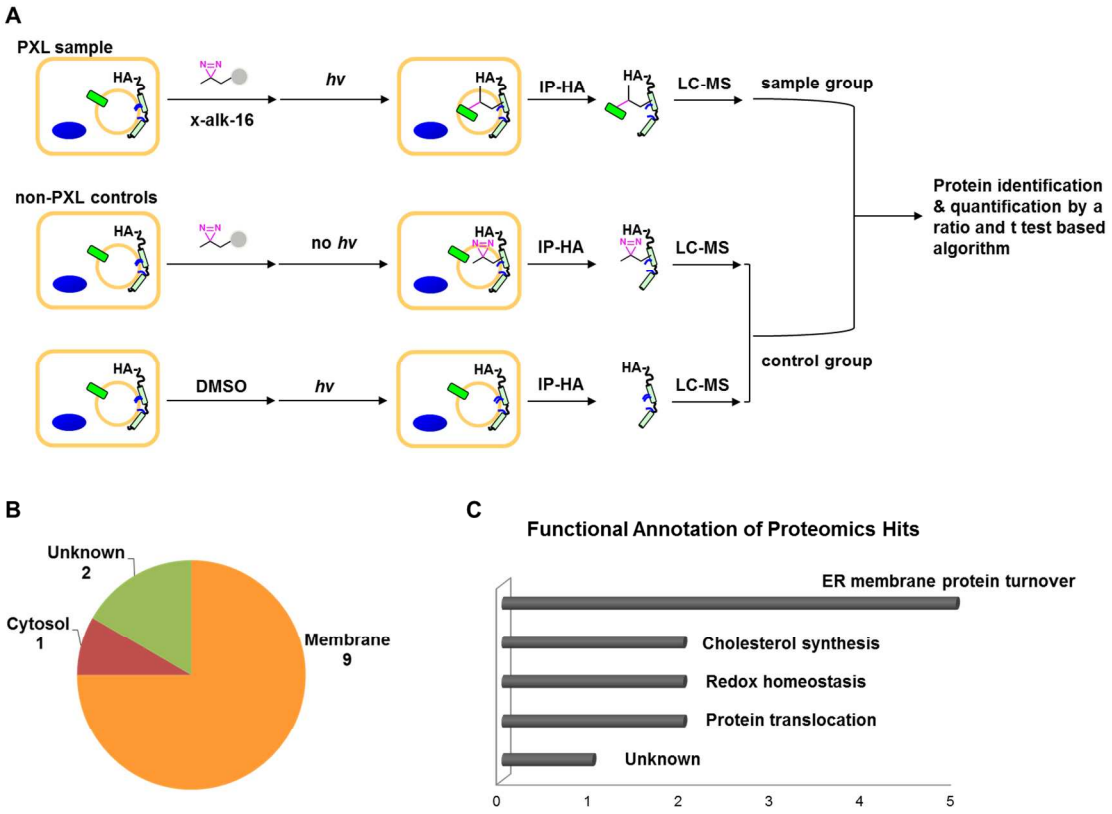
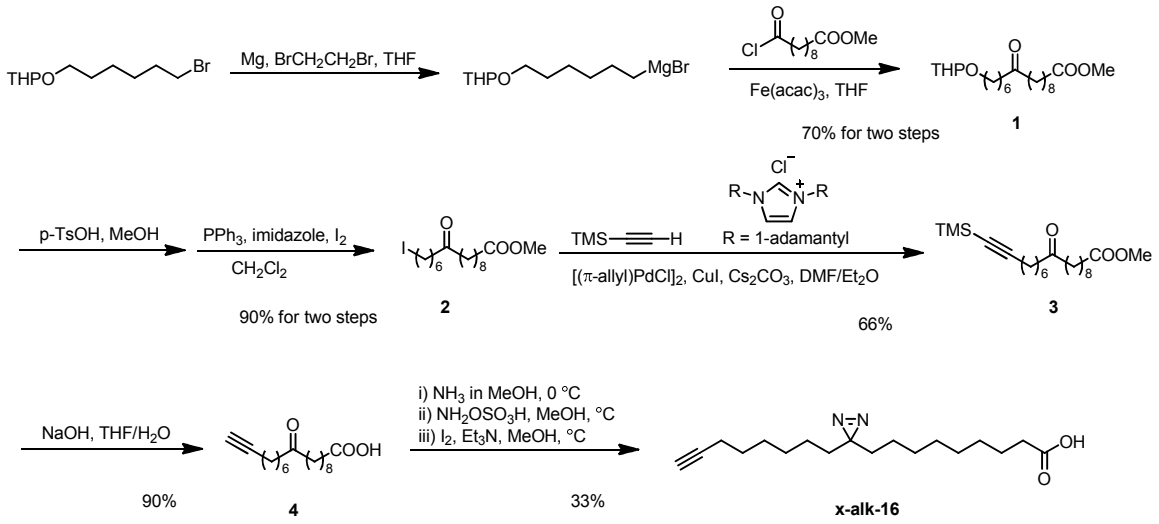


Figure S10. Proteomic analysis of IFITM3-interacting proteins captured by x-alk-16 photocrosslinking in cells. (A) Scheme for x-alk-16-enabled photocrosslinking and label-free quantitative proteomics. (B) Subcellular distribution of IFITM3-interacting candidates from x-alk-16 photocrosslinking proteomic analysis. (C) Functional annotation of x-alk-16-photocrosslinked IFITM3-interacting protein candidates using DAVID.[1] It is noteworthy that VAPA as a known interacting protein of IFITM3 was found to be almost equally present in photocrosslinking sample group and non-photocrosslinking control group. This is because VAPA binds to IFITM3 tightly through co-immunoprecipitation, resulting in high VAPA abundance in the immunoprecipitates of photocrosslinking samples and non-photocrosslinking controls, which would affect the detection of low abundant IFITM3-VAPA photocrosslinking complexes. This observation indicates that our photocrosslinking and proteomics method may be extremely useful for analyzing weak and transient membrane protein interactions, which are normally disrupted during co-immunoprecipitation but can be stabilized by photocrosslinking.

General Methods and Materials

All chemicals were obtained either from Sigma-Aldrich or Acros and were used as received unless otherwise noted. Fisher S704 silica gel (60-200 Mesh, chromatographic grade) was used for flash column chromatography. Analytical TLC was conducted on Merck silica gel plates with fluorescent indicator on glass (5–20 μm, 60 Å), and compounds were visualized by staining with ceric ammonium molybdate (CAM) or basic $KMnO_4$. The ^1H and ^{13}C NMR spectra were obtained on a Bruker Avance DMX600 spectrometer in $CDCl_3$ using tetramethylsilane (TMS) as an internal standard. Chemical shifts are reported in δ ppm, and *J* values are reported in Hz. MALDI-TOF mass spectra were obtained on an Applied Biosystems Voyager-DE. Alk-16, azido-rhodamine, and azido-azo-biotin were synthesized in the laboratory, as previously described.[2]

Synthesis of x-alk-16



Scheme S1. Synthetic scheme for x-alk-16.

*Synthesis of **1***

An oven-dried two-neck round-bottom flask equipped with a condenser was charged with magnesium turnings (50 mmol) and THF (15 mL). A solution of 2-((6-bromohexyl)oxy)tetrahydro-2H-pyran (25 mmol) and 1,2-dibromoethane (2.5 mmol) in THF (30 mL) was added at such a rate that the temperature was maintained below reflux. After addition, the mixture was stirred for additional 30 min at rt. Then the solution was added dropwise into a solution of methyl 10-chloro-10-oxodecanoate (6.2 mL, 27.5 mmol) and iron(III) acetylacetonate (883 mg, 2.5 mmol) in THF at –78 °C under Ar. The resulting mixture was warmed to room temperature and stirred overnight. The reaction was quenched with saturated NH_4Cl solution and then extracted with EtOAc three times. The organic phases were combined, dried over anhydrous Na_2SO_4, and concentrated in vacuo. The residue was purified by silica gel flash chromatography to afford compound **1** (6.7 g, 70% yield). ^1H NMR (600 MHz, $CDCl_3$) δ 4.59 – 4.54 (m, 1H), 3.89 – 3.84 (m, 1H),

3.72 (dt, *J* = 9.5, 6.8 Hz, 1H), 3.66 (s, 3H), 3.53 – 3.46 (m, 1H), 3.37 (dt, *J* = 9.5, 6.8 Hz, 1H), 2.40 – 2.34 (m, 4H), 2.30 (t, *J* = 7.6 Hz, 2H), 1.86 – 1.78 (m, 1H), 1.73 – 1.68 (m, 1H), 1.65 – 1.48 (m, 12H), 1.42 – 1.34 (m, 2H), 1.34 – 1.24 (m, 10H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 211.36, 174.21, 98.84, 67.49, 62.32, 51.40, 42.76, 42.71, 34.05, 30.79, 29.66, 29.60, 29.21, 29.18, 29.10, 29.07, 26.10, 25.53, 24.91, 23.81, 19.71. MALDI-TOF: calcd. for $C_{22}H_{41}O_5$ $[M+H]^+$ 385.3, found 385.4.

*Synthesis of **2***

To a solution of **1** (3.6 g, 9.4 mmol) in MeOH was added *p*-toluenesulfonic acid monohydrate (178 mg, 0.94 mmol) at rt. The solution was stirred for 2 h, and then quenched with saturated $NaHCO_3$. The mixture was extracted with EtOAc, and washed with H_2O. The organic solution was then concentrated to provide a crude product of alcohol, which was re-dissolved in anhydrous CH_2Cl_2. To this solution was added imidazole, PPh_3, and I_2 successively under Ar. The resulting mixture was stirred at rt overnight. Hexane was added into the mixture to precipitate triphenylphosphine oxide, which was removed by filtration. The organic solution was concentrated in vacuo, and the residue was purified by silica gel flash chromatography to afford compound **2** (3.5 g, 90% yield). ^{1}H NMR (600 MHz, $CDCl_3$) δ 3.67 (s, 3H), 3.18 (t, *J* = 7.0 Hz, 2H), 2.41 – 2.36 (m, 4H), 2.30 (t, *J* = 7.5 Hz, 2H), 1.85 – 1.78 (m, 2H), 1.65 – 1.53 (m, 6H), 1.43 – 1.37 (m, 2H), 1.32 – 1.28 (m, 10H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 211.29, 174.30, 51.46, 42.85, 42.56, 34.09, 33.25, 30.25, 29.21, 29.19, 29.08, 28.11, 24.92, 23.82, 23.54, 7.03. MALDI-TOF: calcd. for $C_{17}H_{32}IO_3$ $[M+H]^+$ 411.1, found 411.1.

*Synthesis of **3***

The Sonogashira reaction was performed as previously reported.[3] Briefly, 1,3-bis(1-adamantyl)imidazolium chloride (199 mg, 0.54 mmol), CuI (154 mg, 0.80 mmol), $[(\pi\text{-allyl})PdCl]_2$ (99 mg, 0.27 mmol), Cs_2CO_3 (1.22 g, 3.75 mmol), and iodide **2** (1.1 g, 2.68 mmol) were added in turn to a seal tube equipped with a stir bar. A mixture of Et_2O and DMF (2:1, 6 mL) was added, followed by the addition of trimethylsilylacetylene (0.76 mL, 5.36 mmol). The tube was sealed, and heated to 45 °C for 16 h with vigorously stirring. After that, the crude reaction mixture was filter by Celite, and concentrated. The residue was purified silica gel flash chromatography to afford compound **3** (674 mg, 66% yield). ^{1}H NMR (600 MHz, $CDCl_3$) δ 3.66 (s, 3H), 2.47 – 2.34 (m, 4H), 2.30 (t, *J* = 7.5 Hz, 2H), 2.21 (t, *J* = 7.2 Hz, 2H), 1.74 – 1.54 (m, 8H), 1.54 – 1.35 (m, 2H), 1.35 – 1.09 (m, *J* = 15.4 Hz, 10H), 0.14 (s, 9H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 211.34, 174.23, 107.49, 84.37, 51.41, 42.79, 42.66, 34.06, 29.21, 29.18, 29.07, 28.69, 28.53, 28.40, 24.91, 23.81, 23.67, 19.77, 0.18. MALDI-TOF: calcd. for $C_{22}H_{41}O_3Si$ $[M+H]^+$ 381.3, found 381.2.

*Synthesis of **4***

To a solution of **3** (674 mg, 1.77 mmol) in THF (12 mL) was added a solution of NaOH (425 mg, 17.7 mmol) in H_2O (4 mL). The reaction solution was stirred at rt overnight before quenched with 0.1 M HCl. The mixture was then extracted with CH_2Cl_2 three times. The organic phases were combined, dried over anhydrous sodium sulfate, and then concentrated in vacuo. The residue was purified by silica gel flash chromatography

to afford compound **4** (470 mg, 90% yield). ^{1}H NMR (600 MHz, $CDCl_3$) δ 2.42 – 2.37 (m, 4H), 2.34 (t, *J* = 7.5 Hz, 2H), 2.18 (td, *J* = 7.1, 2.6 Hz, 2H), 1.93 (t, *J* = 2.6 Hz, 1H), 1.66 – 1.60 (m, *J* = 14.9, 7.5 Hz, 2H), 1.59 – 1.49 (m, 6H), 1.44 – 1.38 (m, *J* = 9.6, 7.2 Hz, 2H), 1.34 – 1.24 (m, 10H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 211.60, 179.84, 84.57, 68.21, 42.80, 42.68, 34.01, 29.18, 29.06, 28.98, 28.70, 28.47, 28.25, 24.63, 23.81, 23.68, 18.33. MALDI-TOF: calcd. for $C_{18}H_{31}O_3$ $[M+H]^+$ 295.2, found 295.1.

*Synthesis of **x-alk-16***

To a solution of **4** (400 mg, 1.36 mmol) in MeOH (1 mL) was added an ammonia solution in MeOH (7 N, 20 mL) at 0 °C under Ar. The solution was stirred at that temperature for 3 h. To this solution was then added a solution of hydroxylamine-*O*-sulfonic acid (306 mg, 2.72 mmol) in MeOH (5 mL) dropwise at 0 °C. The resulting mixture was warmed to rt and stirred for 16 h. The white precipitate was removed by filtration and the remaining solution was concentrated by vacuo. The residue was re-dissolved in MeOH (10 mL). To this solution was added Et_3N (0.42 mL, 3 mmol) and a solution of I_2 (440 mg, 1.5 mmol) in MeOH (5 mL) dropwise until the solution stayed red-brown. The reaction mixture was quenched by saturated sodium sulfite solution, and extracted with CH_2Cl_2 three times. The organic solutions were combined, dried over anhydrous sodium sulfate, and concentrated in vacuo. The residue was purified by silica gel flash chromatography to afford x-alk-16 (137 mg, 33% yield). ^{1}H NMR (600 MHz, $CDCl_3$) δ 2.34 (t, *J* = 7.1 Hz, 2H), 2.17 (t, *J* = 6.7 Hz, 2H), 1.93 (s, 1H), 1.66 – 1.59 (m, 2H), 1.53 – 1.47 (m, 2H), 1.37 – 1.21 (m, 16H), 1.11 – 1.04 (m, 4H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 179.70, 84.57, 68.20, 33.95, 32.89, 32.87, 29.72, 29.18, 29.14, 29.07, 28.97, 28.82, 28.69, 28.47, 28.28, 24.62, 23.82, 23.72, 18.34. MALDI-TOF: calcd. for $C_{18}H_{31}N_2O_2$ $[M+H]^+$ 307.2, found 307.2.

Cell culture and transfection

HEK293T cells and MEFs were grown in DMEM (Life Technologies) supplemented with 4.5 g/liter D-glucose, 110 mg/liter sodium pyruvate, and 10% FBS (HyClone, Thermo Scientific) at 37 °C in a humidified incubator with an atmosphere of 5% CO2. For transfection, HEK293T cells were grown on 6-well plates to 90% confluence and transfected with 1 μg of the indicated plasmids per well using Xtremegene 9 (Roche). Cells were generally transfected for about 16 h before metabolic labeling with chemical reporters, as described below. Plasmid constructs for HA-IFITM3, myc-IFITM3, HA-CD9, GFP-CD81, and HA-H-Ras^{G12V} have been described previously.[4] CAV1-HA plasmid was obtained from Addgene (plasmid 27765). VAPA cDNA was purchased from Open Biosystems and PCR cloned into pCMV-myc vector (Clontech) using EcoRI and XhoI restriction sites.

Metabolic labeling and UV irradiation

Cells were metabolically labeled with chemical reporters (alk-16 or x-alk-16) at 50 μM concentrations, unless otherwise stated, for 2 h in DMEM supplemented with 2% charcoal filtered FBS (Omega Scientific). The same volume of DMSO was used in negative controls. For in-cell photocrosslinking experiments, cells labeled with fatty acid reporters were covered with PBS and subjected to UV irradiation at 365 nm on ice for 0–10 min using a Spectrolinker XL-1000 UV crosslinker (Spectronics) at a distance of 3 cm. Cells were then harvested, washed with cold PBS, and flash-frozen in liquid nitrogen before storage at −80 °C.

Preparation of cell lysates

For click labeling of whole cell lysates and immunoblotting, frozen cell pellets were lysed in SDS lysis buffer (4% SDS, 150 mM NaCl, 50 mM triethanolamine pH 7.4, Roche EDTA-free protease inhibitor cocktail, benzonase) with brief vortexing and sonication. For immunoprecipitation, frozen cell pellets were lysed in chilled Brij lysis buffer (1% Brij 97, 150 mM NaCl, 50 mM triethanolamine pH 7.4, Roche EDTA-free protease inhibitor cocktail) with vigorous vortexing. The resulting cell lysates were centrifuged at 1,000 × g for 5 min at room temperature to remove cellular debris. Protein concentrations were determined by the BCA assay (Pierce).

CuAAC click labeling, in-gel fluorescence scanning and affinity purification

The procedure for click labeling of whole cell lysates with azido-rhodamine (az-rho) has been described previously.[2a] Briefly, whole cell lysates (50 μg) diluted with SDS lysis buffer to 44.5 μL were reacted with 5.5 μL freshly prepared click chemistry reaction cocktail containing azido-rhodamine (1 μL, 10 mM stock solution in DMSO), tris(2-carboxyethyl)phosphine hydrochloride (TCEP) (1 μL, 50 mM freshly prepared stock solution in deionized water), tris[(1-benzyl-1H-1,2,3-triazol-4-yl)methyl]amine (TBTA) (2.5 μL, 10 mM stock solution in DMSO/t-butanol) and CuSO4 ·5H2O (1 μL, 50 mM freshly prepared stock solution in deionized water)] for 1 h at room temperature. The click reactions were terminated by the addition of ice-cold methanol (1 mL). The mixtures were placed at −20 °C overnight and then centrifuged at 18,000 × g for 20 min at 4 °C to precipitate proteins. The supernatants from the samples were discarded. The protein pellets were allowed to air-dry for 10 min, resuspended in 35 μL of SDS lysis buffer (4% SDS, 150 mM NaCl, 50 mM triethanolamine pH 7.4), diluted with 12.5 μL 4× reducing SDS-loading buffer (40% glycerol, 200 mM Tris-HCl pH 6.8, 8% SDS, 0.4% bromophenol blue) and 2.5 μL 2-mercaptoethanol. The resulting samples were heated for 5 min at 95 °C before loaded onto 4−20% Tris-HCl gels (Bio-Rad) for SDS-PAGE separation. Generally, 20 μg of protein per gel lane is sufficient for in-gel fluorescence visualization. For in-gel fluorescence scanning, the gels were destained in 40% methanol, 10% acetic acid for at least 1 h, and then scanned on a GE Healthcare Typhoon 9400 variable mode imager with excitation and emission at 532 nm and 580

nm, respectively. After scanning, gels were also stained with Coomassie Brilliant Blue (Bio-Rad).

For affinity purification of alkyne-modified proteins, 2 mg of cell lysates labeled with DMSO, alk-16, or x-alk-16 were used. CuAAC reactions were performed as described above except azido-biotin was substituted for azido-rhodamine. Methanol-precipitated and washed protein pellets were resuspended in 200 µL of 4% SDS (50 mM TEA, 150 mM NaCl, pH 7.4). Equal protein amount for each sample was diluted 1/4 by volume with 50 mM TEA buffer (150 mM NaCl, pH 7.4). 60 µl prewashed streptavidin agarose beads (Invitrogen) were added to each sample. The protein and bead mixtures were incubated for 1 h at room temperature on a nutating mixer. The beads were then washed once with PBS and 0.2% (w/v) SDS, three times with PBS and twice with 250 mM ammonium bicarbonate (ABC). Beads were resuspended in 500 µl 8 M urea, reduced with 10 mM DTT for 30 min, and then alkylated with 50 mM iodoacetamide in the dark for another 30 min. Finally, the beads were washed with 25 mM ammonium bicarbonate and digested with 0.5 µg of trypsin at 37 °C overnight. The supernatant for each sample was collected, dried, and solubilized in 5% acetonitrile/1% formic acid for LC-MS analysis.

Immunoprecipitation

Cell lysates were prepared in Brij 97 lysis buffer for immunoprecipitation. HA-tagged and myc-tagged proteins were immunoprecipitated from ~400 µg of HEK293T cell lysates using anti-HA agarose beads (HA-7, Sigma) or anti-myc agarose beads (Sigma), respectively. After 2 h incubation on a rotator at 4 °C, the beads were washed three times with 1 mL of chilled RIPA buffer (1% Triton X-100, 1% sodium deoxycholate, 0.1% SDS, 150 mM NaCl, 50 mM triethanolamine pH 7.4). The immunoprecipitates were eluted off the beads by addition of 1× reducing SDS-loading buffer with boiling for 5 min, and then loaded onto 4−20% Bio-Rad Criterion Tris-HCl gels for separation by SDS-PAGE and Western blotting analysis. Alternatively, the beads were resuspended in 22 µL of SDS lysis buffer (4% SDS, 150 mM NaCl, 50 mM triethanolamine pH 7.4, Roche EDTA-free protease inhibitor cocktail) and incubated with 3 µL freshly prepared click chemistry reaction cocktail (same as above) at room temperature for 1 h. Then the reaction mixture was diluted with 8.9 µL 4× reducing SDS-loading buffer (40% glycerol, 200 mM Tris·HCl pH 6.8, 8% SDS, 0.4% bromophenol blue) and 1.8 µL 2-mercaptoethanol, heated for 5 min at 95 °C. 20 µL of the supernatant was loaded per gel lane for separation by SDS-PAGE (4−20% Bio-Rad Criterion Tris-HCl gel) and in-gel fluorescence scanning. The rest was loaded onto another gel for Western blot analysis.

Western blot

Proteins separated by SDS-PAGE were transferred to nitrocellulose membranes (50 mM TrisHCl, 40 mM glycine, 0.0375% SDS, 20% MeOH in deionized water, Bio-Rad Trans-Blot Semi-Dry Cell, 20 V, 40 min), which were blocked with PBST (0.05% Tween-20 in PBS) containing 5% nonfat milk for 1 h at room temperature or overnight at 4 °C. The membrane were washed three times with PBST, and then incubated with appropriate antibodies at recommended concentrations. Anti-HA-HRP (3F10, Roche) and anti-myc-HRP (9E10, Roche) were used for anti-HA and anti-myc blots, respectively. GFP, caveolin-1, IFITM3, calnexin, and p53 protein levels were immunoblotted by incubating the blots with mouse anti-GFP monoclonal (JL-8, Clontech), rabbit anti-CAV1 polyclonal (#3238, Cell Signaling), rabbit anti-IFITM3 polyclonal (ab15592, Abcam), rabbit anti-calnexin polyclonal (ab22595, Abcam) and mouse anti-p53 monoclonal (DO-1, Santa Cruz) antibodies, respectively, followed by incubation with goat anti-mouse-HRP or monkey anti-rabbit-HRP secondary antibodies (Jackson Immunoresearch Laboratories). Blots were developed using Bio-Rad Clarity Western ECL substrate and imaged with a Bio-Rad ChemiDoc MP Imager.

Large-scale immunoprecipitation for proteomic analysis

HEK293T cells were cultured in 150 mm dishes to ~80% confluency, and transfected with HA-IFITM3 using Xtremegene 9 (Roche) for 16 h. Cells were then labeled with 50 μM x-alk-16 or same volume of DMSO for 2 h and irradiated with UV at 365 nm for 5 min before harvested and washed with PBS twice. The cell pellets were lysed with chilled RIPA buffer (1% Triton X-100, 1% sodium deoxycholate, 0.1% SDS, 150 mM NaCl, 50 mM triethanolamine pH 7.4) supplemented with Roche EDTA-free protease inhibitor cocktail and benzonase. Cell lysates were centrifuged at 16,000 × g for 5 min at 4 °C to remove cellular debris. Protein concentrations were determined by the BCA assay (Pierce). The clarified cell lysates were incubated with anti-HA magnetic beads (Pierce) at 4 °C for 1 h with end-over-end rotation. The beads were then washed five times with chilled RIPA buffer, six times with PBS and 50 mM ammonium bicarbonate (ABC) buffer, and eluted with 0.5 M NH_4OH twice. The pooled eluates were dried, resuspended in 50 mM ABC buffer, reduced with 10 mM DTT for 30 min, and then alkylated with 50 mM iodoacetamide in the dark for another 30 min. The eluates were digested with trypsin at 37 °C overnight and desalted with C18 StageTips[5] before analyzed by LC-MS.

Mass spectrometry analysis

LC-MS analysis was performed with a Dionex 3000 nano-HPLC coupled to an Orbitrap XL mass spectrometer (ThermoFisher). Peptide samples were pressure-loaded onto a home-made C18 reverse-phase column (75 μm diameter, 15 cm length). A 180-minute gradient increasing from 95% buffer A (HPLC grade water with 0.1% formic acid) and 5% buffer B (HPLC grade acetonitrile with 0.1% formic acid) to 75% buffer B in 133

minutes was used at 200 nL/min. The Orbitrap XL was operated in top-8-CID-mode with MS spectra measured at a resolution of 60,000@m/z 400. One full MS scan (300–2000 MW) was followed by three data-dependent scans of the nth most intense ions with dynamic exclusion enabled. Peptides fulfilling a Percolator calculated 1% false discovery rate (FDR) threshold were reported.

In the proteomic analysis of alk-16- and x-alk-16-labeled proteins, acquired tandem MS spectra were extracted using ProteomeDiscoverer v.1.4.0.288 (Thermo, Bremen, Germany) and queried against Uniprot complete human database concatenated with common known contaminants[6] using MASCOT v.2.3.02 (Matrixscience, London, UK). The abundance of an identified protein was calculated based on the average area of its three most abundant peptides. For a protein considered to be labeled by the reporters, it had to be enriched more than 20 fold above the DMSO control measured by protein abundance with at least two unique peptides.

Label-free quantification of x-alk-16-crosslinked IFITM3 interacting proteins was performed with the label-free MaxLFQ algorithm in MaxQuant software as described.[7] The search results from MaxQuant were analyzed by Perseus (http://www.perseus-framework.org/). Briefly, the non-photocrosslinking control replicates and photocrosslinking sample replicates were grouped correspondingly (Figure S9a). The results were cleaned to filter off reverse hits and contaminants. Only proteins that were identified in all photocrosslinking sample replicates and with more than two unique peptides were subjected to subsequent statistical analysis. LFQ intensities were used for measuring protein abundance and logarithmized. Signals that were originally zero were imputed with random numbers from a normal distribution, whose mean and standard deviation were chosen to best simulate low abundance values below the noise level (Replace missing values by normal distribution – Width = 0.3; Shift = 2.2). Significant proteins that were more enriched in photocrosslinking sample group versus non-photocrosslinking control group were determined by a volcano plot-based strategy, which combined t test p-values with ratio information. A hyperbolic significance curve in the volcano plot corresponding to a given FDR (= 0.0001) and SO value (= 1) was determined by a permutation-based method.[8] The resulting table was exported as Table S2.

Reference:

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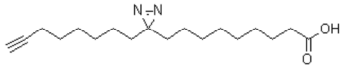
NMR

	Parameter	Value
1	Data File Name	D:/ postdoc/ Raw DATA/ NMR/ NMR for x-alk-16/ TP-3-33p/ 1/ fid
2	Title	TP-3-33p
3	Comment	3-33 pure
4	Origin	Bruker BioSpin GmbH
5	Owner	tpeng
6	Site	
7	Spectrometer	spect
8	Author	
9	Solvent	CDCl3
10	Temperature	298.2
11	Pulse Sequence	zg30
12	Number of Scans	32
13	Receiver Gain	11
14	Relaxation Delay	1.0000
15	Pulse Width	7.5750
16	Acquisition Time	2.1037
17	Acquisition Date	2014-01-18T19:09:57
18	Modification Date	2014-01-18T19:11:14
19	Spectrometer Frequency	600.17
20	Spectral Width	7788.2
21	Lowest Frequency	-1052.7
22	Nucleus	1H
23	Acquired Size	16384
24	Spectral Size	32768



	Parameter	Value
1	Data File Name	D:/ postdoc/ Raw DATA/ NMR/ NMR for x-alk-16/ TP-3-33p/ 2/ fid
2	Title	TP-3-33p
3	Comment	3-33 pure
4	Origin	Bruker BioSpin GmbH
5	Owner	tpeng
6	Site	
7	Spectrometer	spect
8	Author	
9	Solvent	CDCl3
10	Temperature	298.2
11	Pulse Sequence	zgpg30
12	Number of Scans	75
13	Receiver Gain	7290
14	Relaxation Delay	2.0000
15	Pulse Width	10.0000
16	Acquisition Time	0.9830
17	Acquisition Date	2014-01-18T19:14:18
18	Modification Date	2014-01-18T19:16:00
19	Spectrometer Frequency	150.91
20	Spectral Width	33333.3
21	Lowest Frequency	-1575.2
22	Nucleus	13C
23	Acquired Size	32768
24	Spectral Size	65536