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Supporting Information

Investigation of Glycosylphosphatidylinositol (GPI)-Plasma Membrane Interaction in Live Cells and the Influence of GPI Glycan Structure on the Interaction

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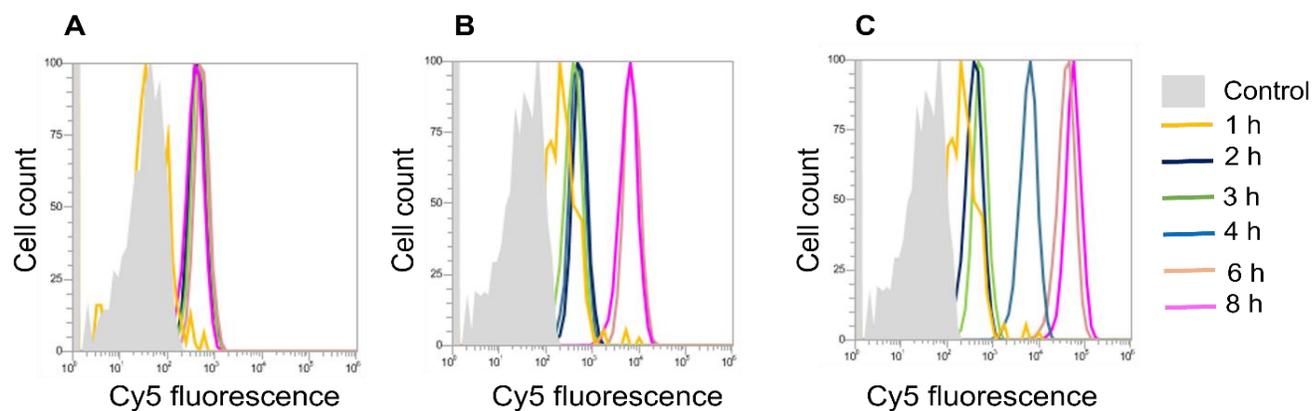


Figure S1: Single parameter FACS histogram of cells incubated with PBS (control, gray-shaded) or probe **3** (**A**), **1** (**B**), and **2** (**C**) for 1, 2, 3, 4, 6, and 8 h (color-coded as shown on the right side of the graphs), which was followed by treatments with biotin-azide conjugate for biotinylation of the probes and then incubation with streptavidin-Cy5 to install the fluorophore. The cells were finally subjected to FACS analysis using a flow cytometer.

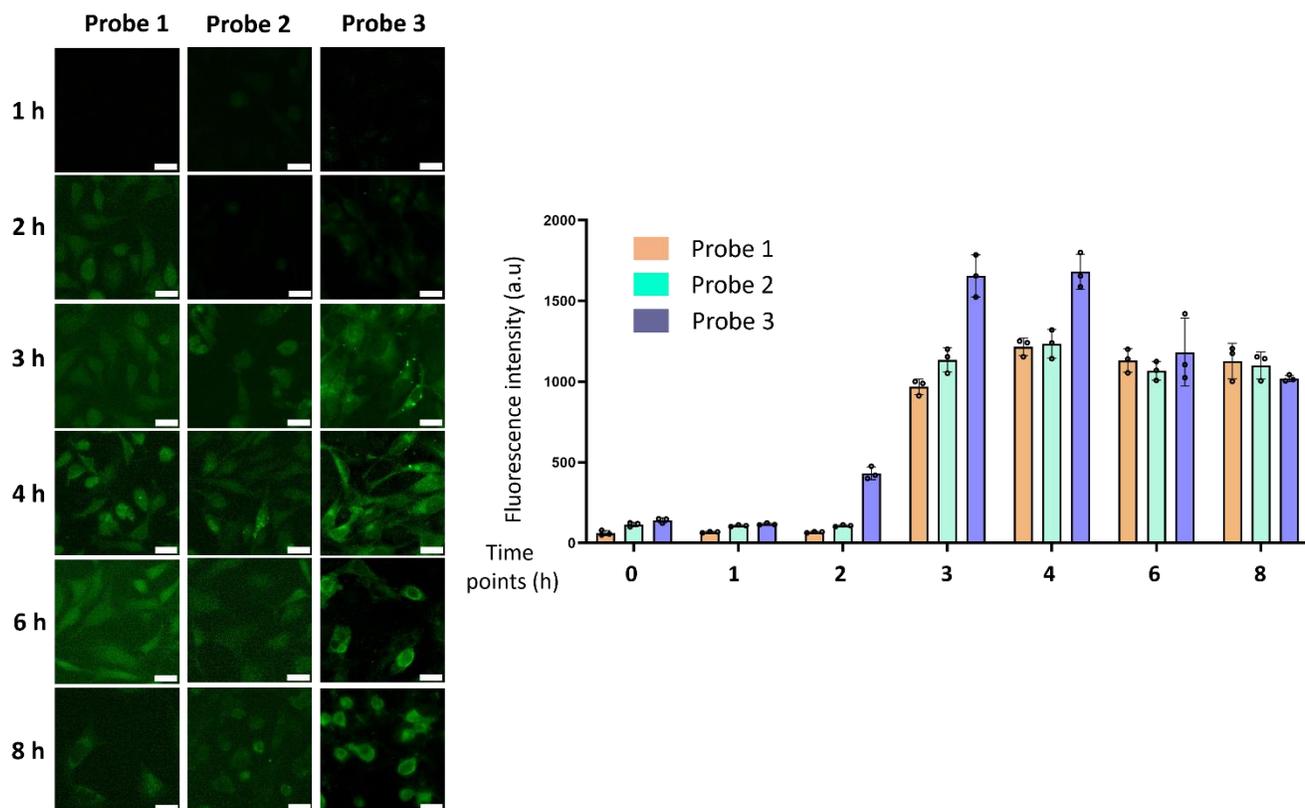


Figure S2: Fluorescence imaging results of the time-dependent incorporation of probes 1-3 by HeLa cells. **(A)** Fluorescent images of HeLa cells incubated with probes 1, 3, and 2 for 1, 2, 3, 4, 6, and 8 h, and then with biotin-azide to biotinylate the alkyne-labeled probes on the cells, which was followed by incubation with streptavidin-A488 to introduce the green fluorophore. The scale bars are 20 μm . **(B)** Bar graphs of the fluorescence intensities of the labeled cells as the average from three distinct frames captured in three independent experiments.

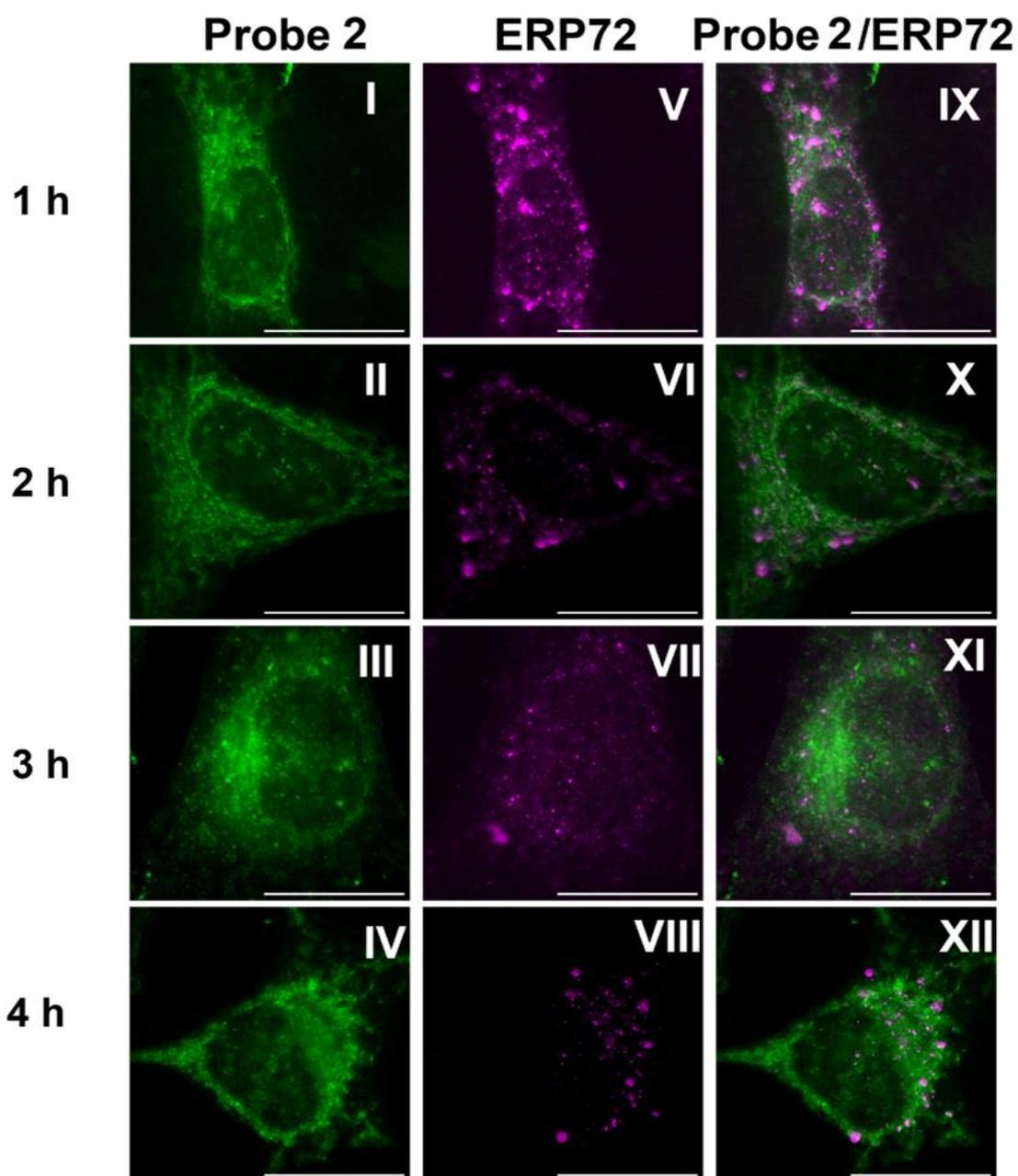


Figure S3: Time-dependent changes in the localization of probe **2** compared to the localization of ER. Fluorescent images of HeLa cells incubated with **2** for 1, 2, 3, and 4 h and then with biotin-azide, which was followed by incubation with streptavidin-A488. The cells were fixed, permeabilized, and finally treated with Cy5-anti-EPR72 antibody conjugate to stain the ER. (I-IV): A488 (green) fluorescence images showing labeled probe **2**; (V-VIII): Cy5 (purple) fluorescence images showing labeled ER; (IX-XII): overlay of green and purple fluorescence images. The scale bars are 20 μm .

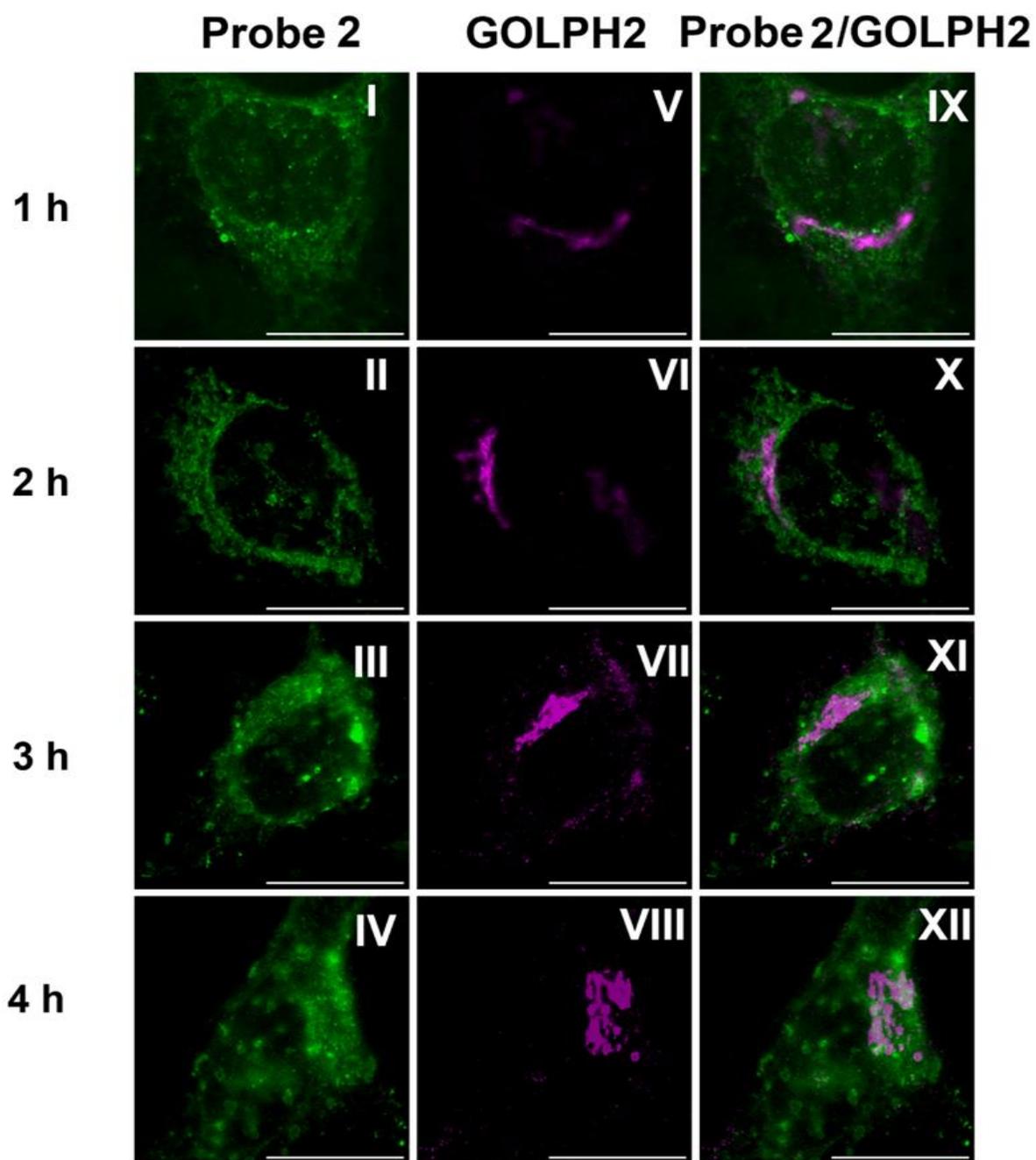


Figure S4. Time-dependent changes in the localization of probe **2** compared to the localization of Golgi. Fluorescent images of HeLa cells incubated with **2** for 1, 2, 3, and 4 h and then with biotin-azide, which was followed by incubation with streptavidin-A488. The cells were fixed, permeabilized, and finally treated with Cy5-anti-GOLPH2 antibody conjugate to stain the Golgi. (I-IV): A488 (green) fluorescence images showing labeled probe **2**; (V-VIII): Cy5 (purple) fluorescence images showing labeled Golgi; (IX-XII): overlay of green and purple fluorescence images. The scale bars are 20 μ m.

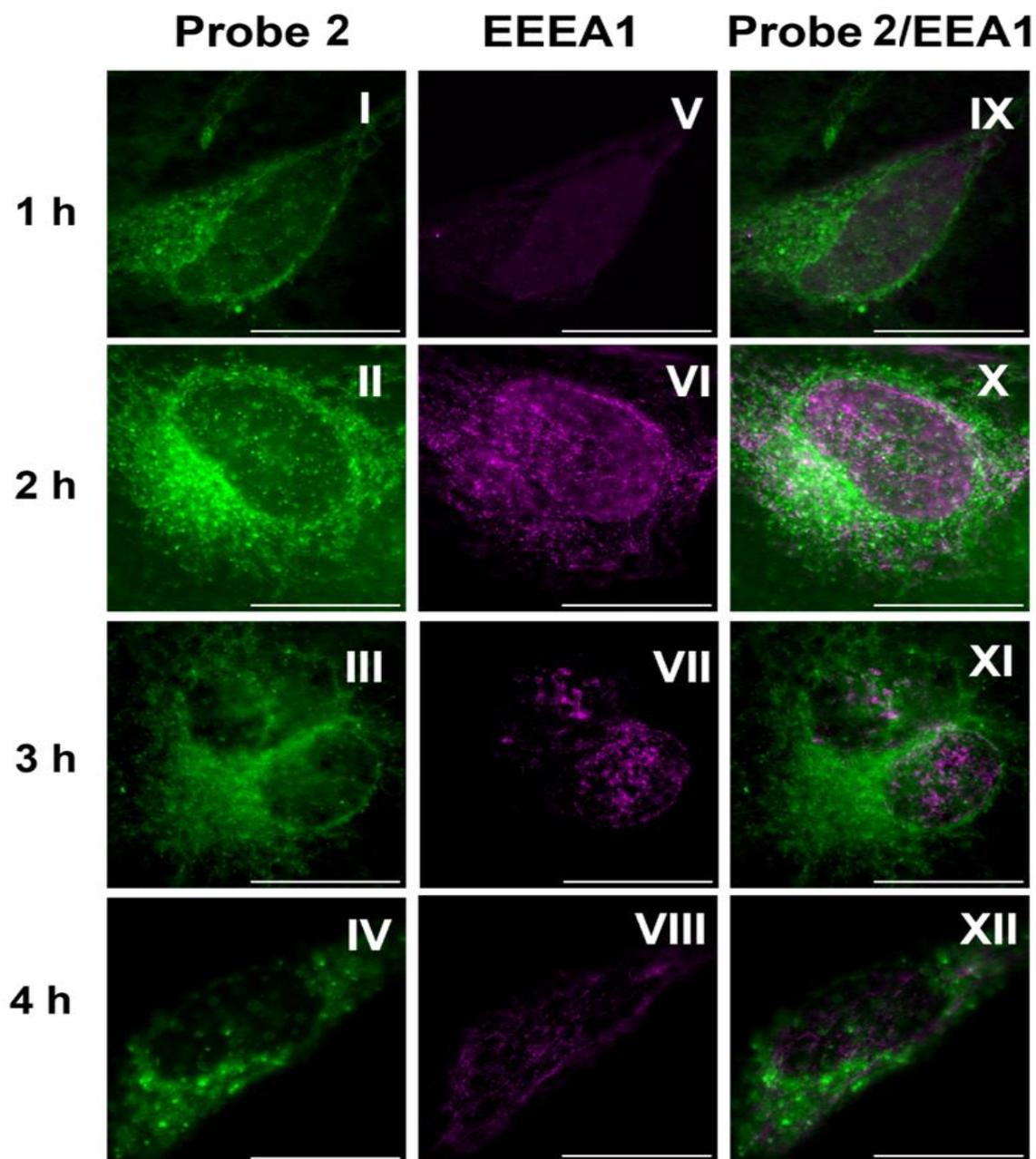


Figure S5. Time-dependent changes in the localization of probe **2** compared to the localization of the early endosome. Fluorescent images of HeLa cells incubated with **2** for 1, 2, 3, and 4 h and then with biotin-azide, which was followed by incubation with streptavidin-A488, cell fixation, permeabilization, and finally treatment with Cy5-anti-EEA1 antibody conjugate to stain the early endosome. (I-IV): A488 (green) fluorescence images showing labeled probe **2**; (V-VIII): Cy5 (purple) fluorescence images showing labeled early endosome; (IX-XII): overlay of green and purple fluorescence images. The scale bars are 20 μm .

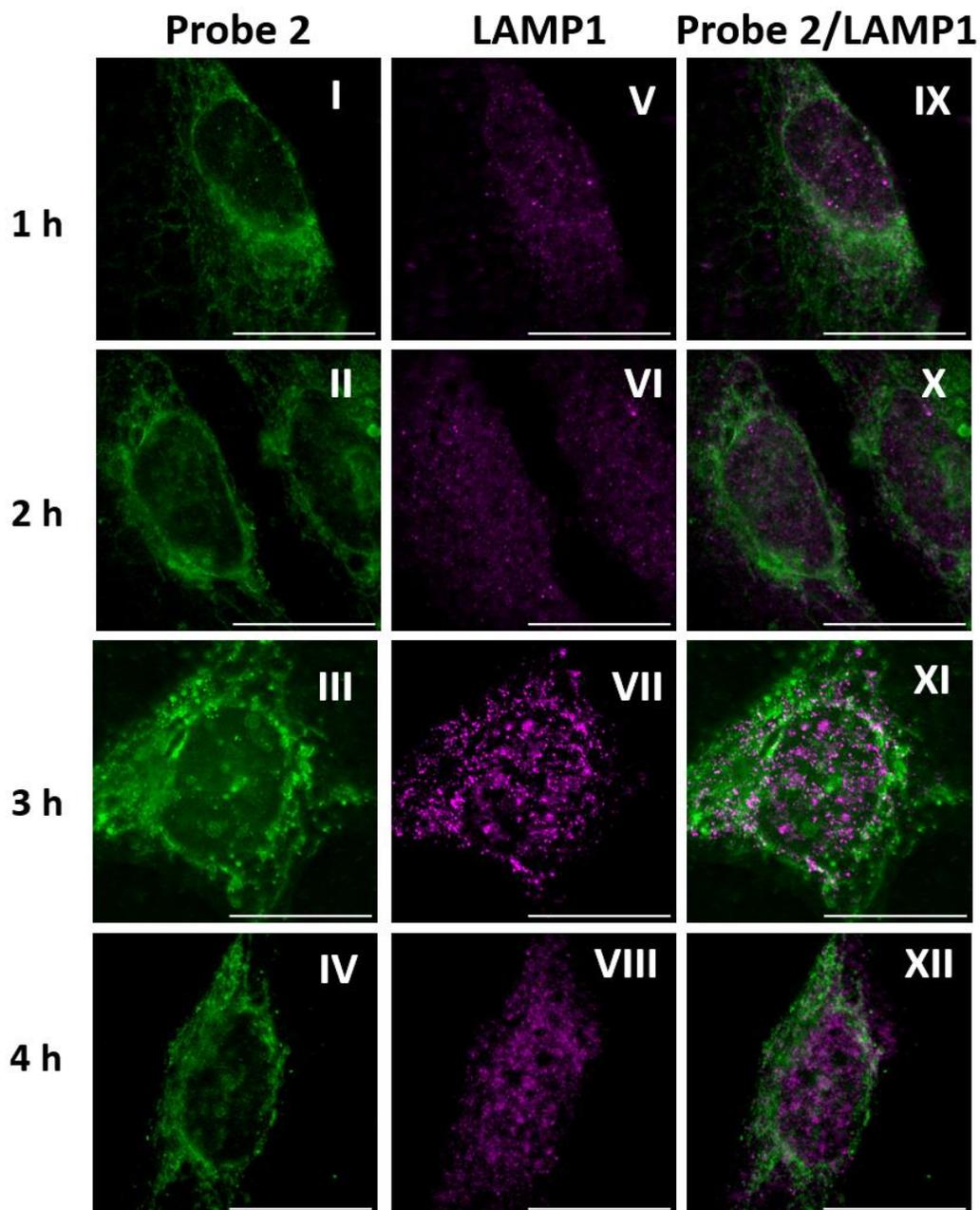


Figure S6. Time-dependent changes in the localization of probe **2** compared to the localization of the lysosome. Fluorescent images of HeLa cells incubated with **2** for 1, 2, 3, and 4 h and then with biotin-azide, which was followed by incubation with streptavidin-A488, cell fixation, permeabilization, and finally treatment with Cy5-anti-LAMP1 antibody conjugate to stain the lysosome. (I-IV): A488 (green) fluorescence images showing labeled probe **2**; (V-VIII): Cy5 (purple) fluorescence images showing labeled lysosome; (IX-XII): overlay of green and purple fluorescence images. The scale bars are 20 μ m.

SDS-PAGE Analysis of Probe-Crosslinked Proteins. The pellets of HeLa cells treated with probes **2** and **3** or PBS (negative control) and UV radiation were lysed in 500 μ L of lysis buffer containing 5.0 μ L of the protease inhibitor (HaltTM Protease inhibitor cocktail, Thermo Fisher Scientific) using a Qsonica probe sonicator (6 pulses, 60% duty cycle, 30 S each, Amp 10). The lysates were centrifuged at 14,000g and 4 $^{\circ}$ C for 5 min, and the supernatant was collected. The protein concentration in the supernatant was determined with a BCA protein assay kit (Thermo Scientific) according to the manufacturer's instructions, with the absorbance at 480 nm measured using a plate reader (BioTek citation 1). Approximately 100 μ g of the proteins was aliquoted to a 1.5 mL tube, which was followed by addition of the freshly prepared solutions of biotin-azide (1 mM in DMSO, 5 μ L), CuSO₄ (150.9 mM in H₂O, 0.66 μ L), sodium ascorbate (150.9 mM in H₂O, 1.52 μ L), and tris(3-hydroxypropyltriazolylmethyl)amine (THPTA, 5 mM in H₂O, 2.0 μ L). Then, doubly distilled water was added to the reaction mixture to reach a final volume of 100 μ L. The reaction mixture was vortexed at rt for 1 h before being quenched by adding 500 μ L of ice-cold acetone and incubation at -20 $^{\circ}$ C for 1 h. The cloudy solution was vortexed and then centrifuged at 21,000g (4 $^{\circ}$ C, 25 min). The proteins were collected and washed with cold acetone (1 mL, 2x) and pelleted. The pellet was dried at rt to remove any residual acetone and reconstituted in DPBS (100 μ L) supplemented with 1% SDS. The resulting mixture was sonicated in a water bath until a homogeneous, transparent solution was achieved. The protein concentration was determined by the BCA assay. A diluted protein solution (~1.0 μ g/ μ L) was prepared for each sample using 1X PBS. Protein solutions (10 μ L) were mixed with SDS loading dye (3.3 μ L, 4x stock solution) and dithiothreitol (DTT, 1 M stock, 0.5 μ L), and the solution was boiled at 95 $^{\circ}$ C and then loaded into the SDS-PAGE gel containing 10% acrylamide (10 μ g protein/gel lane) that was developed for the visualization after Coomassie blue staining or for Western blot. In the latter case, the gel was transferred to a membrane, which was blocked with blocking buffer containing 5% BSA at rt for 1 h and washed with TBST buffer three times. Thereafter, the membrane was incubated with a streptavidin-AP solution (0.1 μ g/mL in PBS, Thermo Fisher Scientific) at rt for 45 min, washed with TBST buffer five times, and incubated with 10 mL of NBT/BCIP solution (Thermo Fisher Scientific) at 4 $^{\circ}$ C with gentle shaking. The blot was further washed with TBST buffer three times and finally photographed. The gel fluorescence and images were processed with the GE Typhoon Trio Image Quant TL image analysis software. The results are shown in Figure S7.

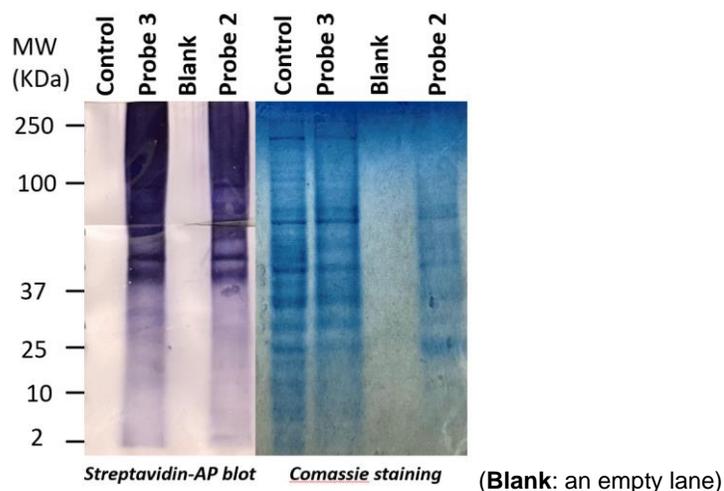


Figure S7. SDS-PAGE results of total proteins of HeLa cells treated probes **2** and **3** or PBS (control) as revealed by Coomassie staining and probe-crossed proteins revealed by Western blot using streptavidin-AP/NBT/BCIP.

Table S1. Unique proteins pulled down by probe 2 but not by probe 3

| Uniprot ID* | Proteins | Cellular locations | Log ₂ FC | Protein names and reported/predicted biological functions |
|--|----------|--|---------------------|---|
| Proteins found in all three experiments | | | | |
| Q8N2G8 | GHDC | Endoplasmic reticulum | 5.11 | GH3 domain-containing protein: an acid-amino acid ligase, showing activities to modify intracellular proteins by adding carboxylic acids to the amino group of proteins. ^[1] |
| Q9BRT6 | LLPH | Nucleus | 3.80 | LLP homolog: LLP homologs are evolutionarily conserved mammalian proteins that bind to polyadenylated RNAs and different ribosomal proteins to participate in various signaling pathways. ^[1-2] |
| P80217 | IFI35 | Cytoplasm, Nucleus | 3.34 | Interferon-induced 35 kDa protein: a protein participating in the innate immune response pathway. ^[1, 3] |
| Q9NW82 | WDR70 | Nucleus | 3.01 | WD repeat-containing protein 70: plays a role in DNA repair, cell cycle progression, and chromatin repair. ^[1, 4] |
| Q13158 | FADD | Plasma membrane | 2.59 | FAS-associated death domain protein: an adaptor protein involved in cell death induced signaling complex and thus in CD95-mediated apoptosis. ^[1] |
| B8JMH0 | Iba57 | Mitochondrial matrix | 2.55 | Putative transferase CAF17 homolog: a protein present in the mitochondria, participating in the iron-sulfur cluster assembly pathway. ^[1, 5] |
| Q14118 | DAG1 | Basolateral plasma membrane | 2.28 | Dystroglycan 1: an extracellular glycoprotein playing an important role in cell adhesion-mediated signaling and cell polarity. ^[1, 6] |
| Q9NUL3 | STAU2 | Cytoplasm, ER | 1.73 | Double-stranded RNA-binding protein Staufen homolog 2: an RNA binding protein required for the microtubule-dependent transport. ^[1, 7] |
| Q9BXB4 | OSBPL11 | Late endosome and Golgi membranes | 1.62 | Oxysterol-binding protein-related protein 11: a protein of the OSBP protein class (cholesterol/oxysterol transfer proteins), which regulates glycolipids and glycosphingolipid metabolism in cells. ^[1, 8] |
| P62068 | UBP46 | Cytoplasm, Nucleus | 1.53 | Ubiquitin carboxyl-terminal hydrolase 46: a deubiquitinating enzyme, which removes ubiquitin molecules from different signaling proteins, e.g., GAD1/GAD67. ^[1, 9] |
| Q14457 | BECN1 | Endosomal or Golgi/trans-Golgi network membranes | ** | Beclin-1: a protein of the phosphatidylinositol-3-phosphate (PI3K) pathway, which regulates the level of PI3K and plays a role in membrane trafficking and endocytic pathways. ^[1, 10] |
| Q6P587 | FAHD1 | Mitochondria, cytoplasm | ** | Mitochondrial acylpyruvase FAHD1: a mitochondrial acylpyruvase, which catalyzes acetylpyruvate hydrolysis. ^[1, 11] |
| Proteins found in any two experiments | | | | |
| P46937 | YAP1 | Cytoplasm, nucleus | 5.45 | YES1 associated transcriptional coactivator YAP1: a transcriptional regulator protein, which controls the transcriptional program involved in cell differentiation and proliferation. ^[1, 12] Scaffolding protein Scribble in cooperation with YES1 and YAP1 proteins regulates different cellular signaling pathways. ^[13] |

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|------------------------|--------|--|------|--|
| P27449 | ATP6C | Cytoplasmic vesicles, clathrin-coated vesicles | 5.24 | V-type proton ATPase 16 kDa proteolipid subunit C: a part of a multisubunit enzyme responsible for translocating proton by hydrolyzing ATP. ^[1] |
| P50135 | HNMT | Cytoplasm | 4.56 | Histamine N-methyltransferase: the primary metabolizing enzyme of histamine, which inactivates histamine by a simple N-methylation reaction. ^[1, 14] |
| Q9UPQ9 | TNRC6B | Cytoplasm | 3.49 | Trinucleotide repeat-containing gene 6B protein: an important signaling protein for the miRNA pathway, which helps in RNA mediated gene silencing. ^[1, 15] |
| Q9H0P0 | NT5C3A | Cytoplasm, ER | 3.25 | Cytosolic 5'-nucleotidase 3A: a nucleotidase, which shows specific activity towards the cytidine monophosphate (CMP) and 7-methylguanosine monophosphate to participate in different signaling pathway. ^[1] |
| P46934 | NEDD4 | Cytoplasm, Cell membrane | 3.03 | E3 ubiquitin-protein ligase NEDD4: a ubiquitin protein ligase, which transfers ubiquitin from a previous protein member to the target substrate and thus controls protein degradation in lysosome. ^[1, 16] This protein also acts as a regulator of protein level in the Hippo pathway. ^[17] NEDD4 also interacts with YAP protein. ^[17-18] |
| O43765 | SGTA | Cytoplasm, nucleus | 2.56 | Small glutamine-rich tetratricopeptide repeat containing protein alpha: a chaperon protein that directs misfolded cytosolic protein to the ER, ^[1, 19] functioning as tail anchored membrane protein. ^[20] It actively interacts with BAG6 protein to regulate the degradation process of misfolded proteins. ^[21] |
| Q13033 | STRN3 | Plasma membrane, Cytoplasm | 2.1 | Striatin-3: a peripheral membrane protein that functions as a partner for different signaling pathway. ^[1, 22] |
| Q5TCQ9 | MAGI3 | Cell membrane | 1.91 | Membrane-associated guanylate kinase 3: a WW and PDZ domain-containing protein acting as scaffolding protein at the cell-cell junction to regulate different signaling pathways including ERK, RhoA, and JNK signaling pathways. ^[1, 23] |
| Q969V6 | MRTFA | Cytoplasm | 1.66 | Myocardin-related transcription factor A: Transcriptional coactivator that regulates the Rho-GTPase induced changes in cellular actin concentration. ^[1, 24] |
| O43148 | RNMT | Nucleus | 1.13 | mRNA cap guanine-N7 methyltransferase: catalytic subunit of methyltransferase that methylates the guanosine residue of mRNAs. ^[1, 25] |
| P49459 | UBE2A | Ubiquitin-conjugating enzyme E2 A | ** | Ubiquitin-conjugating enzyme E2 A: a ubiquitinating enzyme, which catalyzes the covalent attachment of ubiquitin to proteins. ^[1, 26] |
| Q9ULC3 | RAB23 | Cytoplasmic side of plasma membrane | ** | Ras-related protein Rab-23: a small GTPase protein playing a key role in retrograde trafficking and fusion of vesicles from post-Golgi network to the membrane. ^[1, 27] |
| Q96CV9 | OPTN | Golgi, Cytoplasm | ** | Optineurin: a Golgi complex residual protein playing a role in vesicle trafficking from the Golgi network to membrane and exocytosis, and in Rab-mediated vesicle trafficking. ^[1, 28] |
| P15289 | ARSA | ER, Lysosome | ** | Arylsulfatase A: a sulfatase that hydrolyzes cerebroside sulfate. ^[1, 29] |
| O14976 | GAK | Golgi, Trans-Golgi network | ** | Cyclin G-associated kinase: a kinase involved in clathrin-mediated sorting of cytoplasmic vesicles. ^[1, 30] |

Proteins found in one experiment

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| Q9Y5X2 | BAG38035.1 | Early-endosome membrane | 4.7 | Sorting nexin 8: a cargo sorting protein that is involved in intracellular protein transport from early endosome to trans-Golgi network. ^[1, 31] |
| Q8WVT3 | TRAPPC12 | ER-Golgi intermediate compartment | 3.06 | Trafficking protein particle complex subunit 12: an important component of transport protein particle (TRAPP), involved in ER to Golgi trafficking of endocytic vesicles at early stage. ^[1, 32] |
| Q9HCK8 | CHD8 | Nucleus | 2.52 | Chromodomain-helicase—DNA-binding protein 8: a DNA helicase that regulates transcription ^[1] and non coding RNA. ^[33] |
| B2RDT8 | SNX8 | Plasma membrane | 2.23 | cDNA FLJ96764, similar to Homo sapiens sorting nexin 8 (SNX8): a peripheral membrane protein showing phosphatidylinositol binding activity involved in relocation of proteins from early endosome to Golgi. ^[31a, 34] |
| Q9NV06 | DCAF13 | Nucleus | 1.59 | DDB1- and CUL4-associated factor 13: a precursor protein of ribosomal subunits involved in ribosome-related functions. ^[1] |
| Q6P3X3 | TTC27 | Cytoplasm | 0.62 | Tetratricopeptide repeat protein 27: a not well explored protein, which plays role in O-GlcNAcylation of proteins. ^[1, 35] |
| A0A0A6YYL4 | CORO7-PAM16 | Cytoskeleton | ** | Coronin protein: an actin binding protein that regulates the remodeling of actin cytoskeleton, and thus is involved in endocytosis and cell motility. ^[1, 36] |
| A8K4P8 | Unknown | Unknown | ** | cDNA FLJ75337, whose function is unknown |
| A0A455C1X9 | WNK2 | Unknown | ** | Serine/threonine-protein kinase WNK2 isoform X1: a protein with kinase activity, and thus may regulate cell signaling by phosphorylating different proteins. ^[1] |
| B2R9X3 | Unknown | cytoplasm | ** | GDP-mannose 4,6-dehydratase: it is involved in GDP-fucose biosynthesis from GDP-D-mannose. ^[1] |
| Q9UJX5 | ANAPC4 | Nucleus | ** | Anaphase-promoting complex subunit 4: a component of anaphase-promoting complex that regulates cell cycle progression. ^[1, 37] |
| P0CG30 | GSTT2B | Cytoplasm | ** | Glutathione S-transferase theta-2B: a glutathione-S transferase (GST) that conjugates glutathione to electrophilic compounds ^[1, 38] and is a GPI-AP. ^[39] |
| Q9Y4C2 | TCAF1 | Cell membrane | ** | TRPM8 channel-associated factor 1: a membrane protein that positively regulates the plasma membrane ion channel TRPM8 activity. ^[1, 40] Importantly, TRPM8 is associated with GPI-APs for ion channel signaling pathway. ^[41] |
| Q9UNS1 | TIMELESS | Nucleus | ** | Protein timeless homolog: a protein playing an important role in the control of DNA replication. ^[1] |
| Q9UJ78 | ZMYM5 | Nucleus | ** | Zinc finger MYM-type protein 5: Serves in transcriptional regulation related function. ^[1, 42] |
| Q95379 | TNFAIP8 | Cytoplasm | ** | TNF α -induced protein 8: a cytokine that regulates TNF-mediated apoptosis. ^[1, 43] |
| P49247 | RPIA | Cytoplasm | ** | Ribose-5-phosphate isomerase: it catalyzes the conversion of ribose-5-phosphate to ribulose-5-phosphate, and thus participates in pentose phosphate pathway. ^[1, 44] |
| Q6MG49 | BAG6 | Cytoplasm | ** | Large proline-rich protein BAG6: an ATP-dependent molecular chaperone, which regulates the quality control of proteins. ^[1] It |

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| | | | | strongly favors long linear hydrophobic sequences such as ER targeting signals and GPI-anchoring signals. ^[45] |
| <u>P13984</u> | GTF2F2 | Nucleus | ** | General transcription factor IIF subunit 2: a transcription initiation factor protein that binds to RNA polymerase II and is involved in transcription process related regulation. ^[1] |
| <u>Q15047</u> | SETDB1 | Nucleus | ** | Histone-lysine N-methyltransferase SETDB1: a histone methyltransferase that is mainly responsible for methylation of proteins. ^[1, 46] |
| <u>Q13625</u> | TP53BP2 | Cytoplasm | ** | Apoptosis-stimulating of p53 protein 2: a cytoplasmic protein that regulates cell growth and apoptosis. ^[1, 47] |
| <u>Q6DKK2</u> | TTC19 | Mitochondrial inner membrane | ** | Tetratricopeptide repeat protein 19: a mitochondrial protein responsible for mitochondrial respiratory complex formation. ^[1] |
| <u>P19013</u> | KRT4 | Cytoskeleton | ** | Keratin type II cytoskeletal 4: a keratin protein responsible for cytoskeletal stability. ^[1] |
| <u>Q92643</u> | PIGK | ER membrane | ** | GPI-anchor transamidase: an ER membrane protein that mediates GPI anchor attachment to proteins in the ER lumen. ^[1, 48] |
| <u>Q6NVU6</u> | UFSP1 | Cytoplasm | ** | Inactive Ufm1-specific protease 1: an active protease, which regulates the post-translational ubiquitination of proteins. ^[1, 49] |
| <u>A0A9B0U0N5</u> | CDC42BPA | Cytoplasm, cell projection | ** | Non-specific serine/threonine protein kinase: shows protein kinase activity and thus regulates cell signaling pathway. ^[1] |
| <u>Q9UBQ0</u> | VPS29 | Membrane, Endosome membrane | ** | Vacuolar protein sorting-associated protein 29: a component of retromer complex, which regulates the transportation of the vesicles with enriched ceramide, cholesterol, sphingolipids, and GPI-anchored proteins. ^[1, 50] |
| <u>Q9Y5Y5</u> | PEX16 | ER membrane | ** | Peroxisomal membrane protein PEX16: a protein required for peroxisome membrane biogenesis, and thus it plays role in early stage of peroxisome assembly. ^[1, 51] |
| <u>P42858</u> | HTT | Cytoplasmic vesicle membrane | ** | Huntingtin: a cytoplasmic protein that plays a role in microtubule-mediated transport of cytoplasmic vesicles. ^[1, 52] |
| <u>B3KTC8</u> | Unknown | Cytoplasm | ** | cDNA clone CTONG2015804: a protein highly similar to UDP-N-acetylglucosamine pyrophosphorylase (UAP1) that catalyzes the formation of UDP-GlcNAc. ^[1, 53] |
| <u>Q07954</u> | LRP1 | Clathrin-coated vesicles | ** | Pro-low-density lipoprotein receptor-related protein 1: an endocytic receptor that is involved in endocytosis and in phagocytosis of apoptotic cells. ^[1, 54] |
| <u>Q9Y623</u> | MYH4 | Cytoplasm | ** | Myosin-4: Cytoplasmic protein, which shows actin binding activity, and thus regulates actin-mediated vesicle trafficking. ^[1, 55] |
| <u>Q96RL7</u> | VPS13A | ER and endosome membrane | ** | Intermembrane lipid transfer protein VPS13A: an endoplasmic reticulum located protein that binds to phospholipids and thus stabilizes ER-mitochondria contact sites to enable the transfer of lipids between the ER and mitochondria. ^[1, 56] |
| <u>O00233</u> | PSMD9 | Cytoplasm | ** | 26S proteasome non-ATPase regulatory subunit 9: a chaperone protein that plays a role in the assembly of 26S proteasome complex and thus regulates peptide degradation in an ATP/ubiquitin-dependent non-lysosomal pathway. ^[1, 57] |
| <u>Q96JJ7</u> | TMX3 | ER membrane | ** | Protein disulfide-isomerase TMX3: a disulfide isomerase that regulates protein folding containing disulfide bonds. ^[1, 58] |

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| P30260 | CDC27 | Cytoplasm, cytoskeleton | ** | Cell division cycle protein 27 homolog: an E3 ubiquitin ligase that controls the cell cycle by mediating ubiquitination followed by degradation of the target proteins. ^[1, 37] |
| Q9UJA9 | ENPP5 | Plasma membrane | ** | Ectonucleotide pyrophosphatase/phosphodiesterase family member 5: a type-I transmembrane glycoprotein that hydrolyzes NAD and regulates cellular communication. ^[1, 59] |
| Q8NHV4 | NEDD1 | Cytoskeleton | ** | Protein NEDD1: a cytoskeletal protein that regulates the cell cycle progression by mediating the nucleation of microtubule from the spindle. ^[1, 60] |
| P43251 | BTD | Extracellular matrix | ** | Biotinidase: a protein that is involved in biotin-dependent carboxylase degradation. ^[1] |
| O14646 | CHD1 | Cytoplasm | ** | Chromodomain-helicase-DNA-binding protein 1: an ATP-dependent chromatin-remodeling factor that is involved in transcription regulation. ^[1] |
| Q9NX05 | FAM120C | Nucleus | ** | Constitutive coactivator of PPAR-gamma-like protein 2: an RNA binding protein. ^[1] |
| P61254 | RPL26 | Cytoplasm | ** | Large ribosomal subunit protein uL24: a component protein of a large ribosomal subunit that regulates the biosynthesis of proteins. ^[1, 61] |
| Q8IZ07 | ANKRD13A | Cell and late endosome membranes | ** | Ankyrin repeat domain-containing protein 13A: a ubiquitin-binding protein that regulates the internalization of ligand activated cell membrane receptor. ^[1, 62] |
| P51531 | SMARCA2 | Cytoskeleton | ** | Probable global transcription activator SNF2L2: it is involved in transcription related process. ^[1] |
| O75351 | VPS4B | Late endosome membrane | ** | Vacuolar protein sorting-associated protein 4B: it is involved in late step of endosomal trafficking pathway and in the exosomal release of GPI-anchored proteins. ^[1, 63] |
| Q9HCU5 | PREB | ER membrane | ** | Prolactin regulatory element-binding protein: an ER membrane located GTPase that regulates the recruitment of COPII vesicle at the ER exit site to regulate the vesicle trafficking from ER to membrane. ^[1, 64] |
| Q15554 | TERF2 | Nucleus | ** | Telomeric repeat-binding factor 2: a DNA-binding proteins playing a role in telomere maintenance. ^[1] |
| Q13232 | NME3 | Cytoplasm | ** | Nucleoside diphosphate kinase 3: it plays a role in nucleoside triphosphate synthesis and regulates cell differentiation. ^[1] |
| P24468 | NR2F2 | Cytoplasm | ** | COUP transcription factor 2: a ligand-activated transcription factor involved in transcription related regulation. ^[1] |
| O95785 | WIZ | Nucleus | ** | Protein Wiz: showing histone methyl transferase activity. ^[1] |
| Q8IWT6 | LRRC8A | Plasma membrane | ** | Volume-regulated anion channel subunit LRRC8A: an anion channel receptor that regulates the cellular volume by maintaining the extracellular/intracellular osmotic pressure. ^[1, 65] |
| Q9NQX7 | ITM2C | Plasma membrane | ** | Integral membrane protein 2C: a negative regulator protein of amyloid-beta peptide production. ^[1] |
| P19404 | NDUFV2 | Mitochondrial inner membrane | ** | NADH dehydrogenase [ubiquinone] flavoprotein 2: a component of mitochondrial membrane respiratory chain NADH dehydrogenase complex, which is involved in electron transfer processes. ^[1, 66] |
| Q969R2 | OSBP2 | Plasma membrane | ** | Oxysterol-binding protein 2: a lipid binding protein that regulates the lipid transport over the membrane/extracellular matrix junction. ^[1, 67] |

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| <u>J7HWK6</u> | ND4 | Mitochondrial inner membrane | ** | NADH-ubiquinone oxidoreductase chain 4: a core subunit protein of mitochondrial membrane respiratory chain NADH dehydrogenase, playing key role in electron transport chain. ^[1, 68] |
| P42892 | ECE1 | Early endosome | ** | Endothelin-converting enzyme 1: an endosomal membrane protein with a crucial function in the regulation of endothelin, which induces and regulates clathrin-independent GPI-AP trafficking. ^[1, 69] |
| P01112 | HRAS | Cell membrane | ** | GTPase HRas: a GTPase that recycles between plasma membrane and Golgi to play key role in the trafficking of different lipid modified proteins. ^[1, 70] |
| P17612 | PKAC | Cytoplasm, Cell membrane | ** | cAMP-dependent protein kinase catalytic subunit alpha: a protein kinase which phosphorylates different proteins and thus regulates the PLC-mediated cleavage and subsequent endocytosis of GPI-APs. ^[1, 71] |
| P53680 | AP2S1 | Plasma membrane | ** | AP-2 complex subunit sigma: a component of adaptor protein complex 2 (AP2), which regulates the clathrin coated vesicle formation and thus trafficking of GPI-APs. ^[1, 72] |
| Q9H223 | EHD4 | Plasma membrane, Early endosome | ** | EH domain-containing protein 4: an ATPase and membrane-bound protein primarily found in the membrane of early endosomes, which plays a role in membrane organization, particularly adjacent to the cytoskeleton, tubulin formation, and endosomal transport. It regulates GPI-AP trafficking and signaling pathway, but the mechanism is unclear. ^[1, 72a, 73] |

* The underlined proteins were not identified with both probes **1** and **3**.

** Denotes those proteins that are present in the protein mixture but not assigned any numerical value.

Table S2. Statistically significantly enriched proteins ($P \leq 0.05$, $\log_2FC \geq 2$) pulled down by probe 2

| Uniprot ID* | Proteins | Cellular locations | Log ₂ FC | Protein names and reported/predicted biological functions |
|-------------|----------|--------------------------------------|---------------------|--|
| Q9NV70 | EXOC1 | Cell membrane, cytoplasm | 5.02 | Exocyst complex component 1: a subunit of the Exocyst complex that mediates the tethering of secretory vesicle to the plasma membrane. ^[1, 74] |
| Q8N3V7 | SYNPO | Cytoplasm, Cytoskeleton | 4.56 | Synaptopodin: one of the synaptopodin proteins that bind to actin. They are known to stimulate actin polymerization and initiate binding of different signaling proteins in the actin filament for downstream signaling. ^[1, 75] |
| Q9Y613 | FHOD1 | Cytoplasm, Cytoskeleton | 4.36 | FH1/FH2 domain-containing protein 1: one of the proteins responsible for assemble of actin structure. ^[1] |
| O75607 | NPM3 | | 4.35 | Nucleoplasmin-3: a protein playing a role in biosynthesis of ribosome and chromatin remodeling. ^[1] |
| Q9NPP4 | NLRC4 | Cytoplasm | 3.96 | NLR family CARD domain-containing protein 4: a key component of inflammasome complex in mammalian cells and participates in cytokine production process. ^[1] |
| Q99986 | VRK1 | Nucleus, Cytoplasm | 3.90 | Serine/threonine-protein kinase VRK1: a serine/threonine kinase involved in regulation of cell cycle and in the kinase activity of different proteins in cytoplasm. ^[1] |
| O15116 | LSM1 | Cytoplasm | 3.63 | U6 snRNA-associated Sm-like protein LSm1: it plays a role in degradation of different mRNA. ^[1, 76] |
| Q8WWW0 | RASSF5 | Centrosome, microtubule | 3.55 | Ras association domain containing protein 5: a potential interaction partner of Rap1 and different Ras-like small GTPases. ^[1] |
| O95490 | ADGRL2 | Membrane | 3.39 | Adhesion G protein-coupled receptor (GPCR) L2: a member of latrophin subfamily for GPCR, playing a role in regulation of exocytosis. ^[1] It also plays a significant role in cell adhesion and signal transduction in Hela cells. ^[77] |
| P51948 | MNAT1 | Nucleus | 3.14 | CDK-activating kinase assembly factor MAT1: a protein that stabilizes the CDK complexes and is involved in cell cycle control. ^[1] |
| A0A6P8QK47 | FANCA | Nucleus | 3.11 | Fanconi anemia group A protein isoform X1: it removes the interstrand crosslink between the DNA strands and thus helps in DNA strand unwinding required for transcription and replication. ^[1] |
| Q9C0H2 | TTYH3 | Cell membrane, extracellular exosome | 3.08 | Protein tweety homolog 3: a protein of tweety homolog (TTYH), which acts as Ca ²⁺ dependent ion channel regulator. ^[1, 78] |
| Q03013 | GSTM4 | Cytoplasm | 2.81 | Glutathione S-transferase Mu 4: a cytosolic glutathione s-transferase (GST), which can react with hydrophobic electrophiles in the cytoplasm to conjugate glutathione to a wide variety of substrates. ^[1, 79] |
| Q5MIZ7 | P4R3B | Cytoplasm, Cytoskeleton | 2.73 | Serine/threonine-protein phosphatase 4 regulatory subunit 3B: a phosphatase that regulates the activities of different phosphatase, and thus plays a key role in the microtubule organizing center (MTOC). ^[1] It is also an interactor of BAG6 protein pulled down by probe 1. |

| | | | | |
|--------|----------|--|------|--|
| B4DKI9 | MRPS27 | Ribosome | 2.66 | CDNA FLJ54799 like mitochondrial 28S ribosomal protein S27: a MRP27 protein playing a key role in mitochondrial protein synthesis in mammalian cells. ^[1, 80] |
| P78332 | RBM6 | Nucleus | 2.66 | RNA-binding protein 6: playing a role in mRNA splicing. ^[1, 81] |
| Q9Y487 | ATP6V0A2 | Cell and ER membranes | 2.63 | V-type protein ATPase 116 kDA subunit a 2: a subunit of V-ATPase that plays a key role in translocating protons. ^[1] |
| Q96QC0 | PPP1R10 | Nucleus | 2.62 | Serine/threonine-protein phosphatase 1 regulatory subunit 10: a scaffold protein helping form PTW/PP1 phosphatase complex that plays a key role in cell cycle progression. ^[1, 82] |
| Q15054 | POLD3 | Cytoplasm, Nucleus | 2.59 | DNA polymerase delta subunit 3: a component of the DNA polymerase complex that plays a key role in genome replication. ^[1, 83] |
| O00400 | SLC33A1 | Plasma, ER and Golgi membranes | 2.43 | Acetyl-coenzyme A transporter 1: a member of acetyl-CoA transporter family proteins that modify the sialyl residue of gangliosides and glycoproteins. ^[1, 84] |
| Q7Z6E9 | RBBP6 | Cytosol, microtubule organizing center | 2.41 | E3 ubiquitin-protein ligase RBBP6: an E3 ubiquitin-protein ligase playing a key role in ubiquitination of proteins and thus contributes to various malignancies. ^[1] |
| Q59EK0 | AB209811 | Trans-membrane complex assembly | 2.35 | Epsilon isoform of regulatory subunit B56, a protein phosphatase 2A (PP2A) variant: a ubiquitously expressed serine/threonine phosphatase that regulates many cellular functions, e.g., lipid metabolism, glycolysis, etc. ^[1, 85] PP2A class of proteins interacts with YAP to regulate the Hippo signaling pathway. ^[86] |
| Q9NWS0 | PIH1D1 | Nucleus | 2.26 | PIH1 domain-containing protein 1: it plays a role in the assembly of small nucleolar ribonucleoprotein (snoRNP) and participates in many cellular functions. ^[1, 87] |
| Q96GA3 | LTV1 | Cytoplasm | 2.41 | Protein LTV1homolog: it is involved in ribosomal subunit biogenesis and exporting from nucleus. ^[1, 88] |
| Q53GQ2 | COPS7B | Cytoplasm, Nucleus | 2.13 | COP9 signalosome complex subunit 7B: a component protein of COP9 signalosome complex involved in various cellular processes. ^[1, 89] |
| P54278 | PMS2 | Nucleus | 2.12 | Mismatch repair endonuclease PMS2: it plays a role in the DNA mismatch repair system. ^[1, 90] |
| B4DSS4 | AK299890 | * | 2.00 | cDNA FLJ56631: No biological function reported. |

* Cellular localization of the protein is not revealed by Uniprot yet.

Abbreviations:

LLP: Long-term synaptic facilitation protein

DUB: Deubiquitinase or deubiquitinating enzyme

PLC: Phospholipase C

TRPM8: Transient receptor potential cation channel subfamily M (melastatin) member 8

TNF α : Tumor necrosis factor alpha

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