Supporting Information for

Chemoproteomic Profiling of Itaconation by Bioorthogonal Probes in Inflammatory Macrophages

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Figure S1. Synthesis of ITalk. Itaconate anhydride and 7-Octyn-1-ol were incubated at 110 °C for 4 h.
**Figure S2.** Evaluation of ITalk labeling in cell lysates. (A) Concentration-dependent labeling of ITalk in Raw264.7 cell lysates. Raw264.7 cell lysates were treated with different concentrations of ITalk at 37 °C for 1 h. (B) Time-dependent labeling of ITalk in Raw264.7 cell lysates. Raw264.7 cell lysates were treated with ITalk at 37 °C for different times. (C) Blocking free cysteines by iodoacetamide (IA) diminished ITalk labeling in cell lysates. Raw264.7 cell lysates were pre-treated with various concentrations of IA and then labeled with 100 μM of ITalk at 37 °C for 1 h. (D) Competition of ITalk labeling by endogenous itaconate induced by LPS stimulation. Raw264.7 cells were treated with 100 ng/mL LPS and the cell lysates were labeled with 100 μM of ITalk. The labeled lysates were reacted with azide-rhodamine (Rho) via CuAAC and the fluorescent intensity was determined by in-gel fluorescence scanning. Coomassie Brilliant Blue (CBB) staining demonstrates the equal labeling.
**Figure S3.** ITalk recapitulates the function of itaconate in LPS-stimulated Raw264.7 cells. (A) ITalk could be partially hydrolyzed to release itaconate. HEK293T cells were treated with 100 μM OI or ITalk for 4 h. The cellular level of itaconate was quantified by LC-SRM. (B) ITalk significantly increases the protein level of NRF-2 in LPS-stimulated Raw264.7 cells. (C) ITalk significantly inhibits lactate production in LPS-stimulated Raw264.7 cells. Raw264.7 cells were treated with 100 ng/mL LPS and then treated with 1 mM itaconate, 100 μM OI or ITalk, respectively.
Figure S4. Evaluation of ITalk labeling in living Raw264.7 cells. (A) Concentration-dependent labeling of ITalk in living Raw264.7 cells. Raw264.7 cells were treated with different concentrations of ITalk for 2 h. (B) Time-dependent labeling of ITalk in living Raw264.7 cells. Raw264.7 cells were treated with 100 μM of ITalk for different time points. (C) Competitive labeling of ITalk by OI in living Raw264.7 cells. Raw264.7 cells were pre-treated with 1 mM OI and OMS, respectively. Then the cells were labeled with 100 μM of ITalk for 12 h. (D) ITalk modifications are resistant to cleavage by hydroxylamine. Raw264.7 cells were treated with 100 μM of ITalk for 12 h, followed by the click reaction with azide-Rho via CuAAC. The labeled lysates were treated with 5% hydroxylamine and then boiled with the SDS-PAGE loading buffer at 95 °C for 10 min. (E) Octynyl alcohol did not cause detectable protein labeling in living cells. The structure of octynyl alcohol is shown. Raw264.7 cells were treated with 100 μM of octynyl alcohol for 12 h. The labeled lysates were reacted with azide-Rho via CuAAC and the fluorescent intensity was determined by in-gel fluorescence scanning. Coomassie Brilliant Blue (CBB) staining demonstrates the equal labeling.
Figure S5. ITalk labeling did not induce cytotoxicity. Raw264.7 cells were treated with 100 μM of ITalk and OI for 12 h, respectively. The cell viability was determined by MTS cell proliferation assay.
Figure S6. Quantitative chemoproteomic profiling of itaconate substrates by ITalk in inflammatory macrophages. (A) Workflow for the quantitative chemoproteomic profiling of itaconate substrates in inflammatory macrophages. Raw264.7 cells were treated with 100 μM ITalk for 12 h and after cell lysis, the lysates were reacted with azide-biotin, followed with enrichment by streptavidin beads and on-beads trypsin digestion. The peptides from ITalk-treated cells were labeled as “light” while the peptides from vehicle-treated cells were labeled as “heavy” by dimethyl labeling. The labeled proteins were identified and quantified by LC-MS/MS analysis. (B) LPS significantly stimulates IL-1β secretion in Raw264.7 cells. Raw264.7 cells were treated with 10 ng/mL LPS for 12 h and IL-1β secretion was determined by ELISA. (C) Distribution of the enrichment ratio of ITalk-labeled proteins in the second biological replicate. (D) Distribution of enrichment ratio of ITalk-labeled proteins in the third biological replicate. (E) Overlap of the itaconate substrates identified by ITalk in three biological replicates. (F) Identification of known itaconate targets by ITalk. MS1 chromatographic peaks of peptides from KEAP1, ALDOA and LDHA are shown.
Figure S7. Time-resolved chemoproteomic profiling of sensitive itaconate substrates by ITalk. (A) Workflow for the time-resolved chemoproteomic profiling of sensitive itaconate substrates in inflammatory macrophages. Raw264.7 cells were treated with 100 μM ITalk for 1 h and 10 h, respectively. After cell lysis, the lysates were reacted with azide-biotin, followed with enrichment by streptavidin beads and on-beads trypsin digestion. The peptides from the 1 h ITalk-treated cells were labeled as “light” while the peptides from the 10 h ITalk-treated cells were labeled as “heavy” by dimethyl labeling. The labeled proteins were identified and quantified by LC-MS/MS analysis. (B) IL-1β secretion was stimulated by 1 or 10 h of LPS treatment in Raw264.7 cells. Raw264.7 cells were treated with 10 ng/mL LPS for 1 or 10 h, following with the detection of IL-1β secretion by ELISA. (C) Overlap of the sensitive itaconate substrates identified by ITalk in three biological replicates. (D) SDHA is a sensitive itaconate substrate with a quantification ratio of 1.806. The MS1 chromatographic peaks of peptides from SDHA are shown. (E) Biological process analysis of the sensitive itaconate substrates by gene ontology.
Figure S8. Site-specific analysis of the itaconate substrates by ITalk in inflammatory macrophages. (A) Workflow for quantitative chemoproteomic profiling of itaconate substrates in inflammatory macrophages. Raw264.7 cells were treated with 100 μM ITalk for 12 h and the lysates were reacted with azide-AC-biotin, followed by enrichment with streptavidin beads. After on-beads trypsin digestion to release the unmodified peptides, the modified peptides were then specifically released by acid cleavage and analyzed by LC-MS/MS. (B) Overlap of the itaconate modified cysteines identified by ITalk in three biological replicates. (C) Sequence motif analysis of the itaconate modified cysteines. (D) Distribution of the itaconation targets by number of the modification sites. (E) Biological process analysis of the targets with multiple itaconation sites.
Figure S9. The MS/MS spectra of the peptide containing ITalk modification on C63 of WIPI3, C218 of BRI3B, C239 of RIPK3, C334 of RIPK3 and C360 of RIPK3, respectively. The modified cysteines are labeled as red. The corresponding b and y ions are shown. The spectra were generated by the pFind software.1
Figure S10. OI exacerbates the necroptosis induced by TSZ. HT-29 cells were treated with 250 μM OI for 24 h. Then the cells were treated with 20 ng/mL TNF-α, 1 μg/mL SMAC mimic and 50 μg/mL zVAD-FMK for 3 h. (A) The cell viability was determined by MTS cell proliferation assay. (B) The cell viability was determined by CellTox™ Green Cytotoxicity Assay.

Figure S11. 1H NMR spectrum of ITalk (400 MHz, DMSO-d6).
Figure S12. $^{13}$C NMR spectrum of ITalk (400 MHz, DMSO-d6).

Figure S13. HRMS of ITalk
Supporting Information Methods

**Compound Synthesis.** The synthesis of OI and OMS was performed as previously reported³. For the synthesis of ITalk, itaconate anhydride (1.00 g, 8.92 mmol, 1.0 eq) and 7-Octyn-1-ol (1.18 g, 9.36 mmol, 1.05 eq) were heated to 110°C for 4h. After cooling to room temperature, the solution was poured slowly into hexane (30 mL) and the resulting solid filtered off and washed with cold hexane (10 mL). The solid was purified by silica chromatography column with mobile phase (5~6% methanol in methylene chloride) to afford ITalk as a white solid (1062 mg, 50%).

1H NMR (500 MHz, DMSO-d6) δ 12.62 (s, 1H), 6.15 (d, J = 1.5 Hz, 1H), 5.76 (d, J = 1.2 Hz, 1H), 4.00 (t, J = 6.6 Hz, 2H), 3.30 (s, 2H), 2.74 (s, 1H), 2.14 (d, J = 2.6 Hz, 2H), 1.58 – 1.49 (m, 2H), 1.43 (d, J = 7.1 Hz, 2H), 1.37 – 1.27 (m, 4H).

13C NMR (126 MHz, DMSO-d6) δ 170.95, 167.76, 135.36, 128.37, 84.98, 71.63, 64.50, 37.68, 28.43, 28.30, 28.20, 25.23, 18.07.

ESI MS: calcd. for C_{13}H_{19}O_4+ [M+H]+ m/z, 239.12833; found, 239.12772.

**Cell culture.** Raw264.7, HT-29 and A549 cells were obtained from ATCC and maintained in DMEM (Thermo Fisher Scientific) supplemented with 10% (vol/vol) dialyzed FBS (Thermo Fisher Scientific), 100 U/mL penicillin, and 100 mg/mL streptomycin in a humidified atmosphere at 37°C with 5% CO₂. BMDMs were prepared from 6- to 12-week-old C57BL/6J mice and cultured in RPMI-1640 medium supplemented with 10% (vol/vol) dialyzed fetal bovine serum (Thermo Fisher Scientific), 100 U/mL penicillin and 100 mg/mL streptomycin and 20 ng/mL mouse recombinant macrophage colony stimulating factor (315-02, Peprotech). Cortical neurons were prepared from postnatal day 0 at rat pups (strain Sprague-Dawley) and maintained as previously described³.

**Plasmids.** Full-length complementary DNAs of KEAP1, ALDOA, RB13B, LDHA, WIPI3 and RIPK3 were obtained from hORFeome Database and subcloned into a modified pCLHCX retroviral vector. The mutants C63S of WIPI3, C218S of BRI3B and mutants C239S/C334S/C360S of RIPK3 plasmid was generated by using the PCR-based site-directed mutagenesis with TransStart FastPFU DNA polymerase (Beijing TransGen Biotech Co., Ltd).

**Antibodies and Reagents.** The antibodies used for immunoblotting were rabbit monoclonal anti-RIP antibody (cat. no. ab202985, Abcam), rabbit monoclonal anti-RIP3 phospho S232 antibody (cat. no. ab195117, Abcam), rabbit monoclonal anti-MLKL phospho S345 antibody (cat. no. ab196436, Abcam), rabbit monoclonal anti-MLKL (cat. no. #37705, cell signaling), rabbit monoclonal Anti-DDB1 antibody (cat. no. ab109027, Abcam), rabbit monoclonal Anti-Gelsolin antibody (cat. no. ab109014, Abcam), rabbit monoclonal Anti-AK2 antibody (cat. no. ab40793, Abcam), rabbit monoclonal Anti-ATP citrate lyase antibody (cat. no. ab109014, Abcam), mouse monoclonal anti-His antibody (cat. no. HT501, Beijing TransGen Biotech Co., Ltd.) and mouse monoclonal anti-beta-actin antibody (cat. no. HC201, Beijing TransGen Biotech Co., Ltd.). Acid-cleavable azide-biotin tags (DADPS Biotin Azide, cat. no. 1330-5) and Rhodamine Azide (AZ109) were purchased from Click Chemistry Tools. Itaconate (cat.
no. I29204-100G), LPS (L2630-10MG) and methylsuccinic acid (cat. no. M81209-25g) were purchased from Sigma. Dimethyl itaconate (cat. no. BD125425) and 7-Octyn-1-ol (cat. no. BD110929) were purchased from Bidepharm. Itaconic anhydride (cat. no. B25216) was purchased from Alfa Aesar. TNF-α (cat. no. 410-MT-010) was purchased from R&D Systems. zVAD-FMK (cat. no. MB3313) and LCL-161 (SMAC mimetic, cat. no. MB4557) were purchased from Meilunbio.

**Reaction between ITalk and GSH.** 1 mM GSH and 1 mM ITalk were incubated in PBS buffer (pH 7.4) at 37 °C for 2 h, then subjected directly to LC-MS analysis. ITalk-GSH was detected with the mass of 546.21 and a retention time of 1.209 min. LC-MS conditions: flow rate: 0.3 mL/min; eluent: buffer A - H$_2$O (0.1% formic acid), buffer B - acetonitrile (0.1% formic acid); the gradient is gradually changed from 95% buffer A to 100% buffer B in 5 minutes.

**Quantification of GSH reactivity of itaconate derivatives.** 1 mM GSH and 1 mM itaconate derivatives (itaconate, ITalk, OI or DMI) were incubated in PBS buffer (pH 7.4) at 37 °C for 2 h. The samples were analyzed by LC-MS and GSH reactivity was quantified by calculating the peak areas of the remaining GSH in the reaction sample.

**Quantification of intracellular itaconate.** HEK293T cells were plated at 2 x 10$^6$ per well in 6-well plates overnight, and treated with 1mM ITalk or OI for 4 hours, respectively. The cells were washed with PBS gently in the plates for three times and collected by centrifugation. The cells were further washed with PBS for three times by centrifugation. The cell pallets were lysed by sonication in ice-cold PBS containing 0.1% TritonX-100, centrifuged at 20000 g for 30 min to remove cell debris, and protein concentrations were determined by BCA protein assay. After normalizing the protein concentration to 2 mg/ml of 100 µL, 900 µL cold methanol was added to extract the small molecule metabolites on ice. The mixture was incubated at -20 °C for 2 h and was centrifuged at 20000 g for 1 h at 4 °C. The supernatant was collected and analyzed by LC-SRM. The LC-SRM system is composed of an AB SCIEX 5500 triple-quadrupole mass spectrometer and a SHIMADZU DGU-20A liquid chromatography instrument with an Agilent column. The buffer gradient is 100%-0 Buffer A (100% water, 0.1% formic acid) and 0%-100% Buffer B (100% methanol, 0.1% formic acid) for 10 min. The absolute concentration of itaconate was calculated according to the standard curve of itaconate.

**In vitro proteins labeling by ITalk.** For the labeling of proteins in cell lysates, frozen Raw264.7 cells were resuspended in ice-cold PBS buffer containing EDTA-free Pierce Halt™ protease inhibitor cocktail. The cells were lysed by sonication in ice and cell lysates were collected by centrifugation (20,000 g, 30 min) at 4 °C to remove the debris. The protein concentration was determined by using the BCA protein assay kit (Pierce). 50 µL of cell lysates (2 mg/ml) were incubated with 100 µM ITalk at 37 °C for 1 h. The resulting lysates were precipitated by 200 µL methanol, 50 µL chloroform and 150 µL Milli-Q water. The precipitated proteins were centrifuged at 8000 g for 5 min at 4 °C and washed twice with 500 µL cold methanol. For visualizing the probe labeling efficiency by in-gel fluorescence, the precipitated proteins were resuspended in 50 µL PBS containing 0.4% SDS, 1 mM CuSO$_4$, 100 µM TBTA
ligand, 100 μM Rhodamine-azide, and 1 mM TCEP for 1 h at room temperature. The reacted samples were resolved on 10% SDS-PAGE gels and imaged by ChemiDoc XRS+ (Bio-Rad). The gels were then stained by Coomassie brilliant blue to demonstrate equal loading.

In situ proteins labeling by ITalk. For the labeling of itaconate targets in living cells, the Raw264.7 cells were grown to 80% confluence. The cells were treated with 100 μM OI or ITalk for 12 h. The cells were washed with PBS for three times and centrifuged at 1000 rpm for 3 min. The cell pellets were stored at -80 °C. The cell pellets were resuspended in ice-cold PBS buffer containing EDTA-free Pierce Halt™ protease inhibitor cocktail. The cells were lysed by sonication in the ice and cell lysates were collected by centrifugation (20,000 g, 30 min) at 4 °C to remove the debris. The protein concentration was determined by using the BCA protein assay kit. For visualizing the probe labeling efficiency by in-gel fluorescence, 50 μL of lysates (2 mg/mL) were mixed with 1 mM CuSO₄, 100 μM TBTA ligand, 100 μM Rhodamine-Azide, and 1 mM TCEP for 1 h at room temperature. The reacted samples were resolved on 10% SDS-PAGE gels and imaged by ChemiDoc XRS+ (Bio-Rad). The gels were then stained by coomassie brilliant blue to demonstrate equal loading.

Cell viability assays. For evaluation of the cell toxicity of ITalk, 10,000 Raw264.7 cells per well were seeded in 96-well dishes to grow overnight. Then the cells were treated with 100 μM ITalk or OI for 24 h. The cells were washed with pre-warmed PBS and incubated with serum-free medium containing MTS reagent (cat. no. G3582, Promega) for 2h. The absorbance at 490 nm was measured and cell viability under test conditions was reported as a percentage relative to the negative control treatment.

For evaluation of the impact of itaconate on necroptosis, 10,000 HT-29 cells per well were seeded in 96-well dishes to grow overnight. The cells were treated with 250 μM OI for 24 h and then treated with 20 ng/mL TNF-α, 1 μg/mL SMAC mimetic and 50 μg/mL zVAD-FMK for 3 h. The cell viability was determined by MTS as described above. For evaluating the cell viability by CellTox™ Green Cytotoxicity Assay (cat. no. G8741, Promega), the cells were treated with 100 µL of Green Reagent (2X) per well and incubated at r.t. for 1 h. The fluorescence at 485nm/520nm was measured for cell viability calculation.

Measurement of IL-1β production by enzyme-linked immunosorbent assay (ELISA). Raw264.7 cells were initially plated overnight in 6 cm dish. The cells were treated with 10 ng/mL LPS and 1 mM itaconate or 100 μM ITalk or 100 μM OI for 24 h. Afterwards, the condition media were collected and cytokine production was assayed using ELISA kits for IL-1β (cat. no. SEA563Mu) purchased from Cloud-Clone, according to manufacturer’s instructions.

Glucose consumption and lactate production. Raw264.7 cells were cultured in 96 wells overnight. The cells were treated with 10 ng/mL LPS and 1 mM itaconate or 100 μM ITalk or 100 μM OI for 24 h. Glucose consumption in the culture medium were measured using the Glucose (GO) Assay Kit (cat. no. KA4088, Abnova) whereas lactate levels in the culture medium were determined using a Lactate Assay Kit (cat. no. 700510, Cayman).
Quantitative profiling of itaconation targets by ITalk. For the identification of itaconation targets by ITalk, Raw264.7 cells were grown to 80% confluence in 15 cm dish. Raw264.7 cells were treated with 10 ng/mL LPS and 100 μM OI or ITalk for 12 h. The cells were collected by PBS wash for three times and centrifugation at 1000 rpm for 3 min. The cells were lysed in 1 mL ice-cold PBS buffer containing EDTA-free Pierce Halt™ protease inhibitor cocktail with sonication. The cell lysates were collected by centrifugation (20,000 g, 30 min) at 4 °C to remove the debris. The protein concentration was determined by using the BCA protein assay kit. 1 mL cell lysates (2 mg/mL) were reacted with 1 mM CuSO₄, 100 μM TBTA ligand, 100 μM azide-biotin, and 1 mM TCEP for 1 h at room temperature. The resulting click-labeled lysates were centrifuged at 8000 g for 5 min at 4 °C and washed twice with 1 mL cold methanol.

The proteins were resuspended in 1 mL PBS containing 1.2% SDS. 100 μL streptavidin beads (Thermo Fisher Scientific) were washed for three times with 1 mL PBS, and resuspended in 5 mL PBS, which was added to the protein solution. The resulting solution was incubated for 4 h at 29 °C, followed by washing with 5 mL PBS for three times, and 5 mL distilled water for three times. The resulting beads were resuspended in 500 μL PBS containing 6 M urea and 10 mM DTT and incubated at 65 °C for 15 min, followed by addition of 20 mM iodoacetamide for 30 min at 35 °C in the dark. The beads were then collected by centrifugation and resuspended in 200 μL PBS containing 2 M urea, 1 mM CaCl₂ and 10 ng/uL trypsin (Promega). Trypsin digestion was performed at 37 °C with rotation overnight and the beads were washed with 200 μl distilled water for three times.

For dimethyl labeling, per 100 μL peptides were reacted with 4 μL of 4% “light” formaldehyde (Sigma) and “heavy” formaldehyde (¹³CD₂O) (Sigma), respectively. The resulting solution were treated with 4 μL 0.6M sodium cyanoborohydride (Sigma) and incubated at r.t. for 1 h. The reaction was quenched by adding 16 μL 1% ammonia and 8 μL 5% formic acid. The “light” and “heavy” samples were combined and subjected for fractionation. The peptides were separated into 20 fractions by a high-pH reverse phase C18 column (Agela Technologies) using Agilent HPLC system. Mobile phase A: 2% ACN–98% H₂O (adjusted to pH 10 with NH₃·H₂O); B: 98% ACN–2% H₂O (adjusted to pH 10 with NH₃·H₂O). Samples were separated using a 20 min gradient of buffer B at a flow rate of 1.5 mL/min, as follows: 0 min 0% B; 0.1 min 5% B; 1 min 8% B; 7 min 14% B; 12 min 24% B; 16 min 40% B; 18 min 95% B; 20 min 15% B. The columns were operated at 25 °C and the temperature was controlled by a built-in column heater. The 20 fractions were combined into 12 fractions and dried in a SpeedVac.

Time-resolved chemoproteomics for identification of sensitive itaconation targets. For the time-resolved chemoproteomic profiling, Raw264.7 cells were grown to 80% confluence in 15 cm dish. The cells were treated with 10 ng/mL LPS and 100 μM ITalk for 1 or 10 h, respectively. The procedures for cell lysis, click reaction, streptavidin enrichment and trypsin digestion were performed as described above. For dimethyl labeling, the samples from 1 h treatment were labeled as “light” while those from 10 h treatment were labeled as “heavy”. The data analysis was performed as described above.

Identification of itaconation sites by ITalk. For the identification of itaconation sites by ITalk, Raw264.7 cells were grown to 80% confluence in 15 cm dish. Raw264.7 cells were treated with
10 ng/mL LPS and 100 μM ITalk for 12 h. The cells were collected by PBS wash for three times and centrifugation at 1000 rpm for 3 min. The cells were lysed in 1 mL ice-cold PBS buffer containing EDTA-free Pierce Halt™ protease inhibitor cocktail with sonication. The cell lysates were collected by centrifugation (20,000 g, 30 min) at 4 °C to remove the debris. The protein concentration was determined by using the BCA protein assay kit. 1 mL cell lysates (2 mg/mL) were reacted with 1 mM CuSO₄, 100 μM TBTA ligand, 100 μM acid-cleavable azide-biotin (DADPS Biotin Azide, cat. no. 1330-5), and 1 mM TCEP for 1 h at room temperature. The resulting click-labeled lysates were centrifuged at 8000 g for 5 min at 4 °C and washed twice with 1 mL cold methanol. The streptavidin enrichment and on-beads Trypsin digestion were performed as described above. Release of the modified peptides from the beads was carried out by incubating the beads with 200 μL of 2% formic acid/water for 1 h with gentle rotation. After centrifugation, the supernatant was collected. Then the cleavage process was repeated and supernatant was combined. In addition, the beads were washed with 50% acetonitrile/water containing 1% formic acid (400 μL), and the washes were combined with the supernatant to form the cleavage fraction. Sample was dried in a vacuum centrifuge and stored at -30 °C until analysis.

**LC-MS/MS analysis.** Samples were analyzed by LC-MS/MS on Q Exactive series Orbitrap mass spectrometers (Thermo Fisher Scientific) coupled with EasyNano-LC. Under the positive-ion mode, full-scan mass spectra were acquired over the m/z range from 350 to 1800 using the Orbitrap mass analyzer with mass resolution of 70000. MS/MS fragmentation is performed in a data-dependent mode, of which the TOP 20 most intense ions are selected for MS/MS analysis with a resolution of 17500 using collision mode of HCD. Other important parameters: isolation window, 2.0 m/z units; default charge, 2+; normalized collision energy, 28%; maximum IT, 50 ms; and dynamic exclusion, 20.0 s.

**Data analysis.** For the dimethyl labeling-based experiments, LC-MS/MS data was analyzed by ProLuCID with static modification of cysteine (+57.0215 Da). The isotopic modifications (28.0313 and 34.0631 Da for light and heavy labeling, respectively) are set as static modifications on the N-terminal of a peptide and lysines. The ratios of reductive dimethylation were quantified by the CIMAGE software as described previously⁴. For the identification of itaconation sites, LC-MS/MS data was analyzed by ProLuCID with static modification of cysteine (+57.0215 Da) as well as variable modifications of +325.16378 Da on cysteine and +382.18524 Da on lysine or histidine.

**Statistical analysis.** Results are expressed as mean ± s.d. Fold change in relation to control groups of three independent cell culture and subsequent procedures. Student’s t-test was used to compare experimental data. We analyzed the data in GraphPad Prism (GraphPad Software), using the unpaired, two-tailed t-test module. Statistical significance was considered when a p value was below 0.05. *p < 0.05; **p < 0.01; ***p < 0.001. N.S. not significant.

**Data availability.** The data that support the findings of this study are available from the corresponding authors on reasonable request.
Supporting Information Reference: