
Supplementary information

**Membranolytic peptide programs
immunogenic cell death for cancer therapy**

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Membranolytic peptide programs immunogenic cell death for cancer therapy

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Supplementary Materials and Methods

Materials. L-Glutamic acid was purchased from GL Biochem (Shanghai) Ltd. (China). 3-Butenyl alcohol, triphosgene, cysteamine hydrochloride, maleic anhydride, citraconic anhydride (CA), *cis*-aconitic anhydride (AA), 3,4,5,6-tetrahydrophthalic anhydride (TA), 2,3-dimethylmaleic anhydride (DA), and 1,1'-(1,4-phenylenebis[methylene]) bis-pyridinium dibromide (DPX) were purchased from Tokyo Chemical Industry (Japan). 8-Aminonaphthalene-1,3,6-trisulfonic acid disodium salt (ANTS) was purchased from Shanghai Macklin Biochemical Co., Ltd. 1-Hexadecylamine, dodecylamine, hexylamine, 2,2-dimethoxy-2-phenylacetophenone, and propidium iodide (PI) were purchased from Sigma-Aldrich (USA). Anhydrous ethyl acetate (EtOAc), tetrahydrofuran (THF), and hexane were dried by passing through the solvent purification system (Vigor, China) which was connected to a glovebox (Vigor). 1,2-Dioleoyl-*sn*-glycero-3-phosphocholine (DOPC), 1,2-dioleoyl-*sn*-glycero-3-phospho-L-serine, sodium salt (DOPS) and phosphatidylinositol (Pdl) were purchased from AVT (Shanghai) Pharmaceutical Co., Ltd. (China). Roswell Park Memorial Institute (RPMI) 1640 Medium, Dulbecco's modified Eagle medium (DMEM), FastDigest XbaI, FastDigest BamHI, DRAQ7 dye, BODIPY 581/591 C11, LysoTracker Green, CellMask Deep Red stain and β -mercaptoethanol were purchased from Thermo Fisher Scientific (USA). Fetal bovine serum (FBS) was purchased from ExCell Bio (China) or Gibco (USA). Penicillin–streptomycin solution (100 $\times$), trypsin–EDTA solution containing phenol red, 4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid (HEPES, 100 $\times$), MEM nonessential amino acids (100 $\times$), sodium pyruvate (100 $\times$), 4',6-diamidino-2-Phenylindole (DAPI), 2'-(4-ethoxyphenyl)-5-(4-methyl-1-piperazinyl)-2,5'-bis-1H-benzimidazole trihydrochloride trihydrate (Hoechst 33342) and phosphatase inhibitor cocktail were purchased from Beyotime Biotechnology Co., Ltd. (China). Thiazolyl blue tetrazolium bromide (MTT), Cell Counting Kit-8 (CCK-8), doxorubicin hydrochloride (DOX), and mitoxantrone hydrochloride (MTX) were purchased from Meilunbio (China). Polybrene, fluorescein isothiocyanate isomer I (FITC), and ferroceneacetic acid were purchased from Shanghai Macklin Biochemical Co., Ltd. (China). Cycloheximide (CHX) and protease inhibitor cocktail were

purchased from Selleck (USA). Puromycin dihydrochloride hydrate was obtained from BBI Life Sciences (China). 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), collagenase type IV, hyaluronidase and DNase I were purchased from Diamond (China). Tumour necrosis factor alpha (TNF- α , mouse), RSL3, Gly-Phe- β -naphthylamide (GPN), polyethyleneimine (PEI) transfection reagent were purchased from MedChemExpress (MCE, USA). T4 DNA ligase was purchased from New England Biolabs (USA). RNAiso, PrimeScript RT Master Mix, and TB Green Premix Ex Taq™ II were purchased from Takara Biomedical Technology (Japan). Cy5-NHS was purchased from Lumifore Biotech Co., Ltd. (China). GM-CSF, IL-4 and IL-2 were purchased from PeproTech (USA).

Antibodies. Anti-RIPK1 (Cell Signaling Technology, 3493, 1:1000), anti-p-RIPK1 (Ser166) (Cell Signaling Technology, 53286, 1:1000), anti-RIPK3 (Cell Signaling Technology, 95702, 1:1000), anti-p-RIPK3 (Thr231/Ser232) (Cell Signaling Technology, 91702, 1:1000), anti-MLKL (Cell Signaling Technology, 37705, 1:1000), anti-p-MLKL (S345) (Abcam, ab196436, 1:1000), anti-GSDME (Abcam, ab215191, 1:1000), anti-GSDMD (Abcam, ab209845, 1:1000), anti-PARP1 (Abcam, ab191217, 1:1000), anti-caspase-3 (Cell Signaling Technology, 9662, 1:1000), anti-LC3 (Proteintech, 14600-1-AP, 1:2000), anti-SQSTM1/p62 (Abcam, ab109012, 1:10000), anti-GAPDH (Proteintech, 60004-1-Ig, 1:5000), anti- β -actin (Proteintech, 66009-1-Ig, 1:5000), anti- α -tubulin (Proteintech, 11224-1-AP, 1:5000), anti-HMGB1 (Abcam, ab79823, 1:10000), anti-NINJ1 (Thermo Fisher Scientific, PA5-95755, 1:500), anti-CD3 (Cell Signaling Technology, 78588S, 1:200), anti-CD31 (Abcam, ab182981, 1:500), Alexa Fluor 555 Goat Anti-Rabbit IgG (H+L) (Jackson, 111-565-144, 1:100).

Inhibitors. Methyl- β -cyclodextrin (m β CD), 2-deoxy-D-glucose (2-DG), and chlorpromazine hydrochloride (CPZ) were purchased from Shanghai Macklin Biochemical Co., Ltd. (China). Chloroquine diphosphate (CQ) was purchased from Sigma-Aldrich (USA). The following inhibitors were purchased from MedChemExpress (MCE, USA): wortmannin (WMN); lysosomal acidification inhibitors (LAIs): Bafilomycin A1 (BafA), concanamycin A (CMA); lysosomal enzyme

inhibitors (LEIs): cathepsin B inhibitor CA-074 methyl ester (CA-074-Me), aloxistatin (E-64d), pepstatin; inhibitors for necroptosis: necrostatin-1 (Nec-1), GSK-872, GSK-840, necrosulfonamide (NSA), and GW806742X hydrochloride (GW806742); inhibitors for pyroptosis: VX-765, disulfiram (DSF), dimethyl fumarate (DMF), methylcobalamin (MeCbl), and Z-VAD-FMK; inhibitors for ferroptosis: N-acetyl-L-cysteine (NAC), deferoxamine (DFO), liproxstatin-1 (Lip-1), ferrostatin-1 (Fer-1); and inhibitors for autophagy: MRT68921 dihydrochloride (MRT), SBI-0206965 (SBI), Spautin-1.

Characterisation. The proton nuclear magnetic resonance (^1H -NMR) spectra of the peptides were recorded using Bruker Magnet System 400'54 Ascend spectrometer at 400 MHz (Bruker, Germany) using deuterated chloroform (CDCl_3) or deuterium oxide (D_2O) as the solvent. The total correlation spectroscopy (TOCSY) NMR spectra of the peptides were recorded using Bruker AVANCE NEO spectrometer at 600 MHz (Bruker, Germany) using deuterated methanol (CD_3OD) and D_2O as solvents. MestReNova (Version 12.0.0-20080, Spain) was used for the analysis of NMR data. The conformation of peptides was detected on circular dichroism (CD) spectrometer (Applied Photophysics Ltd., UK) using a quartz cell with an optical path length of 0.05 cm. The size and zeta potential of $\text{aMP}_{\text{C16-CA50}}$ (0.08 mg ml^{-1}) at different pH ($0.1 \times$ phosphate-buffered saline, PBS) were determined via dynamic light scattering (DLS) using a Zetasizer system (Malvern Panalytical Technologies Corporation, UK). Sections for TEM were cut with an ultramicrotome (Leica EM UC7; Leica, Germany). Morphological analysis by transmission electron microscopy (TEM) was performed using a Tecnai G2 Spirit (FEI, the Netherlands) and a Talos L120C (Thermo Fisher Scientific, USA), both at an accelerating voltage of 120 kV. Fluorescence and absorbance were measured using Infinite M Plex microplate spectrophotometer (TECAN, Switzerland). Confocal microscopy imaging was carried out using an IXplore SpinSR spinning-disk confocal microscope (Olympus, Japan). The biochemical indices were detected using an automatic biochemistry analyzer (Hitachi Automatic Analyzer 3100, Japan). Flow cytometry analysis was performed using BD LSRFortessa, BD

FACSCelesta, or BD FACSCanto II flow cytometers (BD Biosciences, USA), and the results were analyzed using FlowJo v10 software (FlowJo, USA). Isothermal titration calorimetry (ITC) assays were performed using a MicroCal PEAQ-ITC system (Malvern Panalytical, UK). Western blot images were captured with a ChemiDoc imaging system (Bio-Rad, USA) and analyzed using Image Lab. H&E images were acquired using a KF-FL-005 digital pathology scanner (KFBIO, China) and evaluated via CaseViewer software (v2.1; 3DHISTECH, Hungary). Immunofluorescence images were acquired using a TissueFAXS imaging system (TissueGnostics, Austria) and quantified using HALO software (Indica Labs, USA). Bioluminescence was detected using an IVIS Lumina imaging system (PerkinElmer, USA) and analyzed with Living Image software (v4.4).

Simulations. The initial configurations of the helical peptides were constructed by attaching the synthesized side chains onto the backbones using the code developed in our laboratory. The Bax Group PDB Server (<http://spin.niddk.nih.gov/bax/nmrserver/pdbutil>) was employed for constructing the backbones of helical peptides, and the Automated Topology Builder server (<https://atb.uq.edu.au/>)⁶¹⁻⁶³ was used for generating the side-chain coordinates. The peptides were modeled using the GROMOS 54A7 united-atom force field⁶⁴. Partial charges for the backbone atoms were assigned from the standard GROMOS 54A7 force field, while those for the side chains were calculated using GAMESS-US at a B3LYP/6-31* level of theory⁶⁵.

After preparation, the helical peptide MP_{C16} was immersed in a water box (6.2 nm × 6.2 nm × 9.0 nm) containing NaCl (~200.0 mM) to maintain charge neutrality using PACKMOL⁶⁶. The prepared system was first energy minimized using the steepest descent method for 5,000 steps to remove high-energy overlaps in the initial solvated peptide system followed by a short relaxation of 1 ns under the NVT ensemble, with the peptide backbone being fixed. Subsequently, a steered molecular dynamics (MD) simulation was carried out by applying an artificial stretching potential onto the terminal Ca atoms to produce an elongated configuration. Considering that the energy

landscape of peptide folding is extremely complicated and that several trapped states could potentially exist on the landscape, an incompletely elongated extended configuration was employed as the starting structure for further simulations. This configuration was relaxed for 5 ns using the NPT ensemble, with the terminal Ca atoms restrained with a force constant of $1,000 \text{ kJ mol}^{-1} \text{ nm}^{-2}$. A 100-ns free MD simulation using the NPT ensemble was then performed to examine the stability of the elongated structure and the propensity of the peptide to refold from an unstructured coil structure to the helical configuration. The SPC water model⁶⁷ was employed in the present study. Periodic boundary conditions were applied, and the Particle Mesh Ewald method was employed for long-range electrostatic interactions⁶⁸. Coulomb and Van der Waals interactions were truncated at 1.0 nm. The LINCS algorithm was used for constraining the bonds involving hydrogen atoms in both the solvent and the solute⁶⁹. Product simulations were conducted in the NPT ensemble at 298 K, and Parrinello–Rahman pressure coupling schemes⁷⁰ were used with a reference pressure of 1 atm. Temperature regulation was accomplished using the V-rescale thermostat with a relaxation time constant of 0.1 ps⁷¹. Equations of motion were numerically integrated using a leap-frog algorithm with a 2.0 fs time step⁷². All simulations were performed using the GROMACS (V2020) program package⁷³, and the software package VMD was employed for trajectory visualization and analysis⁷⁴.

Evaluation of dye leakage from liposomes. The liposome leakage assay using MPs was conducted according to a modified previously reported procedure⁷⁵, utilizing the liposome-encapsulated fluorescent probe ANTS and quencher DPX. A lipid film was prepared using a mixture of lipids, with DOPC and DOPS at a molar ratio of 9:1. The film was hydrated with a dye solution (10 mM Na_2HPO_4 buffer) containing DPX and ANTS (19.0 and 5.35 mg ml^{-1} , respectively). The dispersion was subjected to 10 cycles of freeze–thaw processes using liquid nitrogen and a water bath maintained at 37°C and subsequently extruded 20 times through a polycarbonate membrane filter (400 nm) using a liposome extruder (Hamilton Company, USA). Finally, liposome purification was accomplished *via* gel filtration using Sephadex G50 column (Beijing Separation

Technology Co., Ltd., China). The MPs, including MP_{C16}, aMP_{C16}-DA₅₀, aMP_{C16}-TA₅₀, aMP_{C16}-CA₃₀, aMP_{C16}-CA₅₀, aMP_{C16}-CA₇₀, aMP_{C16}-AA₅₀, aMP_{C12}-CA₅₀, aMP_{C6}-CA₅₀, and iMP, were preincubated under different pH conditions before use, and then incubated with dye-encapsulated liposomes in equal volumes to obtain a final concentration of 160 µg ml⁻¹ of MPs. Following 1 h of incubation, dye leakage was monitored using a microplate reader with excitation and emission wavelengths of 360 and 530 nm, respectively. Liposome treated with buffers served as negative controls and those incubated with 0.1% Triton X-100 were employed as the positive controls (considered as 100% dye leakage). The percentage of dye leakage for each sample was calculated using the formula $[(F_{\text{sample}} - F_{\text{negative control}})/(F_{\text{positive control}} - F_{\text{negative control}})] \times 100\%$.

Dye leakage assay from giant unilamellar vesicles (GUVs). GUVs were prepared as previously reported procedures with modifications⁷⁶⁻⁷⁸. Briefly, solutions of the lipids DOPC and DOPS (5 mg ml⁻¹) in chloroform were mixed at a volumetric ratio of 19:1. The lipid mixture was gently spread onto the conductive surface of indium tin oxide (ITO) electrodes, and then dried under vacuum for 2 h to remove chloroform. To create the setup, a round silicone frame was placed between two lipid film-coated ITO electrodes. The chamber was gently filled with sucrose solution (100 mM) containing calcein (80 µM). A sinusoidal AC electric field (4.5 V, 100 Hz) was applied for 2 h to induce GUV formation. The GUV-containing solution was collected, diluted 20-fold with glucose solution (120 mM), and stained with CellMask Deep Red (1:1000; Thermo Fisher) to obtain GUVs containing lipid membranes stained with CellMask Deep Red and the lumen with calcein. Subsequently, the MPs that were preincubated overnight under different pH conditions were added to the GUV-containing solution to obtain a final concentration of 20 µg ml⁻¹ of MPs. Calcein dye leakage from GUVs was monitored for 30 min after the addition of MPs using the 40× objective lens of the IXplore SpinSR spinning-disk confocal microscope. The images presented herein depict either calcein dye leakage from the GUV, captured when the CellMask Deep Red-stained membrane morphology was still visible, or the calcein-loaded GUVs

(without any dye leakage) after 30 min of incubation with the MPs.

Synthesis of FITC-, Cy5- and ferrocene-labelled peptides. MP_{C16} and FITC (1 equivalent of MP_{C16}) were dissolved in DMSO, and the mixture was stirred at room temperature for 12 h. MP_{C16}-FITC was purified by dialysis with molecular weight cut-off (MWCO) of 1 kDa against distilled water for 24 h followed by lyophilization. aMP_{C16}-CA₅₀-FITC was synthesized by mixing MP_{C16}-FITC with citraconic anhydride and triethylamine (5 and 10 equivalents relative to MP_{C16}-FITC, respectively) in DMSO. The mixture was stirred at room temperature for 12 h. aMP_{C16}-CA₅₀-FITC was obtained upon dialysis (MWCO = 1 kDa) against distilled water for 24 h followed by lyophilization. Other FITC-labelled aMPs were synthesized using a similar procedure with 3,4,5,6-tetrahydrophthalic anhydride or 2,3-dimethylmaleic anhydride as the starting materials. And Cy5-labelled aMPs were synthesized in a similar procedure with Cy5-NHS as the labelling reagent.

For the synthesis of ferrocene-labelled aMP_{C16}-CA₅₀ (aMP_{C16}-CA₅₀-Fe), ferrocenylacetic acid, EDCI, and NHS (1, 1.1, and 1.1 equivalents relative to MP_{C16}, respectively) were dissolved in methanol, and the mixture was stirred at room temperature for 4 h. MP_{C16} was dissolved in DMF and added to the reaction mixture, followed by stirring at room temperature for 24 h. Ferrocene-labelled MP_{C16} (MP_{C16}-Fe) was obtained by dialysis (MWCO = 14 kDa) against distilled water for 24 h, followed by lyophilization. aMP_{C16}-CA₅₀-Fe was synthesized by mixing MP_{C16}-Fe with citraconic anhydride and triethylamine (5 and 10 equivalents of MP_{C16}-Fe, respectively) in DMSO. The mixture was stirred at room temperature for 12 h. aMP_{C16}-CA₅₀-Fe was obtained upon dialysis (MWCO = 1 kDa) against distilled water for 24 h followed by lyophilization.

Cell culture. The MC38, Panc02, EMT6, SKOV3 and LoVo cells were sourced from the National Collection of Authenticated Cell Cultures, Chinese Academy of Sciences (Shanghai, China). B16-F10, HEK293T and HeLa cells were sourced from the American Type Culture Collection (ATCC). A549 and L929 cells were sourced from Qiansheng Biotechnology (Guangzhou, China) and iCell Bioscience (Shanghai, China),

respectively. EO771 and MB49 cells were kindly provided by Prof. Jianing Chen and Prof. Bo Wang, respectively (both from Sun Yat-sen Memorial Hospital, Sun Yat-sen University, China). HepG2 and U2OS cells were kindly provided by Prof. Hongrui Wang (Xiamen University, China). MC38-OVA and A549-NY-ESO-1 cells were established and kindly provided by Prof. Xiaojun Xia and Prof. Penghui Zhou, respectively, from Sun Yat-sen University Cancer Center (SYSUCC, China). The cells were cultured in DMEM or RPMI 1640 media supplemented with 10% FBS and penicillin–streptomycin, and maintained at 37°C under conditions of 5% CO₂. Media at pH 7.4 or 6.8 were prepared by dissolving DMEM powder (13.37 g l⁻¹) and HEPES (5.96 g l⁻¹) in ultrapure water obtained from a Millipore water purification system, followed by pH adjustment using hydrochloric acid (6 M) or sodium hydroxide (5 M).

Construction of fluorescent-tagged proteins expressing cells. The expression vector for green fluorescent protein (GFP)-tagged Galectin-3 (Gal3-GFP) was constructed by amplifying the GFP coding sequence (CDS) *via* PCR using the primers 5'-CCTACTCTAGAATGGTGAGCAAGGGCGAGGAG-3' and 5'-GCCATGAATTCCTTGTACAGCTCGTCCATGCCG-3'; the Galectin3v1 CDS was amplified using the primers 5'-ACAAGGAATTCATGGCAGACAATTTTCGCTCC-3' and 5'-GCCGCGGATCCTTATATCATGGTATATGAAGCAC-3'. Subsequently, the GFP and Galectin3v1 amplicons were inserted into the XbaI–EcoRI and EcoRI–BamHI restriction sites, respectively, of the pCDH-EF1-IRES-Puro vector followed by ligation using T4 DNA ligase.

The expression vector for mCherry-tagged lysosomal-associated membrane protein 1 (LAMP1-mCherry) was constructed by amplifying mCherry CDS *via* PCR using the primers 5'-CCTACTCTAGAATGGTGAGCAAGGGCGAGGAGGAC-3' and 5'-GCCATGGATCCTTACTTGTACAGCTCGTCCATGCCG-3'; the LAMP1 CDS was amplified using the primers 5'-ACAAGGAATTCATGGCGGCCCCCGGCAGCGCC-3' and 5'-GCCGCGGATCCTTAGATAGTCTGGTAGCCTGCGTG-3'. Subsequently, the mCherry and LAMP1 amplicons were inserted into the XbaI–EcoRI and EcoRI–BamHI restriction sites, respectively, of the pCDH-EF1-IRES-Puro vector followed by

ligation using T4 DNA ligase.

The expression plasmid for mCherry-tagged Cathepsin B (CTSB-mCherry) was constructed by amplifying the CTSB CDS *via* PCR using the primers 5'-CCTACTCTAGAATGTGGCAGCTCTGGGCCTCCC-3' and 5'-ACCATCTCGAGGATCTTTTCCCAGTACTGATC-3', while the mCherry CDS was amplified using the primers 5'-AGATCCTCGAGATGGTGAGCAAGGGCGAGGAG-3' and 5'-GCCGCGGATCCTTACTTGTACAGCTCGTCC-3'. Both amplicons were subsequently inserted into the pCDH-EF1-IRES-Puro vector by XbaI–XhoI and XhoI–BamHI restriction sites followed by ligation using T4 DNA ligase.

The expression plasmid for EYFP and luciferase containing the T2A peptide linker (EYFP-T2A-luciferase) was constructed by amplifying EYFP CDS *via* PCR using the primers 5'-GCCTACTCTAGAATGGTGAGCAAGGGCGAGGAGC-3' and 5'-CTCGACGTCACCGCATGTTAGCAGACTTCCTCTGCCCTCCTTGTACAGCTC GTCCATGC-3'; the luciferase CDS was amplified using the primers 5'-TGCTAACATGCGGTGACGTCGAGGAGAATCCTGGCCCAATGGAAGACGCC AAAAACA-3' and 5'-GCCGCGGATCCTTACACGGCGATCTTTCCGCCCTTC-3'. Both the products were ligated *via* overlap extension PCR and subsequently inserted into the pCDH-EF1-IRES-Puro vector by XbaI–BamHI sites followed by ligation with T4 DNA ligase.

The expression plasmid encoding chicken ovalbumin (OVA) was constructed by amplifying the OVA CDS *via* PCR using the following primers: 5'-CCTACTCTAGAATGGGCTCCATCGGTGCAGCAAG-3' and 5'-GCCGCGGATCCTTAAGGGGAAACACATCTGCC-3'. The amplicon was subsequently inserted into the pCDH-EF1-IRES-Puro vector by XbaI–BamHI sites followed by ligation using T4 DNA ligase.

HEK293T cells were transiently transfected with the expression plasmids together with the viral packaging plasmids psPAX2 and pMD2.G. The plasmids for Gal3-GFP, LAMP1-mCherry, CTSB-mCherry, and OVA were transfected using the calcium

phosphate method, whereas the EYFP-T2A-luciferase plasmid was transfected using Lipofectamine 3000. The supernatants containing the recombinant lentiviruses were collected and added to the cultures of MC38, Panc02, EO771, and A549 cells in the presence of polybrene ($10 \mu\text{g ml}^{-1}$). Limited dilution method and puromycin ($2 \mu\text{g ml}^{-1}$) selection were employed for the selection of stable clones.

The pCDH-CMV-mCherry-GFP-Homo-LC3B-EF1-Puro viral solution was purchased from Guangzhou IGE Biotechnology Co., Ltd. MC38 cells were seeded at a density of 0.5×10^4 cells per well in a 96-well plate. An appropriate amount of viral solution was added at a multiplicity of infection (MOI) of 10, based on a cell-to-virus ratio of 1:10. Polybrene was also added to a final concentration of $8 \mu\text{g ml}^{-1}$. After 6 h of infection, the medium was replaced with high-glucose DMEM containing 10% FBS. At 48 h after the medium change, puromycin was added at a concentration of $8 \mu\text{g ml}^{-1}$ to obtain stably transduced cells. Limiting dilution was performed to isolate monoclonal colonies with uniform fluorescence, and the MC38_{mCherry-GFP-LC3} cell line was successfully established.

MC38 cells expressing OVA (MC38_{OVA}) was a kind gift from Prof. Xiaojun Xia of Sun Yat-sen University Cancer Center (China). The NY-ESO-1-specific TCR-T cells and NY-ESO-1-expressing A549 (A549_{NY-ESO-1}) cells were obtained from Prof. Penghui Zhou of Sun Yat-sen University Cancer Center (China). The MC38_{GFP/mCherry} cell line, which stably expresses MyrPalm-GFP on the cell membrane and mCherry in the cytoplasm, was generated as described previously by our group²⁹.

Generation of CRISPR–Cas9 knockout cell lines. To generate the indicated knockout cell lines using the CRISPR-Cas9 system, gene-specific sgRNA sequences were cloned into the lentiCRISPRv2 vector. The sgRNA targeting sequences were as follows: *Mkl1*, 5'-GCCCAAATATACGCAAGATGT-3'; *Gsdme*, 5'-GCGGGGCTATTGGGACAGTCG-3'; *Nin1*, 5'-GCAAGCCGTCGCGGGAACCGG-3'. A non-targeting sgRNA was used as the control (sgNTC: GCGCGATAGCGCAATATAC). The plasmids for *Mkl1*, *Gsdme*, and *Nin1* knockout, as well as the control plasmid, were purchased from Guangzhou IGE Biotechnology

Co., Ltd. HEK293T cells were transiently transfected with the expression plasmids along with the viral packaging plasmids psPAX2 and pMD2.G using polyethylenimine (PEI). The supernatants containing recombinant lentiviruses were collected and added to the cultures of MC38 cells in the presence of polybrene ($8 \mu\text{g ml}^{-1}$). At 8 h after infection, the medium was replaced with high-glucose DMEM containing 10% FBS. 48 h after the medium change, puromycin was added at a concentration of $8 \mu\text{g ml}^{-1}$. After puromycin selection, stable knockout cell lines were established either directly from bulk cell populations or via single-cell clonal selection. The efficiency of gene deletion was assessed by western blotting.

Assay to evaluate the cellular uptake of aMP_{C16}-CA₅₀. Tumour cells were seeded into 24-well plates (at a density of 2×10^5 cells/well) and incubated overnight at 37°C . The culture medium was replaced with fresh medium of pH 7.4 or 6.8 containing different concentrations of FITC-labelled aMP_{C16}-CA₅₀. The cells were incubated at 37°C for the indicated time periods. Subsequently, the cells were washed, trypsinized, and harvested to prepare single-cell suspensions for flow cytometry analysis using a BD FACSCanto II flow cytometer.

Cytotoxicity assay. The cells were seeded into 96-well plates (at a density of 10,000–15,000 cells/well) and incubated overnight at 37°C . The medium was replaced with fresh medium of pH 7.4 or 6.8 containing different concentrations of the aMPs. At the predetermined time points, the cell viability subjected to the various treatments was evaluated by following the standard procedures for MTT or CCK-8 assays and measuring absorbance at 490 or 450 nm, respectively. The cell culture samples incubated with media of the corresponding pH but without any added aMPs were employed as the control representing 100% survival rate, while the wells without cells were set as the blank. The percentage of cell viability was calculated using the formula $(A_{\text{sample}} - A_{\text{blank}})/(A_{\text{control}} - A_{\text{blank}}) \times 100\%$.

The effect of low temperature on the cytotoxic effects of aMPs was evaluated by incubating the cells at 4°C . The effect of inhibition of endocytosis on the cytotoxicity of aMPs was evaluated by pretreating the tumour cells with the following endocytosis

inhibitors for 30 min at 37°C: methyl- β -cyclodextrin (a lipid raft-mediated endocytosis inhibitor, 5 μ M), wortmannin (a macropinocytosis inhibitor, 10 nM), chlorpromazine (an inhibitor of clathrin-mediated endocytosis, 6 μ M), and 2-deoxy-D-glucose (an inhibitor of energy-dependent endocytosis, 20 mM). The cells were subsequently incubated with aMPs in the continued presence of the respective endocytosis inhibitors.

The role of lysosomes in mediating the cytotoxic effects of aMPs was evaluated by the coincubation of tumour cells with aMPs and the inhibitors of lysosomal enzymes (CA-074-Me, the broad-spectrum cysteine protease inhibitor alocistatin (E-64d), and an inhibitor of aspartyl proteases pepstatin A) or lysosomal acidification (CQ, CMA, and BafA).

Analysis of released intracellular contents after treatment with MPs. MC38 cells were seeded into 24-well plates (at a density of 2×10^5 cells/well) and incubated overnight. The cells were then treated with MPs ($160 \mu\text{g ml}^{-1}$) at pH 6.8 in DMEM medium without FBS and incubated at 37°C. At the indicated time points, the culture supernatants were collected by centrifuging at $400 \times g$ for 5 min and used for subsequent analysis. The released intracellular proteins in the supernatants were separated using sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) on 12% gels followed by silver staining to visualize the molecular weight range of the proteins. The Fast Silver Stain Kit (Beyotime, China) was utilized according to the manufacturer’s protocol. Released lactate dehydrogenase (LDH) was quantified using an LDH test kit (Beyotime, China) following the manufacturer’s instructions. The culture supernatants from untreated cells and the cells treated with LDH release reagent were employed as negative and positive (corresponding to 100% LDH leakage) controls, respectively. The percentage of LDH released was calculated as $([A490_{\text{sample}} - A490_{\text{negative control}}]/[A490_{\text{positive control}} - A490_{\text{negative control}}]) \times 100\%$. The level of high-mobility group box 1 (HMGB1) in the culture supernatants was detected by western blotting using rabbit monoclonal antibody against HMGB1 (ab79823, Abcam, UK). Uncropped immunoblot and gel images are shown in Supplementary Fig. 1.

Visualization of the release of intracellular contents in MC38 cells. MC38_{GFP/mCherry}

cells were cultured overnight in glass-bottom cell culture dishes at 37°C followed by treatment with aMP_{C16}-CA₅₀ (160 µg ml⁻¹) at pH 6.8 for 10 h. The cells were subsequently imaged with the IXplore SpinSR spinning-disk confocal microscope equipped with a 60× oil-immersion objective after the addition of DRAQ7™ (0.6 µM). To investigate the release of CTSB from lysosomes, MC38_{CTSB-mCherry} cells were cultured overnight at 37°C in glass-bottom cell culture dishes followed by treatment with aMP_{C16}-CA₅₀ (160 µg ml⁻¹) at pH 6.8 for 12 h. To investigate the release of peptide from lysosomes, MC38_{Gal3-GFP} cells were cultured overnight at 37°C followed by treatment with aMP_{C16}-CA₅₀-Cy5 or aMP_{C16}-TA₅₀-Cy5 (160 µg ml⁻¹) at pH 6.8 for 12 h and 2 h, respectively. Free aMP_{C16}-CA₅₀-Cy5 or aMP_{C16}-TA₅₀-Cy5 in the medium was then washed out and replaced with fresh culture medium supplemented with PI (1 µg ml⁻¹), followed by confocal imaging with the spinning disk confocal microscope IXplore SpinSR equipped with a 60× oil-immersion objective.

LMR and PMR monitoring after temporally controlled treatment with aMP_{C16}-CA₅₀ and MP_{C16}. MC38_{Gal3-GFP} cells were treated with aMP_{C16}-CA₅₀ (80, 160 or 320 µg ml⁻¹) at pH 6.8 in the presence of PI (1 µg ml⁻¹). After 2 h treatment, time-lapse imaging was performed as mentioned in “Detection of LMR”. Then, MP_{C16} was added to the culture medium at a final concentration of 160 µg ml⁻¹ at the respective time points of 4, 8, 12, 16, 20, and 24 h post aMP_{C16}-CA₅₀ treatment. Time-lapse imaging was terminated when almost all cells in the imaging field of view exhibited PI staining.

Immunoblotting analysis of necroptosis, pyroptosis, and apoptosis. MC38 cells (1 × 10⁶ cells/well) were cultured overnight at 37°C in 6-well plates followed by treatment with aMP_{C16}-CA₅₀ (160 µg ml⁻¹) at pH 6.8 for 6, 12, 18, and 24 h. Cells were collected and lysed with lysis buffer containing 100 mM Tris (pH 6.8), 1 mM EDTA, 2% SDS, protease inhibitors, and phosphatase inhibitors. The cell lysate was then heated at 98°C for 10 min and centrifuged at 12,000 rpm for 10 min to collect the supernatant. The concentration of proteins was determined using the BCA method. Subsequently, the supernatant was mixed with 1/4 volume of 5× loading buffer (recipe: 100 mM Tris-HCl (pH 6.8), 2% SDS, 50% glycerol, 500 mM dithiothreitol, 0.05% bromophenol blue),

heated at 98°C for 10 min, and then subjected to SDS-PAGE analysis. MC38 cells treated with TNF- α (20 ng ml⁻¹), CHX (100 μ g ml⁻¹), and Z-VAD-FMK (40 μ M) for 18 h were used as the positive control for necroptosis activation. MC38 cells treated with TNF- α (20 ng ml⁻¹) and CHX (10 μ g ml⁻¹) for 8 h were used as the positive control for the stimulation of GSDME activation. MC38 cells treated with DOX (20 μ g ml⁻¹) for 24 h were used as the positive control for PARP1 and caspase-3 activation, while the same DOX treatment extended to 48 h was used as the positive control for GSDMD activation. Uncropped immunoblot images are shown in Supplementary Fig. 1.

The impact of aMP_{C16}-CA₅₀ on autophagic flux. MC38_{mCherry-GFP-LC3} cells were cultured overnight at 37°C in glass-bottom cell culture dishes, followed by treatment with aMP_{C16}-CA₅₀ at pH 6.8 for 6, 12, 18, and 24 h. The cells were imaged using the IXplore SpinSR confocal microscope equipped with a 60X oil-immersion objective. The number of red only fluorescent puncta (autolysosomes) and yellow fluorescent puncta (autophagosomes) in the cells was counted. The ratio of red fluorescent puncta to the sum of red and yellow fluorescent puncta was calculated. This ratio was used to compare the autophagy status at different time points following aMP_{C16}-CA₅₀ treatment.

The impact of aMPs on LysoTracker staining. MC38 cells were treated with aMP_{C16}-CA₅₀ or aMP_{C16}-TA₅₀ at pH 6.8 for varying concentrations and durations. The cells were incubated with LysoTracker Green (Thermo Fisher, 1:10000) for 30 min and then analyzed by flow cytometry. DAPI (0.5 μ g ml⁻¹) was added before the flow cytometry analysis.

TEM observation of aMP-treated cells. MC38 and Panc02 cells were exposed to a mixture of aMP_{C16}-CA₅₀ and aMP_{C16}-CA₅₀-Fe (4:1, molar ratio; the total peptide concentration of 320 μ g ml⁻¹) at pH 7.4 or 6.8 for 16 h or 24 h. The cells were harvested, fixed with 2.5% glutaraldehyde overnight at 4°C, and washed thrice with PBS (10–15 min each). The cells were then fixed with 1% osmium tetroxide for 2 h, washed thrice with PBS (10–15 min each), and subjected to dehydration with an ethanol gradient (25%, 50%, 75%, 90%, and 100% ethanol) and twice with anhydrous acetone, with ~10 min in each solution at room temperature. The cells were then soaked in

acetone/embedding solution (3:1 ratio for 0.5 h followed by 1:1 ratio for 4 h), and finally in embedding solution overnight at 4°C. The samples were encapsulated in the embedding plate and baked at 37°C for 24 h and at 60°C for another 48 h. Subsequently, 100-nm thick sections were cut with an ultramicrotome and stained with uranyl acetate for 20 min and lead citrate for 12 min. The samples were visualized using a Tecnai G2 Spirit (FEI) or a Talos L120C transmission electron microscope.

ITC detection. ITC was used to assess the interaction between MP_{C16} and phospholipids (DOPS or PdI). Thermograms were recorded at 25°C with stirring at 750 rpm and at a reference power of 10 $\mu\text{cal s}^{-1}$. For the titration of DOPS, MP_{C16} solution (0.35 mM) were loaded into the ITC syringe, and DOPS solution (4.69 mM) were loaded into the ITC cell. 25 injections of 50 μl were performed separated by 150 s with 60 s of initial delay. For the titration of PdI, MP_{C16} solutions (0.18 mM) were loaded into the ITC syringe, and PdI (0.16 mM) were loaded into the ITC cell. 19 injections of 47.5 μl were performed separated by 150 s with 60 s of initial delay. ITC assays were measured on a MicroCal PEAQ-ITC system. Data were analyzed in MicroCal PEAQ-ITC Analysis Software (v1.1.0.1262).

Size change of lipid nanoparticles upon MP_{C16} titration. The size change of lipid nanoparticle during titration with MP_{C16} was detected by DLS using a Zetasizer system. For the titration of DOPS, MP_{C16} solutions (10 or 20 μl , 0.35 mM) were added dropwise each time to DOPS solution (1.5 ml, 4.69 mM). For the titration of PdI, MP_{C16} solutions (5 or 10 μl , 0.17 mM) were added dropwise each time to PdI solution (1.5 ml, 0.64 mM). The particle size was measured three times at 25 °C after being thoroughly mixed.

Preparation of bone marrow–derived dendritic cells (BMDCs). BMDCs were generated as previously described^{79,80}. Briefly, bone marrow cells were isolated from the tibias and femurs of C57BL/6J mice, washed, treated with red blood cell lysis buffer (ACK Lysing Buffer; Lonza, Switzerland), and passed through a 70- μm cell strainer. The cells were then cultured in 24-well plates using RPMI 1640 complete medium supplemented with GM-CSF and IL-4 (20 and 10 ng ml^{-1} , respectively). The medium was replaced after 3 days. On day 6 or 7, the non-adherent cells (BMDCs) were

collected.

Isolation of T cells from mice. Spleen tissues were collected from OT-I (C57BL/6-Tg(TcraTcrb)1100Mjb/J) TCR transgenic mice and mechanically disaggregated. The cell suspensions were filtered using 70- μ m cell strainers. The CD3⁺ T cells were purified using MojoSort Mouse CD3 T Cell Isolation Kit (BioLegend, USA) following the manufacturer's instructions and cultured in T-cell culture medium containing RPMI 1640 supplemented with IL-2 (20 ng ml⁻¹), 10% FBS, 1% penicillin–streptomycin, 1 $\times$ HEPES, 1 $\times$ MEM nonessential amino acids, 1 $\times$ sodium pyruvate and β -mercaptoethanol (50 μ M)^{81,82}.

Flow cytometry analysis of samples from mice. The tumour tissues, tdLNs, spleen, and blood samples were collected from the mice at the indicated time points following treatment. The tumour tissues were cut into small pieces and enzymatically digested with RPMI 1640 medium containing collagenase type IV (1 mg ml⁻¹), hyaluronidase (0.1 mg ml⁻¹) and DNase I (0.1 mg ml⁻¹) under gentle agitation at 37°C. The dissociated cell suspensions were subsequently passed through a 70- μ m nylon mesh to obtain single-cell suspensions. The cells were harvested *via* centrifugation and resuspended in 40% Percoll (GE Healthcare, USA) solution in PBS. The resuspended cells were then centrifuged at 800 $\times$ g for 20 min, and the tumour-infiltrating leukocytes were obtained from the pellet. Spleen and tdLNs were mechanically dissociated by passing through a 70- μ m stainless steel mesh to obtain single-cell suspensions.

For staining of cell surface markers, the cells were washed with PBA buffer (0.5% BSA in PBS), blocked with anti-mouse CD16/32 antibodies (BioLegend, clone 93, 101301, 1: 200) on ice for 20 min, and then incubated on ice for 30 min with the antibodies (all of which were obtained from BioLegend, USA), including anti-CD45-APC/Cy7 (clone 104, 1:200), anti-CD11b-BV785 (clone M1/70, 1:200), anti-CD3-FITC (clone 17A2, 1:100), anti-CD3-BV711 (clone 17A2, 1:100), anti-CD8-Alexa Fluor 700 (clone 53-6.7, 1:200), anti-CD8-PE/Cy7 (clone 53-6.7, 1:200), anti-CD4-BV510 (clone RM4-5, 1:200), anti-CD4-PerCP/Cy5.5 (clone RM4-5, 1:200), anti-NK-1.1-PE/Dazzle 594 (clone S17016D, 1:200), anti-NKp46-BV650 (clone 29A1.4, 1:200), anti-CD11c-PE (clone N418, 1:200), anti-Gr-1-PerCP/Cy5.5 (clone RB6-8C5, 1:200), anti-F4/80-APC

(clone BM8, 1:200), anti-F4/80-Alexa Fluor 700 (clone BM8, 1:200), anti-I-A/I-E-Alexa Fluor 700 (clone M5/114.15.2, 1:200). For surface staining of non-tumoural stromal cells isolated from tumour tissues, antibodies including anti-CD31-PE/Cy7 (clone 390, Invitrogen, 1:200), anti-CD140 β -PE (clone APB5, BioLegend, 1:200) were used. DAPI (1 $\mu\text{g ml}^{-1}$) was added to the samples before flow cytometry analysis to distinguish between live and dead cells.

To evaluate the expression of IFN- γ , perforin, and granzyme B, a cell stimulation cocktail containing protein transport inhibitors (Invitrogen, USA) was added to the single-cell suspensions and incubated for 4 h. The cells were first stained with Zombie Aqua Fixable (BioLegend, 423102) for 25 min, followed by blocking. The cells were harvested and blocked with anti-CD16/32 antibodies (BioLegend, clone 93, 101301, 1:200). After staining for cell surface markers, the cells were washed, fixed with Fixation Buffer (BD Biosciences, USA) in the dark on ice for 20 min, and then washed twice with Perm/Wash buffer (BD Biosciences, USA). The cells were subsequently stained with anti-IFN- γ -PE/Cy7 (clone XMG1.2, 1:200), anti-perforin-PE (clone S16009B, 1:200), and anti-granzyme B-APC (clone QA16A02, 1:200) on ice for 45 min. Isotype control antibodies used included: Rat IgG1 κ isotype control-PE/Cy7 (BioLegend, clone RTK2071, 400416, 1:200), Rat IgG2 $\alpha\kappa$ isotype control-PE (BioLegend, clone RTK2758, 400508, 1:200), Mouse IgG1 κ isotype control-APC (BioLegend, clone MOPC-21, 400142, 1:200). Flow cytometry analysis was carried out using a LSRFortessa flow cytometer (BD Biosciences, USA) and BD FACSDiva software, and the results were analyzed using FlowJo v.10 (FlowJo, USA).

RNA-seq analysis. RNA isolation and bulk RNA sequencing were conducted at the Beijing Genomics Institute (BGI, China) using the MGISEQ-2000RS platform. Sequencing reads were filtered with SOAPnuke (v2.2.1)⁸³ and high-quality reads were aligned with the mouse reference genome (GRCm38) using Bowtie2⁸⁴. Gene expression levels were quantified using RNA-seq by Expectation Maximization (RSEM v1.3.1)⁸⁵. For differential expression analysis, the estimated read counts generated by RSEM were used as input for the DESeq2 R package (v1.34.0)⁸⁶. Statistical significance was determined using a two-sided Wald test. The expression levels for individual genes were normalized to fragments per kilobase of exon model

per million mapped reads (FPKM) for downstream visualization and enrichment analyses. The genes with a fold change in expression of > 2 and P -value of < 0.05 were defined as differentially expressed genes (DEGs). The volcano plots were generated using OmicStudio tools (<https://www.omicstudio.cn/tool>). Gene Ontology (GO) enrichment analysis was performed using the Database for Annotation, Visualization, and Integrated Discovery (DAVID, <https://david.ncifcrf.gov/>)⁸⁷. Statistical significance for GO terms was assessed using a modified Fisher's exact test (EASE score)^{88,89}, and terms with a P -value < 0.05 were considered significantly enriched. GSEA v4.3 software was run on the MSigDB Hallmarks^{90,91} using the gene expression dataset (FPKM) format, classic enrichment statistics, "meandiv" normalization, and for 1000 permutations. Gene sets with false discovery rates (FDRs) equal to 0 after 1000 permutations are depicted as having an infinite $-\log_{10}(q\text{-val})$.

RNA isolation and qRT-PCR. Total RNA was isolated from tumour tissues or cancer cells using the Trizol reagent (Takara, Japan) according to the manufacturer's instructions. Reverse transcription was performed to generate cDNA using PrimeScript™ RT Master Mix (Takara, Japan). The levels of expression of the target genes were quantified by qRT-PCR using TB Green® Premix Ex Taq™ II (Takara, Japan) and specific primers. The qRT-PCR analysis was performed on a LightCycler real-time PCR system (Roche, Switzerland). The relative gene expression (following normalization to GAPDH expression and relative to the expression of the gene in the corresponding control) was calculated by the $2^{-\Delta\Delta C_t}$ method. Furthermore, the relative gene expression levels were normalized using the z-score scale. The color indicates scaled normalized expression levels. Primer sequences used for qRT-PCR are provided in Supplementary Table 2.

Establishment and treatment of bilateral tumour models. To evaluate whether local intratumoural treatment could induce systemic antitumour effects, bilateral tumour models of subcutaneous MC38 colon adenocarcinoma were established by subcutaneously inoculating female C57BL/6J mice with MC38 tumour cells (1×10^6 cells) on the right flank (injected tumour) on days -3 or -4 , and with MC38 cells (1×10^6 cells) on the left flank (distal tumour) on day 0. Bilateral tumour models of orthotopic EMT6 triple-negative breast cancer were established by inoculating female

BALB/c mice with EMT6 cells (3×10^5 cells) in the second right breast pad (injected tumour) on day -3, and in the second left breast pad (distal tumour) on day 0.

To determine whether the antitumour effect on distal tumours was specific to homologous tumours, female C57BL/6J mice were subcutaneously inoculated with: (i) a left-flank MC38 tumour only, (ii) bilateral MC38 tumours, or (iii) a right-flank EO771 tumour and a left-flank MC38 tumour. MC38 or EO771 cells (1×10^6 cells) were inoculated into the right flank on day -4 and MC38 cells (1×10^6 cells) into the left flank on day 0.

To assess the role of T cells in antitumour efficacy in this bilateral subcutaneous MC38 tumour model, CD4⁺ and CD8⁺ T cells were depleted by intravenous administration of monoclonal antibodies specific for mouse CD4 (BioXCell, clone GK1.5, BE0003-1) or mouse CD8 α (BioXCell, clone 2.43, BE0061) at a dose of 2 mg kg⁻¹ at the indicated time points. Intravenous administration of mouse IgG (BioXCell, clone LTF-2, BE0090) was employed as a control.

Mice were randomised into treatment groups such that the mean volume of right-flank tumours was similar across groups, and treatment was then initiated. At the indicated time points, therapeutic agents, including aMPs, GPN, DOX or MTX, were injected (*via* the i.t. route) into the right flank tumours (injected tumour), and α PD-L1 antibody (Bioxcell, clone 10F.9G2, BE0101) was injected (i.v.) at a dosage of 0.75 mg kg⁻¹.

Prophylactic tumour vaccination. MC38, Panc02, or EO771 (6×10^5 cells/well) were seeded into 24-well plates and treated with MPs ($160 \mu\text{g ml}^{-1}$) for 24 h. MPs-treated cell suspensions (100 μl) were injected subcutaneously into the dorsal region of mice on day -14 and day -7. On day 0, live MC38, Panc02, or EO771 cells (5×10^5 cells) were inoculated subcutaneously into mice. To test whether *Mkl*, *Gsdme* or *Ninjl* deficiency affects the immunogenicity of aMP_{C16}-CA₅₀-treated tumour cells, MC38-sgNTC MC38-sg*Mkl*, MC38-sg*Gsdme* and MC38-sg*Ninjl* cells were treated with aMP_{C16}-CA₅₀ ($160 \mu\text{g ml}^{-1}$) for 24 h and used for subcutaneous immunization. As controls, the corresponding knockout cells were subjected to freeze-thawing before vaccination. Mice were immunized on days -14 and -7 and challenged with wild type MC38 cells

(5×10^5 cells) on day 0.

Intravenous administration of aMP_{C16}-CA₅₀ and α PD-L1 combination therapy.

The subcutaneous tumour models were established by subcutaneously inoculating female C57BL/6J mice with MC38 or Panc02 cancer cells (1×10^6). The orthotopic EMT6 tumour model was established by inoculating female BALB/c mice with EMT6 tumour cells (3×10^5) in the second right breast pad. Upon attaining average tumour volumes of approximately 50 mm³ for the MC38- and Panc02 tumours, and 70 mm³ for the EMT6 tumours, the mice were divided into groups such that the mean tumour volumes across the different groups were similar. The peritoneal metastasis model of MC38 tumour cells was established by intraperitoneally injecting female C57BL/6J mice with luciferase-expressing MC38 tumour cells (2×10^5 ; MC38-Luc). The mice were randomly divided into four groups 5 days after the transplantation of MC38-Luc cells. At the indicated time points, the mice were administered aMP_{C16}-CA₅₀ and α PD-L1 *via* the i.v. route. aMP_{C16}-CA₅₀ was administered at a dose of 15 mg kg⁻¹ in all tumour models. α PD-L1 was administered at a dose of 0.75 mg kg⁻¹ in the MC38, EMT6 and MC38-Luc tumour models, and 1 mg kg⁻¹ in the Panc02 tumour model. Tumour volumes were monitored as described in the “Animals” section of the Methods.

***In vivo* toxicity of aMP_{C16}-CA₅₀.** Female ICR mice (6–8 weeks, 25–30 g) were randomly divided into different groups ($n = 5$ or 6 per group). The ICR mice were administered (*via* the i.v. route) with various doses of MPs (5% glucose solution), with one administration per mouse. The mice were monitored post-treatment, and the maximum tolerated dose (MTD) was determined. Similarly, the female ICR mice were randomly divided into two groups ($n = 5$ per group) and administered (*via* the i.v. route) aMP_{C16}-CA₅₀ (15 mg kg⁻¹ in 5% glucose solution). The liver and serum samples were collected after 24 h. The serum samples were employed for determining the concentrations of aspartate aminotransferase (AST), alanine transaminase (ALT), total bilirubin (TBIL), urea, creatinine (CREA), and uric acid (UA). The *in vivo* toxicity of the combination therapy of aMP_{C16}-CA₅₀ (i.v., 15 mg·kg⁻¹) and α PD-L1 (0.75 mg·kg⁻¹) was evaluated in MC38 tumour-bearing mice. At the tumour monitoring endpoint, the serum samples were collected and subjected to serum biochemistry analysis mentioned above. Major organs (including heart, liver, lung, spleen, and kidney) were fixed with

4% paraformaldehyde, embedded in paraffin, and cut into slices (6 μm) for hematoxylin–eosin (H&E) staining. The images were captured using a KF-FL-005 digital pathology scanner.

Cytotoxicity of aMP_{C16}-CA₅₀ in tumour tissues. MC38 or MC38_{GFP} cells were injected into the right posterior back of C57BL/6 mice at a dose of 1×10^6 cells per mouse. Treatment was initiated when the tumours reached approximately 150 mm³. MC38 tumour-bearing mice were administered aMP_{C16}-CA₅₀ or MP_{C16} (125 μg per mouse) via intratumoural injection. Following three consecutive days of intratumoural peptide injection, tumour tissues were harvested. Flow cytometry was performed to quantify the number of viable immune cells and non-immune cells in the tumour tissues. In addition, the tumours were excised and fixed in 4% PFA. H&E staining and immunofluorescence staining for the vascular marker CD31 and the T cell marker CD3 were performed on consecutive tumour sections to evaluate tumour necrosis and damage to non-cancerous cells in the tumour microenvironment following treatments.

***In vivo* distribution and tumour accumulation of aMP_{C16}-CA₅₀.** MC38 cells were injected into the right posterior back of C57BL/6 mice at a dose of 1×10^6 cells per mouse. aMP_{C16}-CA₅₀-Cy5 was intravenously injected into the mice at a dose of 15 mg kg⁻¹ when tumours reached approximately 150 mm³. Mice were euthanized at different time points to collect tissues, including the heart, liver, spleen, lung, kidney, and tumour. These tissues were then subjected to fluorescence imaging using the IVIS Lumina III *in vivo* imaging system, and the fluorescence intensity was quantified in each organ and tumour.

Cellular uptake of aMP_{C16}-CA₅₀ in tumour tissues. Mice were intratumourally injected with aMP_{C16}-CA₅₀-Cy5 or MP_{C16}-Cy5 (125 μg per mouse) when tumours reached approximately 150 mm³. Tumour tissues were excised at 12 h and 24 h after administration and dissociated into single-cell suspensions. The uptake of aMP_{C16}-CA₅₀-Cy5 or MP_{C16}-Cy5 by various cell types in the tumour tissues was assessed by flow cytometry.

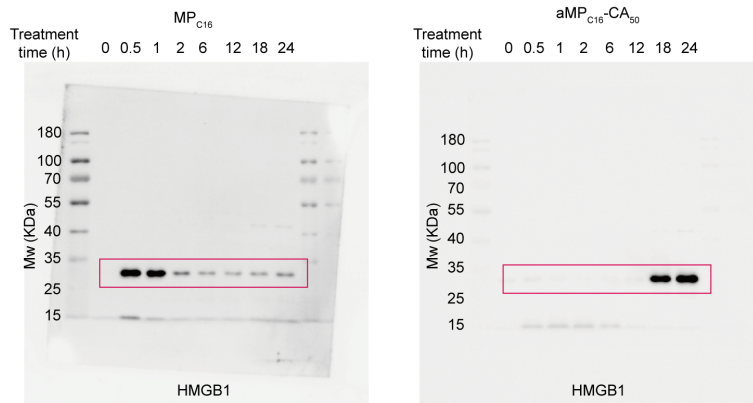
scRNA-seq and data analysis

Library preparation. MC38 tumour-bearing mice were administered intravenously with aMP_{C16}-CA₅₀ and αPD-L1 at doses of 15 and 0.75 mg kg⁻¹, respectively. Tumour tissues were collected 7 days after treatment, cut into small pieces, and enzymatically digested with collagenase type IV (1 mg ml⁻¹), hyaluronidase (0.1 mg ml⁻¹) and DNase I (0.1 mg ml⁻¹) to generate single-cell suspensions. Subsequently, CD45⁺ cells were isolated via magnetic-activated cell sorting (MACS) using anti-CD45 microbeads and MS columns (Miltenyi Biotec, Germany). The single-cell gene expression libraries were prepared using the Chromium Next GEM Single Cell 3' HT Kit v3.1 (Dual Index) according to manufacturer's protocol. The quality and integrity of the library DNA fragments were checked by an Agilent 2100 Bioanalyzer. Sequencing was performed on an Illumina platform (NovaSeq X Plus) by Gene Denovo Biotechnology Co., Ltd. (Guangzhou, China), with a sequencing depth of approximately 80,000 reads per cell. 10X Genomics Cell Ranger software (v8.0.0)⁹² was used to process the clean sequencing data in FASTQ format, align reads to the mouse reference genome (mm10), and summarize unique molecular identifier (UMI) counts in feature-barcode matrices. Genes detected in fewer than three cells were excluded in the quality control (QC) process. Cells with fewer than 200 or more than 6000 UMIs, as well as cells with mitochondrial gene percentages greater than 20% were also excluded for subsequent analyses. Doublets identified by DoubletFinder (v2.0.4)⁹³ were removed. The expression matrix was then normalized using the LogNormalize method in Seurat v5.1.0 for downstream cluster analysis.

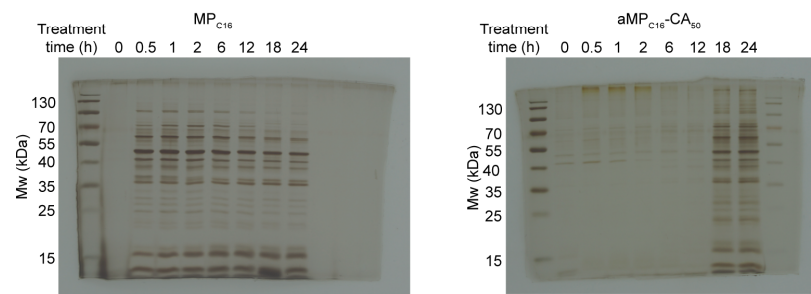
scRNA-seq data visualization. Dimension reduction and unsupervised clustering were performed according to the standard workflow in Seurat v5.1.0⁹⁴. For clustering and visualization, the FindClusters function in Seurat was applied to obtain cell clusters in various resolutions and Uniform Manifold Approximation and Projection (UMAP) was used to reduce the dimensionality of the data. Cell types in clusters were defined using the following marker genes: CD8⁺ T cells (*Cd3e*⁺ *Cd8a*⁺), CD4⁺ T cells (*Cd3e*⁺ *Cd4*⁺ *Foxp3*⁻), Treg cells (*Foxp3*⁺), γδT cells (*Trdv4*⁺), NK cells (*Cd3e*⁻ *Ncr1*⁺), B cells (*Cd79a*⁺), macrophages (*Adgre1*⁺), neutrophils (*S100a8*⁺ *Csf3r*⁺), conventional DCs

(*Cd209a*⁺), migratory DCs (*Ccr7*⁺ *Fscn1*⁺), pDCs (*Siglech*⁺ *Tcf4*⁺), mast cells (*Cpa3*⁺). To quantify single-cell pathway activity, gene set variation analysis (GSVA) was performed with default parameters in each annotated immune subset using the R package GSVA (v1.50.5)⁹⁵. Hallmark gene sets were retrieved from the GSEA database (MSigDB, v7.5.1)⁹⁰. Single-cell GSVA scores were aggregated to the treatment group level by mean calculation, and then z-score normalized independently per pathway for intra-subset activity comparison. Z-scored GSVA scores were visualized as heatmaps using the pheatmap package⁹⁶. For gene expression heatmaps, average expression levels were calculated for each treatment group within each annotated cell subset and then z-score normalised by gene to display relative expression patterns. Additionally, the Interferon-Stimulated Genes (ISG) signature was obtained from the published ISG list (Supplementary Table 3)⁹⁷, Antigen Processing-Cross Presentation gene set for conventional dendritic cells (cDCs) was obtained from Reactome (R-MMU-1236975)⁹⁸, and T cell-mediated Cytotoxicity gene set for CD8⁺ T cells was obtained from Gene Ontology (GO:0001913)⁹⁹. Gene signature scores were quantified using the AUCell (v1.28.0)¹⁰⁰ with aucMaxRank set to 5%. Group-wise comparisons of signature scores were examined using the Kruskal-Wallis H test, followed by Dunn's test with Benjamini-Hochberg correction for pairwise post-hoc comparisons. For visualization, AUCell scores were normalized to z-scores and violin plots were generated to display score distributions. All analyses were conducted in R (v4.3.0).

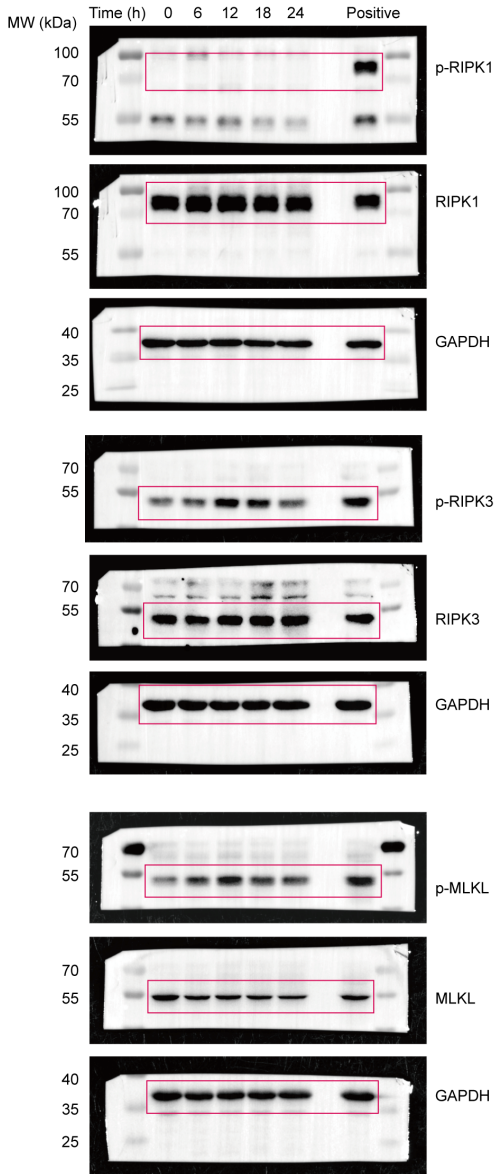
SI Fig. 1a: Uncropped immunoblots corresponding to Fig. 1p



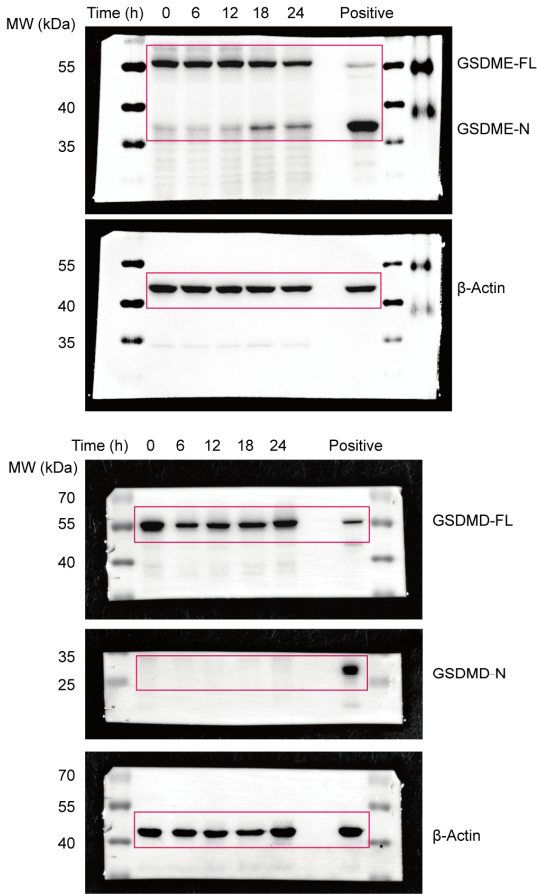
SI Fig. 1b: Uncropped gels corresponding to Fig. 1q



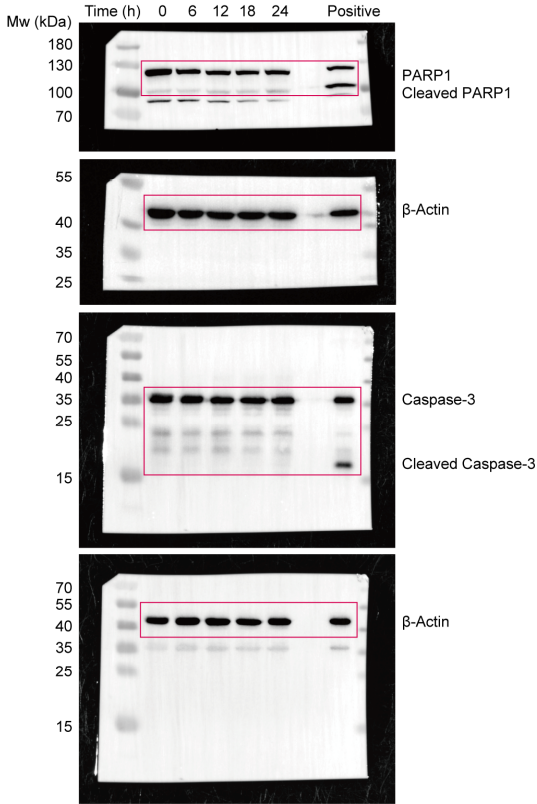
SI Fig. 1c: Uncropped immunoblots corresponding to Extended Data Fig. 1a



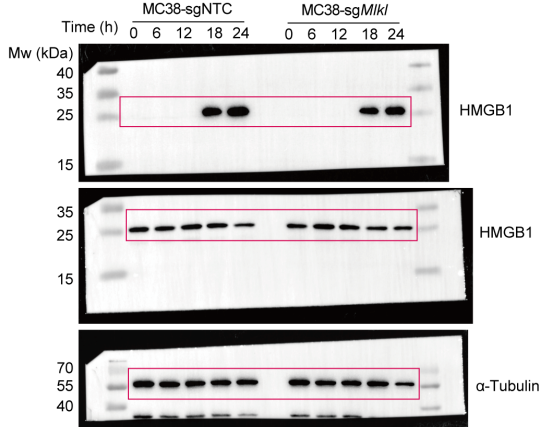
SI Fig. 1d: Uncropped immunoblots corresponding to Extended Data Fig. 1b



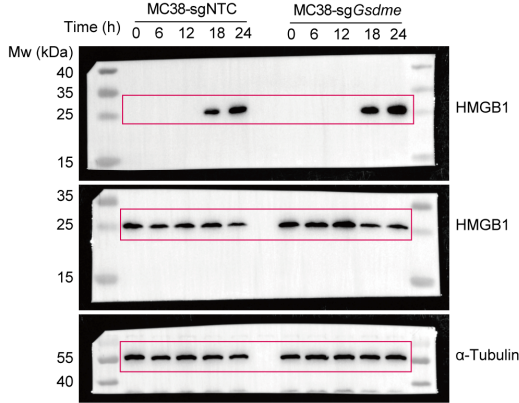
SI Fig. 1e: Uncropped immunoblots corresponding to Extended Data Fig. 1e



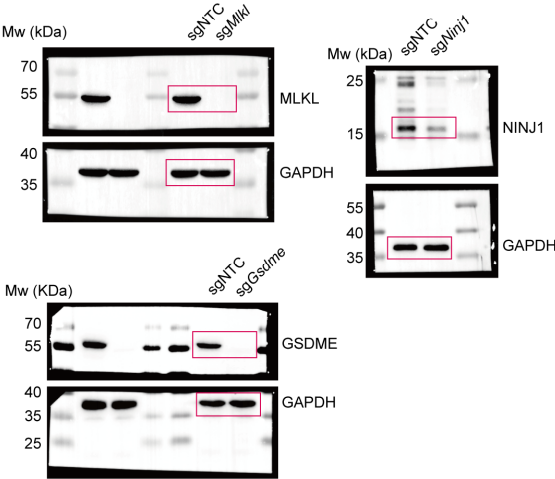
SI Fig. 1g: Uncropped immunoblots corresponding to Extended Data Fig. 1n



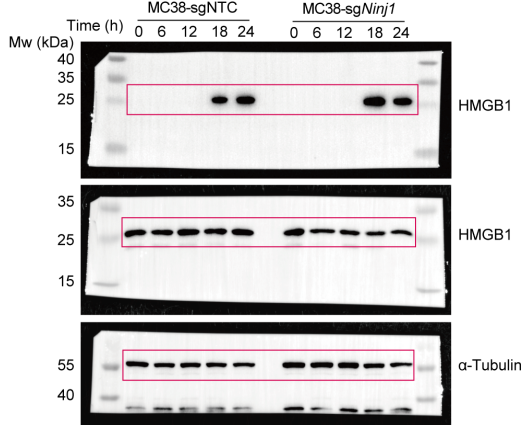
SI Fig. 1h: Uncropped immunoblots corresponding to Extended Data Fig. 1o



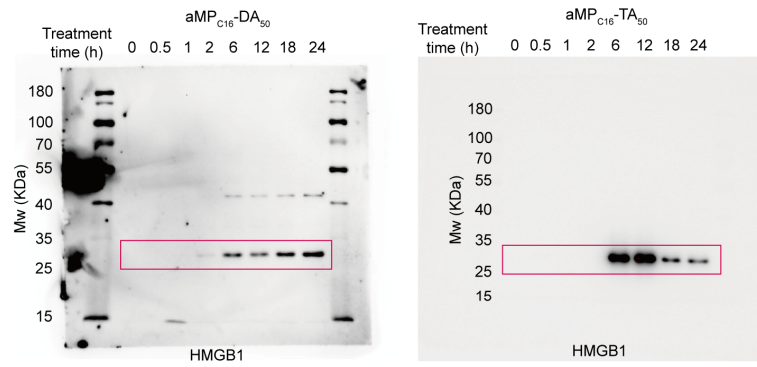
SI Fig. 1f: Uncropped immunoblots corresponding to Extended Data Fig. 1i, 1j, 1k



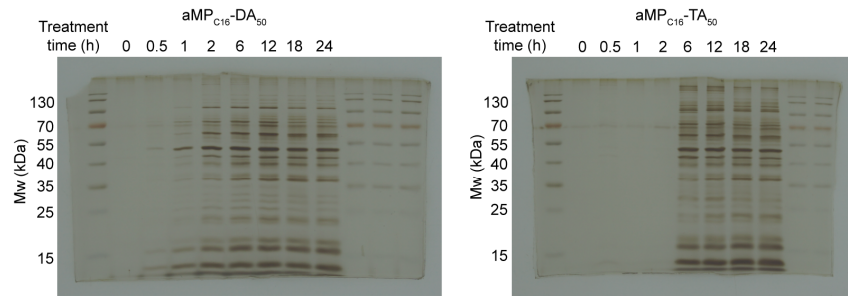
SI Fig. 1i: Uncropped immunoblots corresponding to Extended Data Fig. 1p



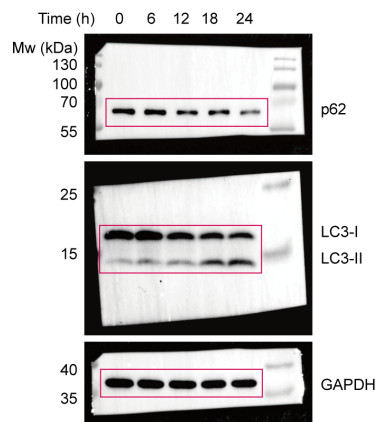
SI Fig. 1j: Uncropped immunoblots corresponding to Extended Data Fig. 6c



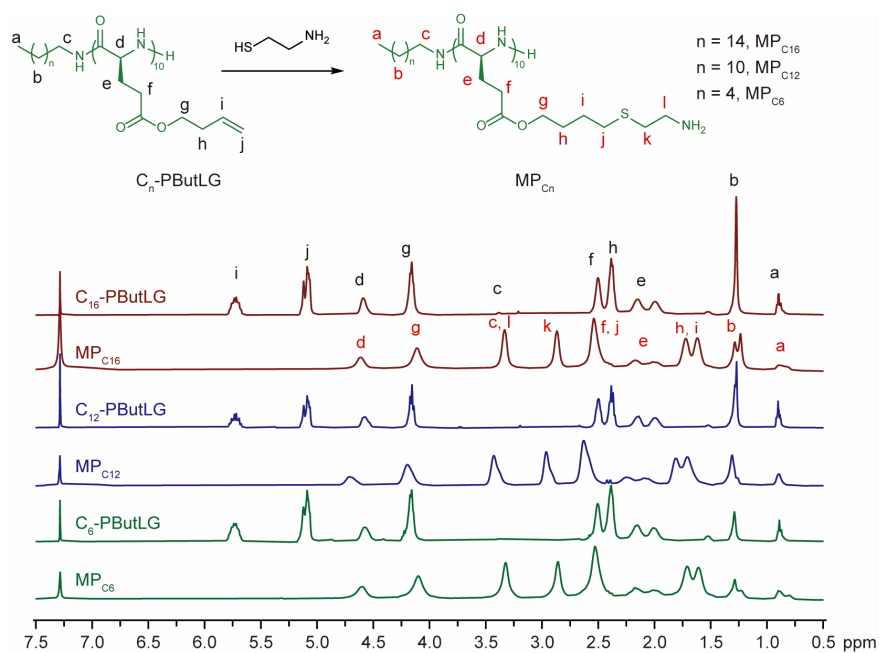
SI Fig. 1k: Uncropped gels corresponding to Extended Data Fig. 6d



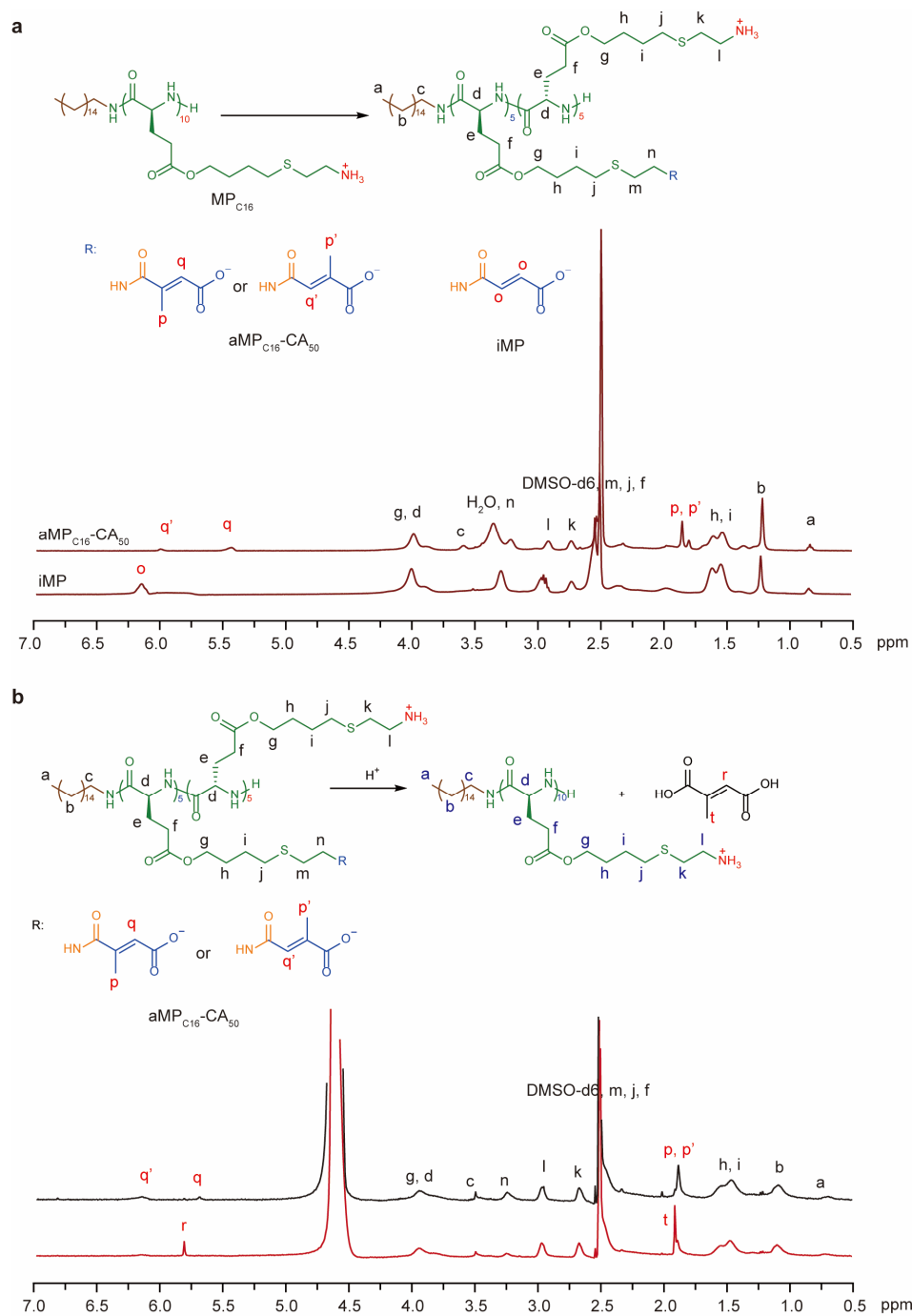
SI Fig. 1l: Uncropped immunoblot corresponding to Supplementary Fig. 11a



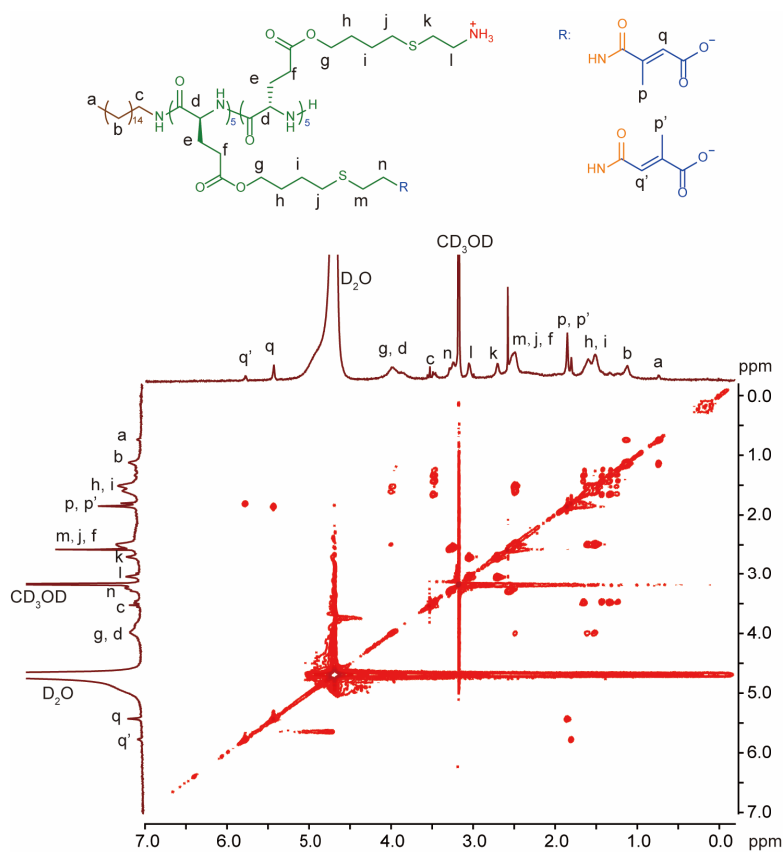
Supplementary Figure 1. Uncropped gel and blot images. **a**, Uncropped immunoblots corresponding to Fig.1p. **b**, Uncropped gels corresponding to Fig.1q. **c**, uncropped immunoblots corresponding to Extended Data Fig. 1a. **d**, Uncropped immunoblots corresponding to Extended Data Fig. 1b. **e**, Uncropped immunoblots corresponding to Extended Data Fig. 1e. **f**, Uncropped immunoblots corresponding to Extended Data Fig. 1i,1j,1k. **g**, Uncropped immunoblots corresponding to Extended Data Fig. 1n. **h**, Uncropped immunoblots corresponding to Extended Data Fig. 1o. **i**, Uncropped immunoblots corresponding to Extended Data Fig. 1p. **j**, Uncropped immunoblots corresponding to Extended Data Fig. 6c. **k**, Uncropped gels corresponding to Extended Data Fig. 6d. **l**, Uncropped immunoblot corresponding to Supplementary Fig. 11a.



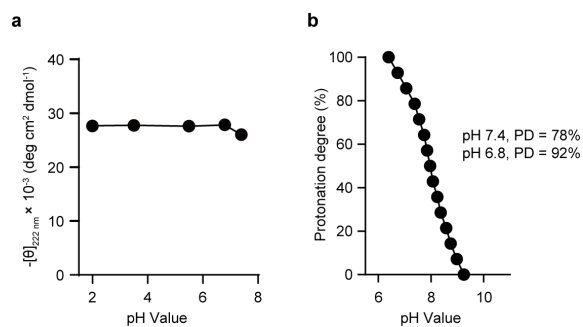
Supplementary Figure 2. Synthesis routes and ¹H-NMR spectra (in CDCl₃) of MP_{C₁₆}, MP_{C₁₂}, and MP_{C₆}.



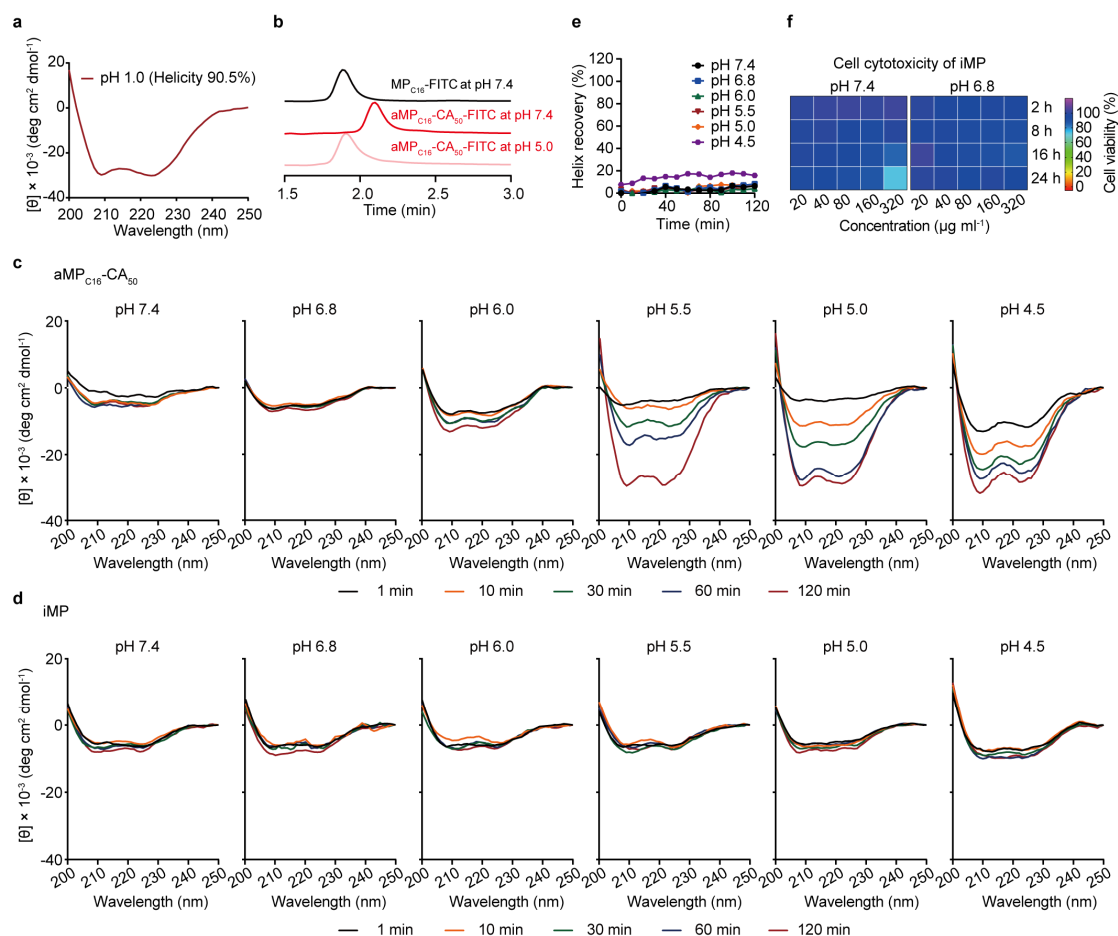
Supplementary Figure 3. Synthesis routes and structural characterization of aMPC₁₆-CA₅₀ and iMP. **a**, Synthesis routes and ¹H-NMR spectra (in DMSO-d₆) of aMPC₁₆-CA₅₀ and iMP. **b**, ¹H-NMR spectra of aMPC₁₆-CA₅₀ upon incubation with DMSO-d₆, D₂O, and DCl (400, 200, and 20 μl, respectively) for 1 (black) and 120 (red) min.



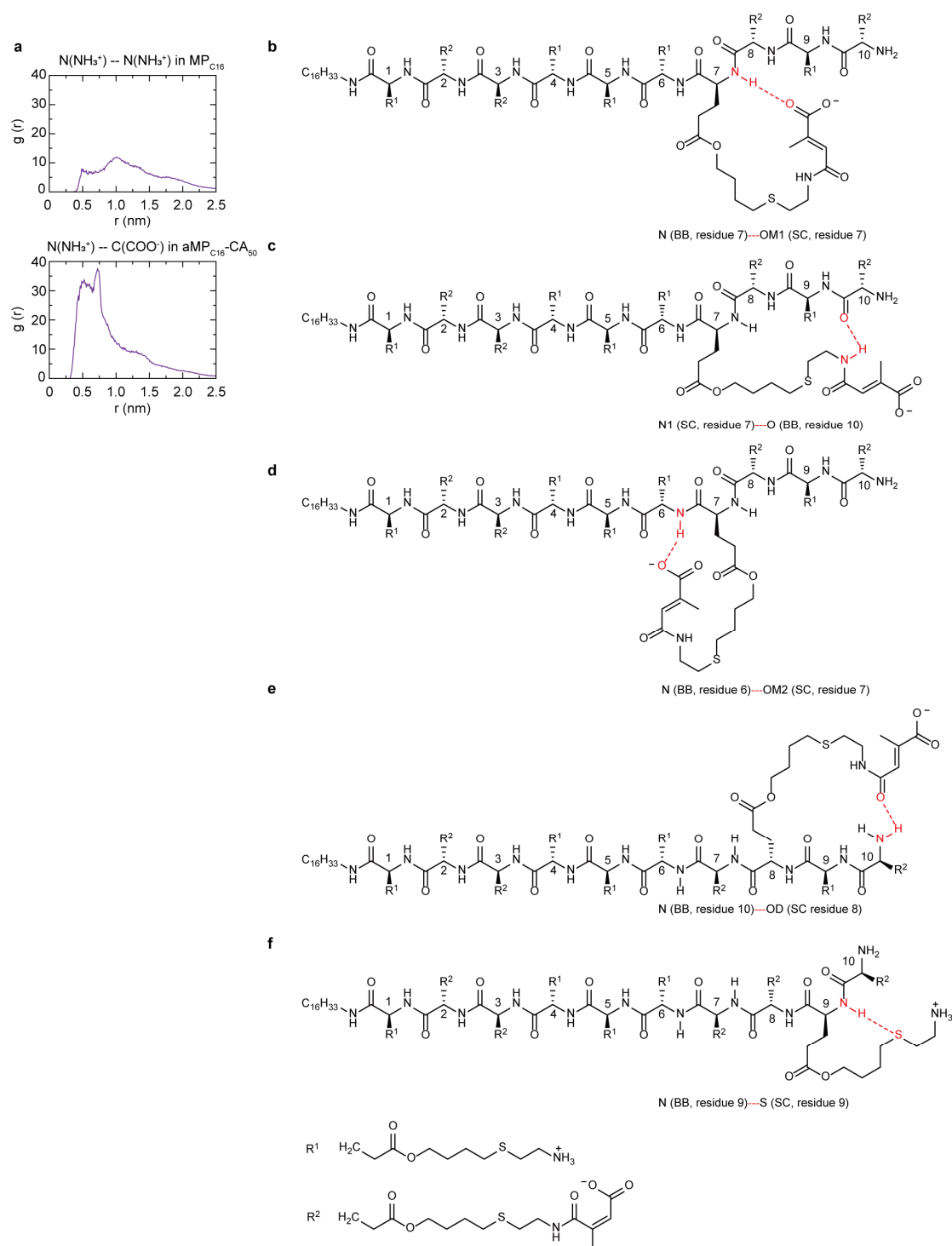
Supplementary Figure 4. ¹H-¹H TOCSY NMR spectrum of aMPC₁₆-CA₅₀. CD₃OD and D₂O (1:3 v/v) were used as the solvent.



Supplementary Figure 5. Helicity (a) and protonation degree (b) of MPC₁₆ under different pH conditions. Concentration of MPC₁₆, 100 µg ml⁻¹.

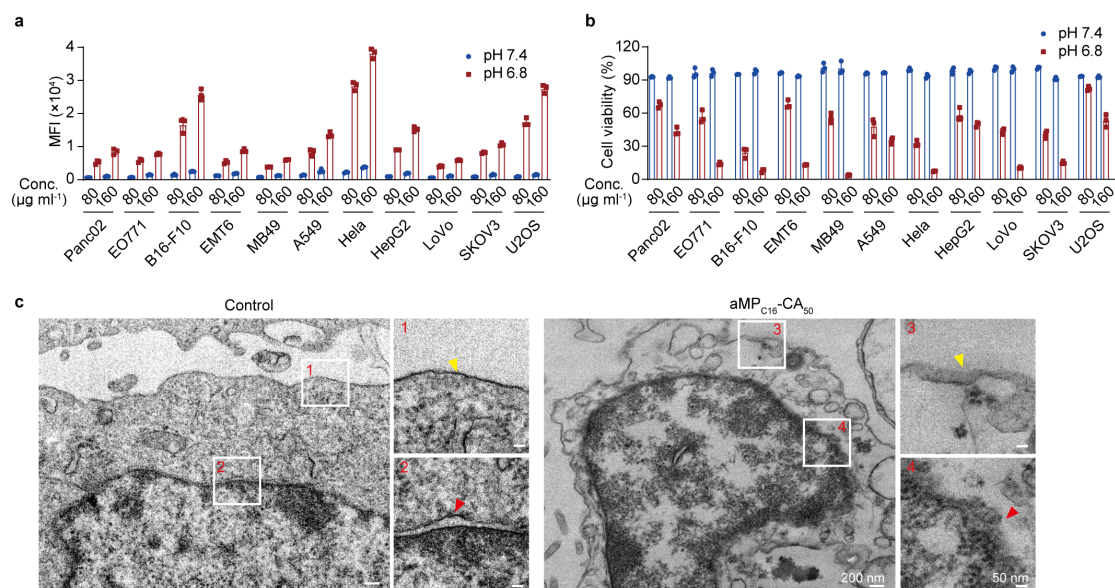


Supplementary Figure 6. pH responsiveness of aMPC₁₆-CA₅₀ and iMP. **a**, CD spectrum of aMPC₁₆-CA₅₀ at pH 1.0. **b**, High-performance liquid chromatography (HPLC) analysis of the transition of aMPC₁₆-CA₅₀-FITC incubated at pH 5.0 for 1 h. **c,d**, CD spectra of aMPC₁₆-CA₅₀ (**c**) and iMP (**d**) incubated in phosphate buffer (PB) of different pH for varying durations. **e**, Time-dependent helicity recovery of iMP upon incubation in PB of different pH. **f**, Concentration- and time-dependent cytotoxicity of iMP against MC38 cells at pH 7.4 and 6.8. Peptide concentration, 100 μ g ml⁻¹ (**a-e**).

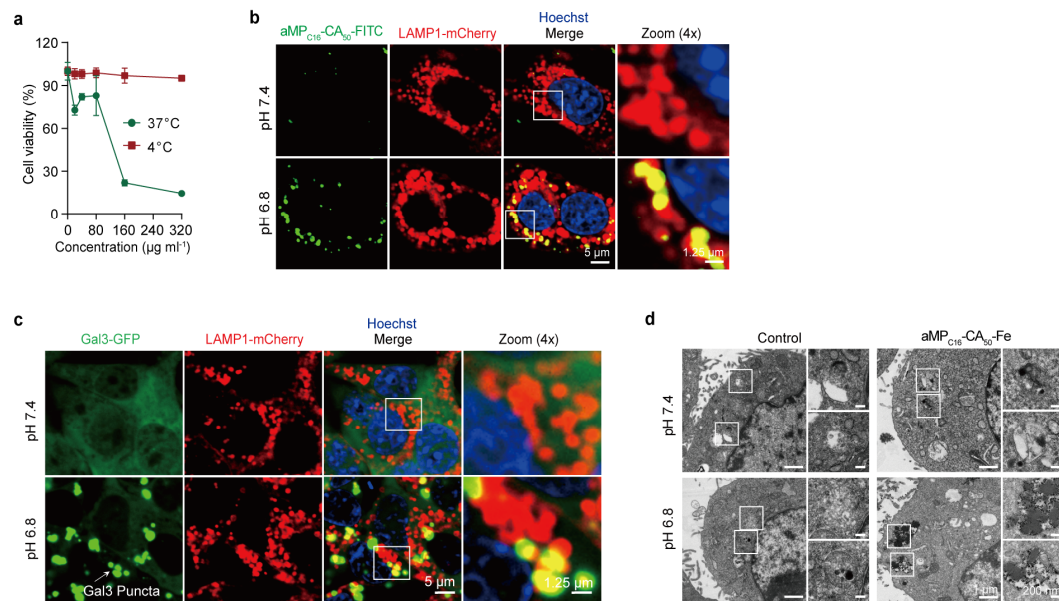


Supplementary Figure 7. The interplay among side chains, as well as their interactions with the backbone. **a**, Radial distribution function, $g(r)$, for the distance between the nitrogen atoms of the NH_3^+ groups ($N[NH_3^+] - N[NH_3^+]$) in MP_{C16} , and the distance between the nitrogen and carbon atoms ($N[NH_3^+] - C[COO^-]$) of the amino and carboxyl groups, respectively, in $aMP_{C16-CA50}$. **b–f**, Chemical structure of $aMP_{C16-CA50}$ revealing high-occupancy hydrogen bonds (HBs). **b**, HB between the nitrogen atom of backbone amide (residue 7) and the oxygen atom of side-chain carboxyl group (residue 7). **c**, HB between the nitrogen atom of side-chain amide group (residue 7) and the carbonyl oxygen of backbone amide (residue 10). **d**, HB between the nitrogen atom

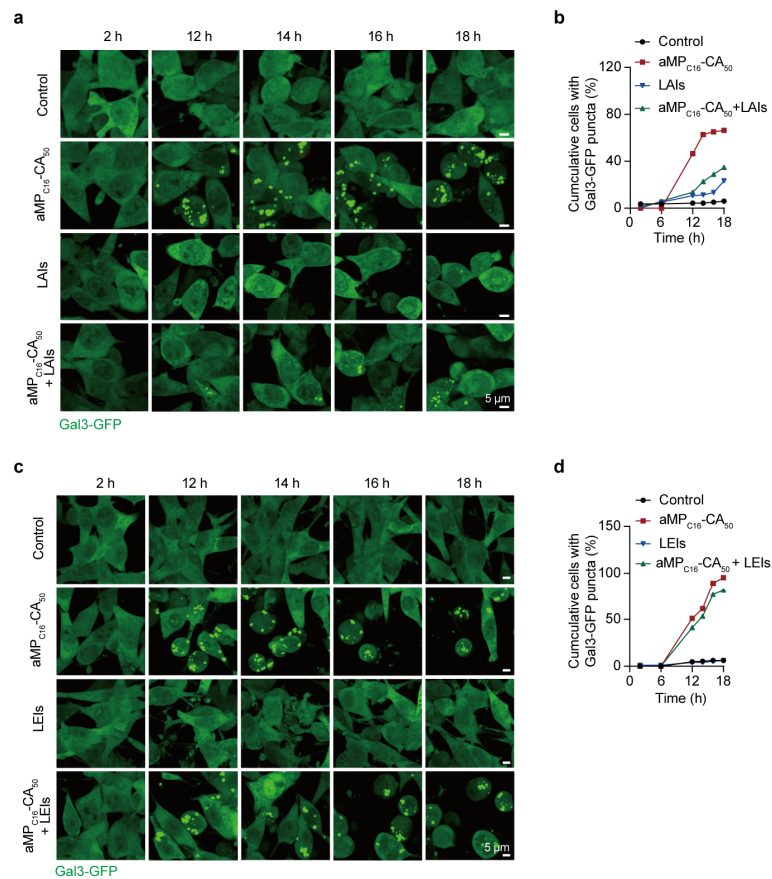
of backbone amide (residue 6) and oxygen atom of the side-chain carboxyl group (residue 7). **e**, HB between the nitrogen atom of backbone amide (residue 10) and the carbonyl oxygen atom of the side-chain amide (residue 8). **f**, HB between the nitrogen atom of backbone amide (residue 9) and the sulfur atom of side-chain (residue 9). N1 (SC, residue 7) represents the nitrogen atom of the side-chain amide group in residue 7, and O (BB, residue 10) represents the oxygen atom of the backbone amide in residue 10. N and O represent the nitrogen and oxygen atoms of the backbone amide, respectively. N1 represents the nitrogen atom of side-chain amide or amine group. OM1 and OM2 represent the carbonyl oxygen in the negatively-charged carboxyl groups while OD is the carbonyl oxygen of amide in the side chains of aMP_{C16}-CA₅₀.



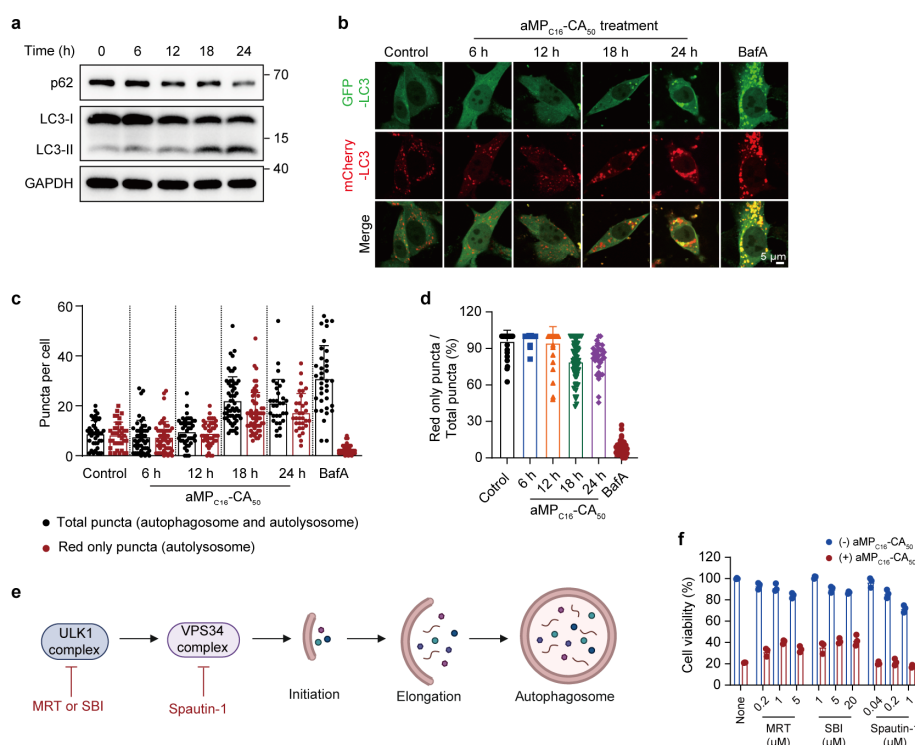
Supplementary Figure 8. pH-dependent cytotoxicity of aMP_{C16}-CA₅₀. **a**, pH- and concentration-dependent uptake of aMP_{C16}-CA₅₀-FITC in a series of tumour cells after 12 h of incubation at pH 7.4 and 6.8. MFI, mean fluorescence intensity. **b**, pH- and concentration-dependent cytotoxicity of aMP_{C16}-CA₅₀ against a series of tumour cells after 24 h of incubation at pH 7.4 and 6.8. **c**, TEM images of MC38 cells treated without or with aMP_{C16}-CA₅₀-Fe (320 $\mu\text{g ml}^{-1}$) for 24 h at pH 6.8. Yellow arrowheads indicate the cell membrane, and red arrowheads indicate the nuclear envelope. Scale bars, 200 and 50 nm for regular and magnified images, respectively. Representative TEM images are shown, and at least six images were taken for each sample. In **a,b**, data are mean $\pm$ s.d., $n = 3$ biological replicates.



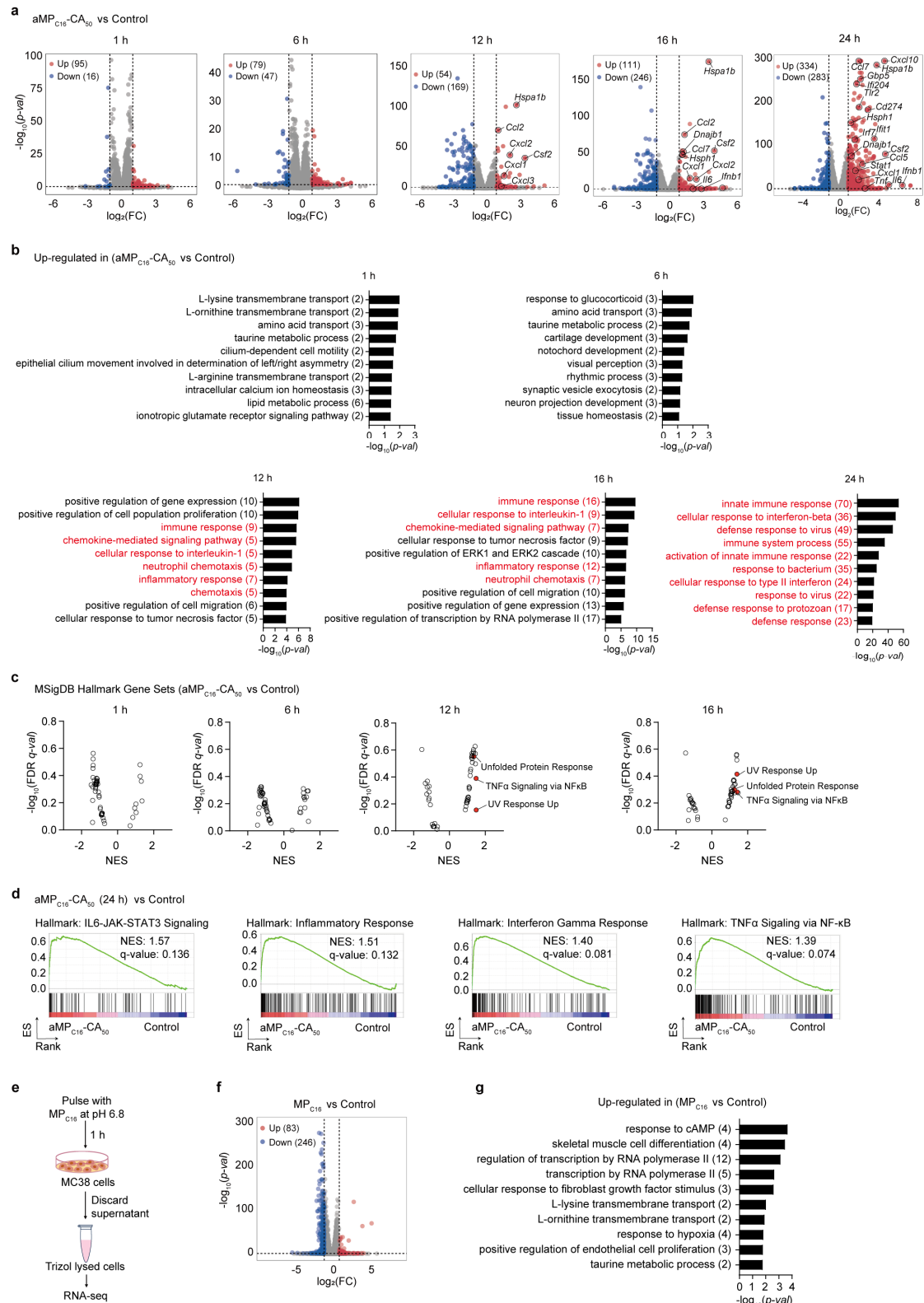
Supplementary Figure 9. pH-dependent LMR of aMP_{C16}-CA₅₀ in Panc02 cells. **a**, Concentration-dependent cytotoxicity of aMP_{C16}-CA₅₀ against Panc02 cells upon incubation at 37 or 4°C for 24 h at pH 6.8. **b**, Confocal imaging of Panc02_{LAMP1-mCherry} cells incubated with aMP_{C16}-CA₅₀-FITC (160 μg ml⁻¹, green) for 1 h at pH 7.4 or 6.8. **c**, Confocal imaging of Panc02_{LAMP1-mCherry&Gal3-GFP} cells incubated with aMP_{C16}-CA₅₀ (160 μg ml⁻¹) for 20 h at pH 7.4 or 6.8. Scale bars in **b,c**, 5 and 1.25 μm for regular and magnified images, respectively. **d**, TEM imaging of Panc02 cells treated with aMP_{C16}-CA₅₀-Fe (320 μg ml⁻¹) for 16 h at pH 7.4 or 6.8. Scale bars, 1 μm and 200 nm for regular and magnified images, respectively. In **a**, data are mean ± s.d., *n* = 3 biological replicates. In **b,c**, representative images from two independent experiments. In **d**, Representative TEM images are shown and at least six images were taken for each sample.



Supplementary Figure 10. Effect of LAIs and LEIs on the Gal3-GFP puncta formation during aMP_{C16}-CA₅₀ treatment. Confocal imaging of MC38_{Gal3-GFP} cells treated with aMP_{C16}-CA₅₀ (160 µg ml⁻¹) at pH 6.8 in the absence or presence of LAIs (**a,b**) or LEIs (**c,d**) at varying durations. **a,c**, Representative images. Scale bar, 5 µm. **b,d**, Percentage of cells exhibiting Gal3-GFP puncta. LAIs concentration: BafA, 40 nM; CMA, 40 nM; CQ, 80 µM (**a,b**). LEIs concentration: CA-074-Me, 1 µM; E-64d, 80 µM; pepstatin, 80 µM (**c,d**). In **a,c**, representative images from two independent experiments.

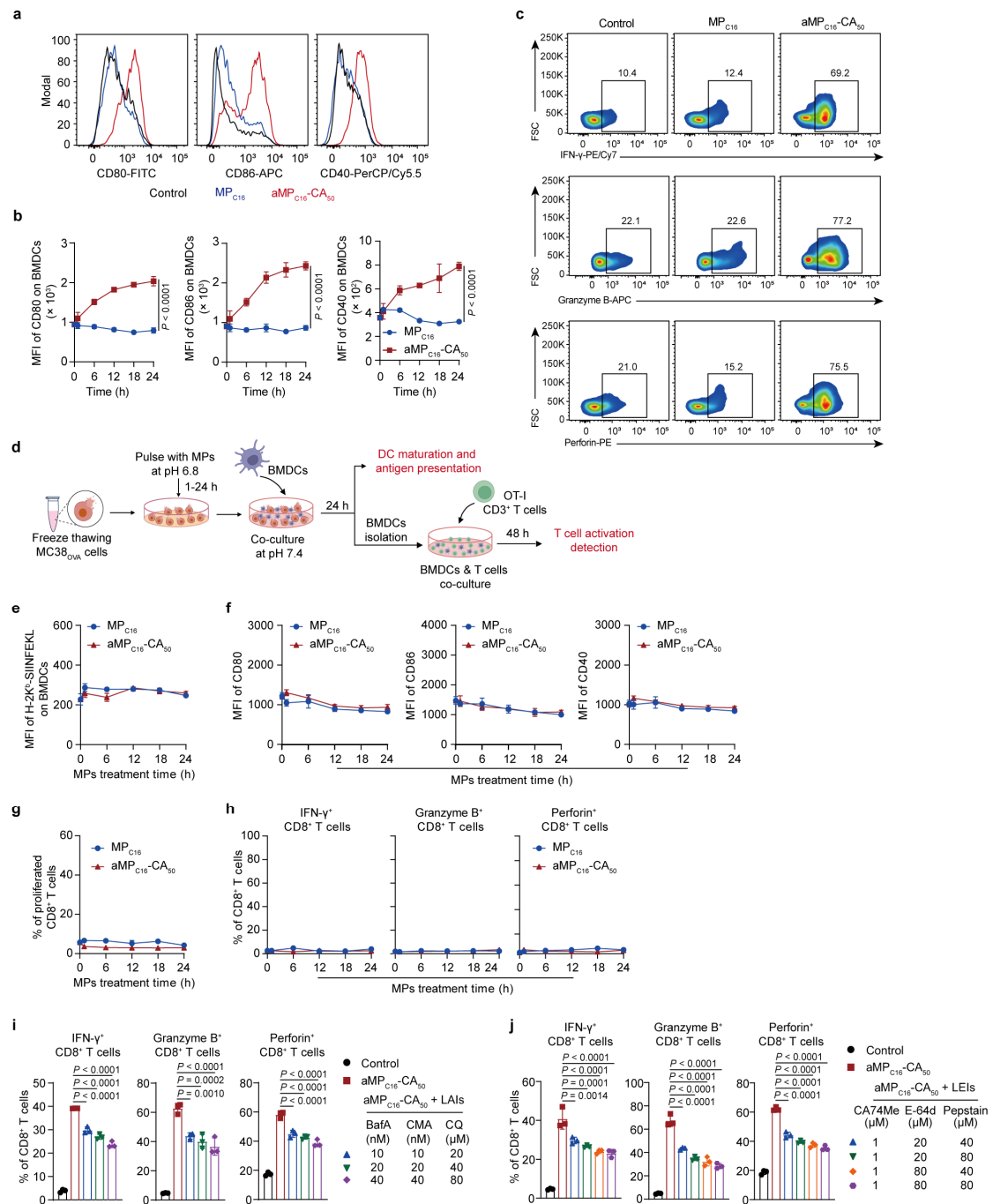


Supplementary Figure 11. Role of autophagy in aMPC₁₆-CA₅₀-induced cytotoxicity in MC38 cells. **a**, Immunoblotting of p62, LC3-I, and LC3-II in MC38 cells treated with aMPC₁₆-CA₅₀ (160 $\mu\text{g ml}^{-1}$) for varying durations. **b–d**, Autophagic flux analysis in MC38^{GFP-mCherry-LC3} cells, which express tandem-tagged GFP-mCherry-LC3 probe. MC38^{GFP-mCherry-LC3} cells were treated with aMPC₁₆-CA₅₀ (160 $\mu\text{g ml}^{-1}$) for the indicated durations, and LC3 puncta were quantified based on GFP and mCherry fluorescence. BafA (0.2 μM) was used as a control for blocking autophagy flux. **b**, Representative confocal images. Scale bar, 5 μm . **c**, Quantification of total puncta (autophagosome and autolysosome) and red-only puncta (autolysosome). **d**, Ratio of red-only puncta to total puncta. **e**, Schematic illustration of the target of autophagy inhibitors. Autophagy inhibitors MRT68921 (MRT), SBI-0206965 (SBI), and Spautin-1 were used. **f**, Viability of MC38 cells after treatment without or with aMPC₁₆-CA₅₀ (160 $\mu\text{g ml}^{-1}$) for 24 h in the absence or presence of autophagy inhibitors ($n = 3$ biological replicates). In **b**, representative images from two independent experiments. In **c,d,f**, data are mean $\pm$ s.d.. In **c,d**, cell numbers for each group (left to right) were $n = 38, 47, 43, 56, 32$ and 36 . Uncropped gel images are shown in Supplementary Fig. 1. Diagrams in **e** were created using BioRender, Xiong.M. <https://BioRender.com/p8xag3c>.



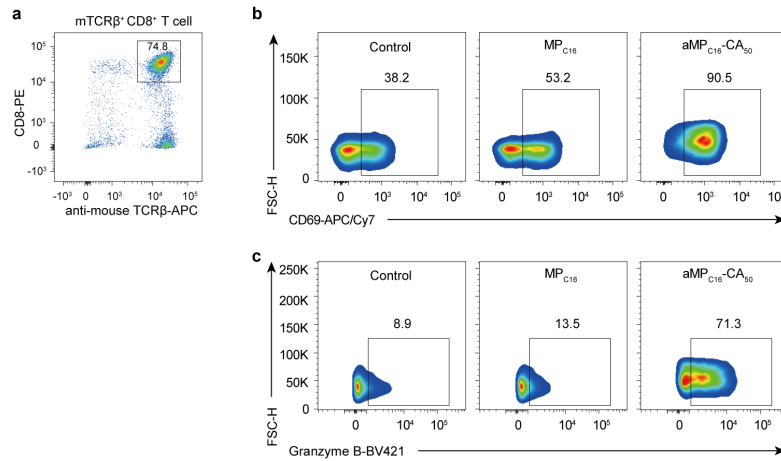
Supplementary Figure 12. RNA-seq analysis of aMP_{C16}-CA₅₀ or MP_{C16}-treated MC38 cells as compared to control cells. a–d, RNA-seq analysis of MC38 cells following treatment with aMP_{C16}-CA₅₀ (160 $\mu\text{g ml}^{-1}$) at pH 6.8 for varying durations, compared to untreated control. **a, Volcano scatter plots comparing gene expression levels. **b**, GO analysis of the upregulated genes. **c**, GSEA of transcriptional profiles**

using the hallmark gene sets from MSigDB. **d**, Enriched gene sets using GSEA. NES: normalized enrichment scores. FDR: false-discovery rate. **e–g**, RNA-seq analysis of MC38 cells following treatment with MP_{C16} (160 $\mu\text{g ml}^{-1}$) at pH 6.8 for 1 h, compared to untreated control. **e**, Schematic representation of the sample preparation strategy. **f**, Volcano scatter plots comparing gene expression levels. **g**, GO analysis of the upregulated genes. In **a,f**, upregulated (fold change >2 , $P < 0.05$) and downregulated (fold change <0.5 , $P < 0.05$) genes are depicted as red and blue dots, respectively. In **b,g**, the top 10 most significant enriched GO biological processes are shown, with immune and stress response-related processes highlighted in red. Statistical significance was determined using DESeq2 (two-sided Wald tests, no multiple-comparison adjustment) (**a,f**), DAVID (one-sided Fisher's exact test, EASE, no multiple-comparison adjustment) (**b,g**), and permutation testing with FDR for GSEA (**c,d**). Diagrams in **e** were created using BioRender, Xiong.M. <https://BioRender.com/p8xag3c>.

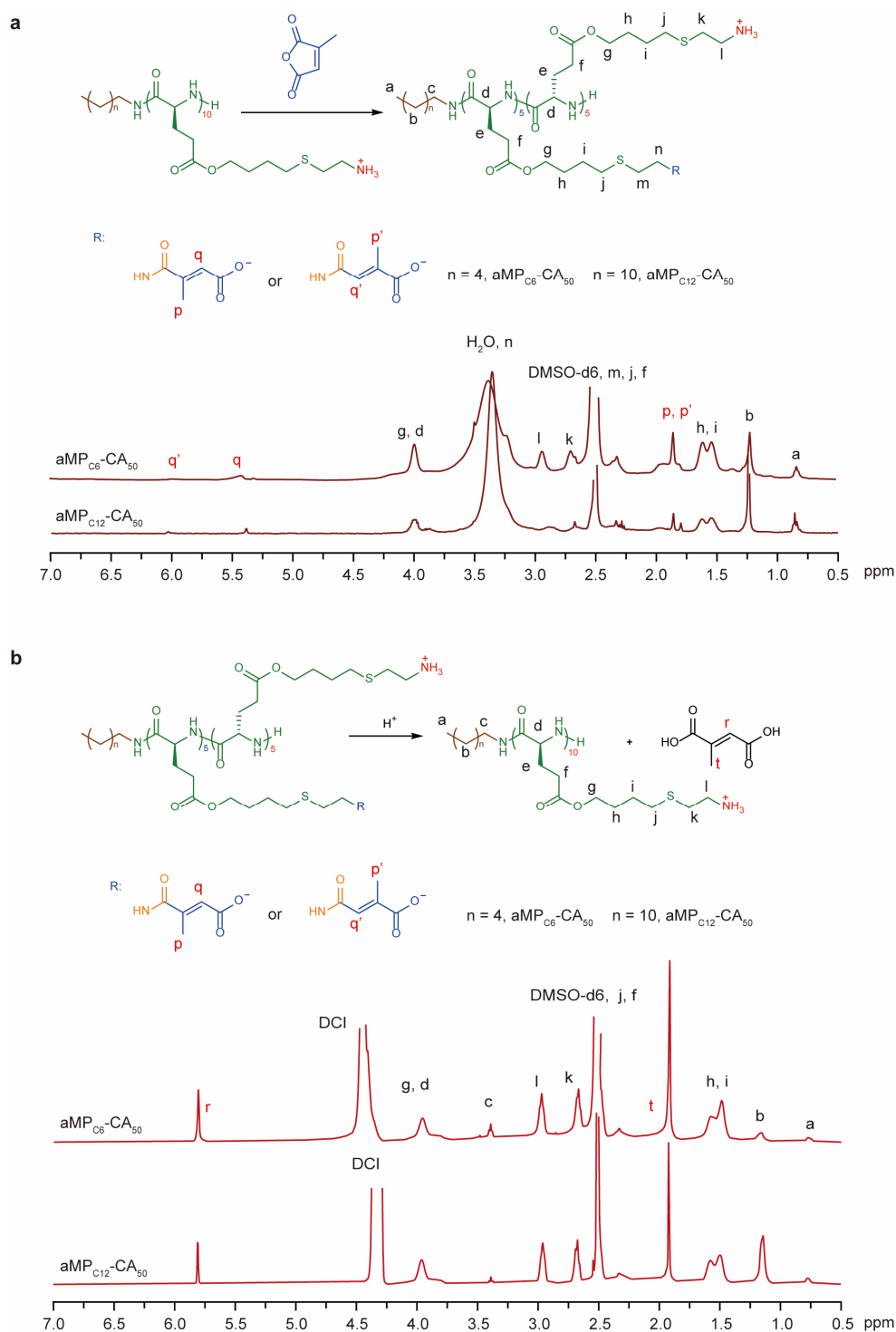


Supplementary Figure 13. $aMP_{C16-CA50}$ effectively enhances the ability of viable $MC38$ cells, but not lysed cells, to stimulate antigen cross-presentation and T cell activation. **a–c**, $MC38_{OVA}$ cells were treated with $aMP_{C16-CA50}$ or MP_{C16} ($160 \mu\text{g ml}^{-1}$) for varying durations at pH 6.8. BMDCs were co-incubated with the pretreated $MC38_{OVA}$ cells, and subsequent with OT-I T cells following the schedule depict in Fig. 3d. **a,b**, Flow cytometry analysis of CD80, CD86, and CD40 expression on BMDCs. **a**, Representative histograms from groups treated with MP_{C16} or $aMP_{C16-CA50}$ for 24 h. **c**, Representative plots from the flow cytometry analyses of IFN- γ , granzyme B, and perforin expression in $CD8^+$ T cells from groups treated with MP_{C16} or $aMP_{C16-CA50}$ for 24 h. **d–h**, Effect of MP_{C16} or $aMP_{C16-CA50}$ treatment on lysed $MC38_{OVA}$ cells. **d**, Experimental design. The $MC38_{OVA}$ cells were lysed *via* freeze–thaw process and then

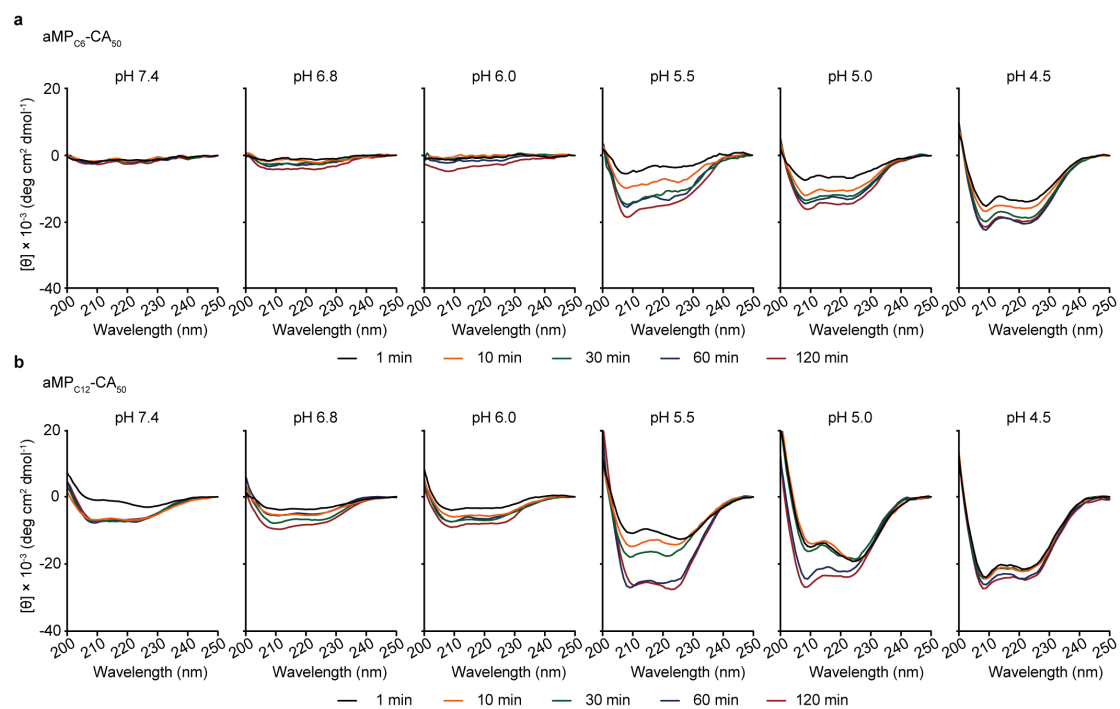
treated with aMP_{C16}-CA₅₀ or MP_{C16} (160 µg ml⁻¹) for varying durations at pH 6.8. **e–h**, Flow cytometry analysis of H-2K^b–SIINFEKL expression (**e**), and CD80, CD86, and CD40 expression (**f**) on BMDCs. Flow cytometry analysis of CD8⁺ T-cell proliferation (**g**) and IFN-γ, granzyme B, and perforin expression in CD8⁺ T cells (**h**). **i,j**, Effect of LAIs (**i**) and LEIs (**j**) on inhibiting the ability of aMP_{C16}-CA₅₀-treated MC38_{OVA} cells to activate OT-I CD8⁺ T cells. In **b,e–j**, data are mean ± s.d., *n* = 3 biological replicates. Statistical significance was determined using two-way ANOVA (**b**) and one-way ANOVA (**i,j**) with Tukey’s multiple-comparison test. Diagrams in **d** were created using BioRender, Xiong.M. <https://BioRender.com/p8xag3c>.



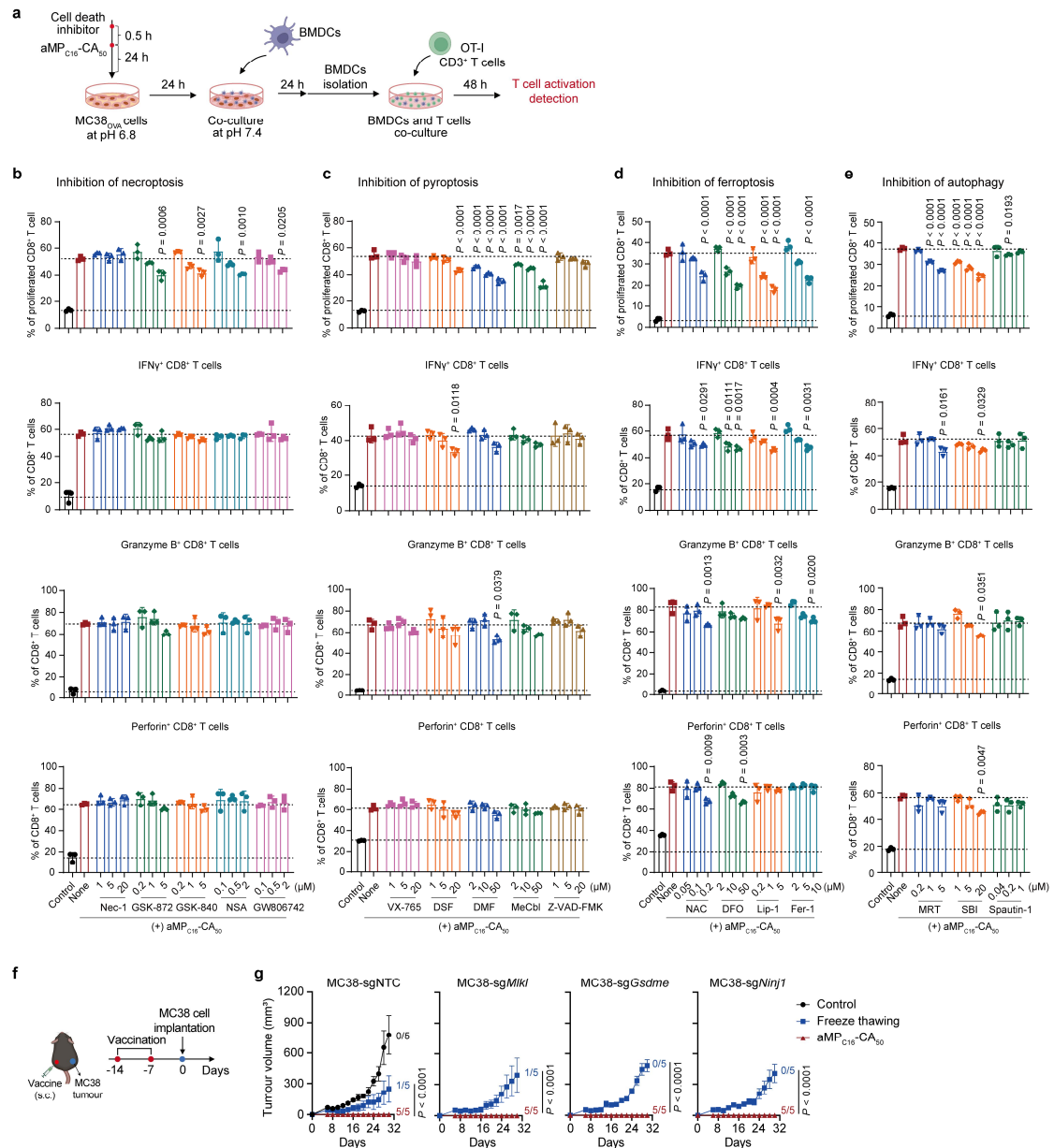
Supplementary Figure 14. Flow cytometry analysis of NY-ESO-1-specific TCR-T cells. **a**, Staining of NY-ESO-1-specific TCR-T cells with anti-mouse TCRβ-chain antibody and anti-human CD8 antibody. **b,c**, Representative plots showing CD69 (**b**) and Granzyme B (**c**) expression in NY-ESO-1-specific TCR-T cells. A549_{NY-ESO-1} cells were treated with aMP_{C16}-CA₅₀ or MP_{C16} (160 μg ml⁻¹) for 24 h and subsequently co-cultured with HLA-A*02:01 DCs from healthy donors. These DCs were then cocultured with NY-ESO-1₁₅₇₋₁₆₅-TCR engineered T cells.



Supplementary Figure 15. Synthesis routes and structural characterization of aMP_{C₆}-CA₅₀ and aMP_{C₁₂}-CA₅₀. **a**, Synthesis routes and ¹H-NMR spectra (in DMSO-d₆) of aMP_{C₆}-CA₅₀ and aMP_{C₁₂}-CA₅₀. **b**, ¹H-NMR spectra of aMP_{C₆}-CA₅₀ and aMP_{C₁₂}-CA₅₀ upon incubation with DMSO-d₆, D₂O, and DCI (400, 200, and 20 μl, respectively).

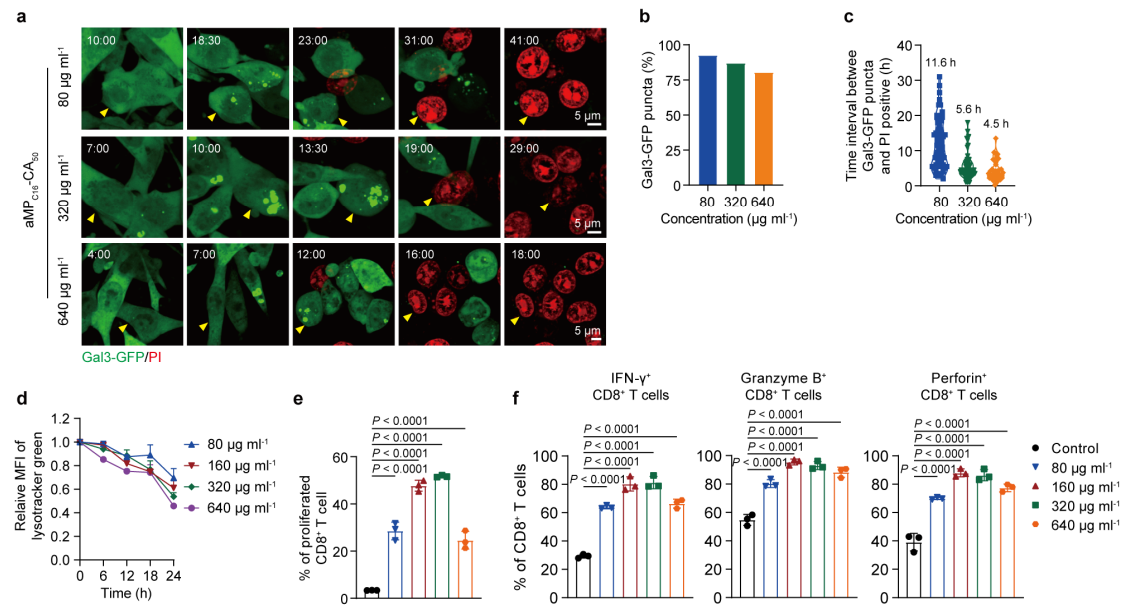


Supplementary Figure 16. Time-dependent CD spectra of $\text{aMP}_{\text{C6}}\text{-CA}_{50}$ (a) and $\text{aMP}_{\text{C12}}\text{-CA}_{50}$ (b) at different pH. Peptide concentration, $100 \mu\text{g ml}^{-1}$.

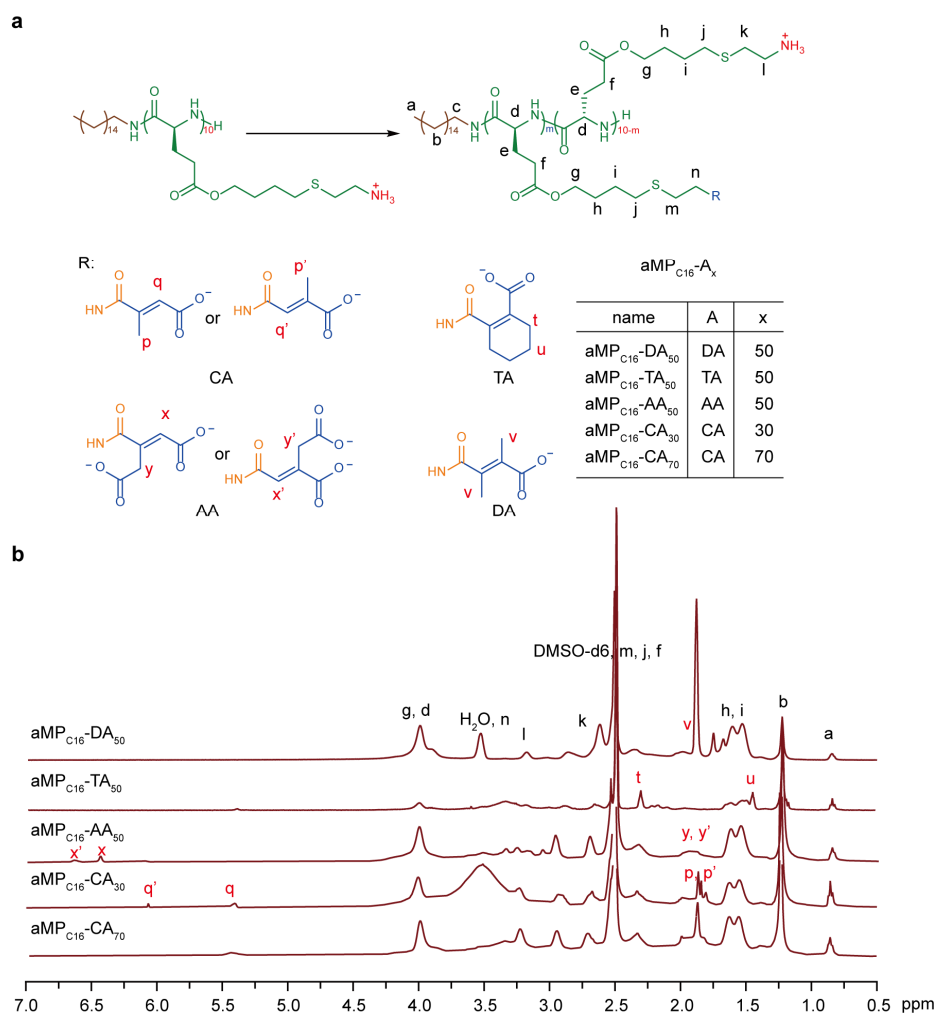


Supplementary Figure 17. The impact of regulated cell death pathways on the immunogenicity of aMP_{C16}-CA₅₀-treated tumour cells. a–e, Effect of cell death pathway inhibitors on the activation of CD8⁺ T cells. **a**, Experimental design. MC38_{OVA} cells were treated with aMP_{C16}-CA₅₀ (160 μg ml⁻¹) for 24 h in the presence or absence of inhibitors, followed by co-incubation with BMDCs. BMDCs were then isolated and co-cultured with OT-I T cells. CD8⁺ T cell proliferation and the expression of IFN-γ, perforin, and granzyme B in CD8⁺ T cells were assessed by flow cytometry. Inhibitors of necroptosis (**b**), pyroptosis (**c**), ferroptosis (**d**), and autophagy (**e**) were tested. Nec-1, necrostatin-1¹⁰¹; NSA, necrosulfonamide¹⁰²; GW806742, GW806742X hydrochloride¹⁰³; DSF, disulfiram¹⁰⁴; DMF, dimethyl fumarate¹⁰⁵; MeCbl, methylcobalamin¹⁰⁶; NAC, N-Acetyl-L-cysteine¹⁰⁷; DFO, deferoxamine¹⁰⁸, Lip-1, liproxstatin-1¹⁰⁹; Fer-1, ferostatin-1¹⁰⁹; MRT, MRT68921 dihydrochloride¹¹⁰; SBI, SBI-0206965¹¹¹. **f,g**, The impact of *Mkl* or *Gsdme* knockout, or incomplete *Ninj1* knockout on the vaccination efficacy of aMP_{C16}-CA₅₀-treated MC38 cells. **f**,

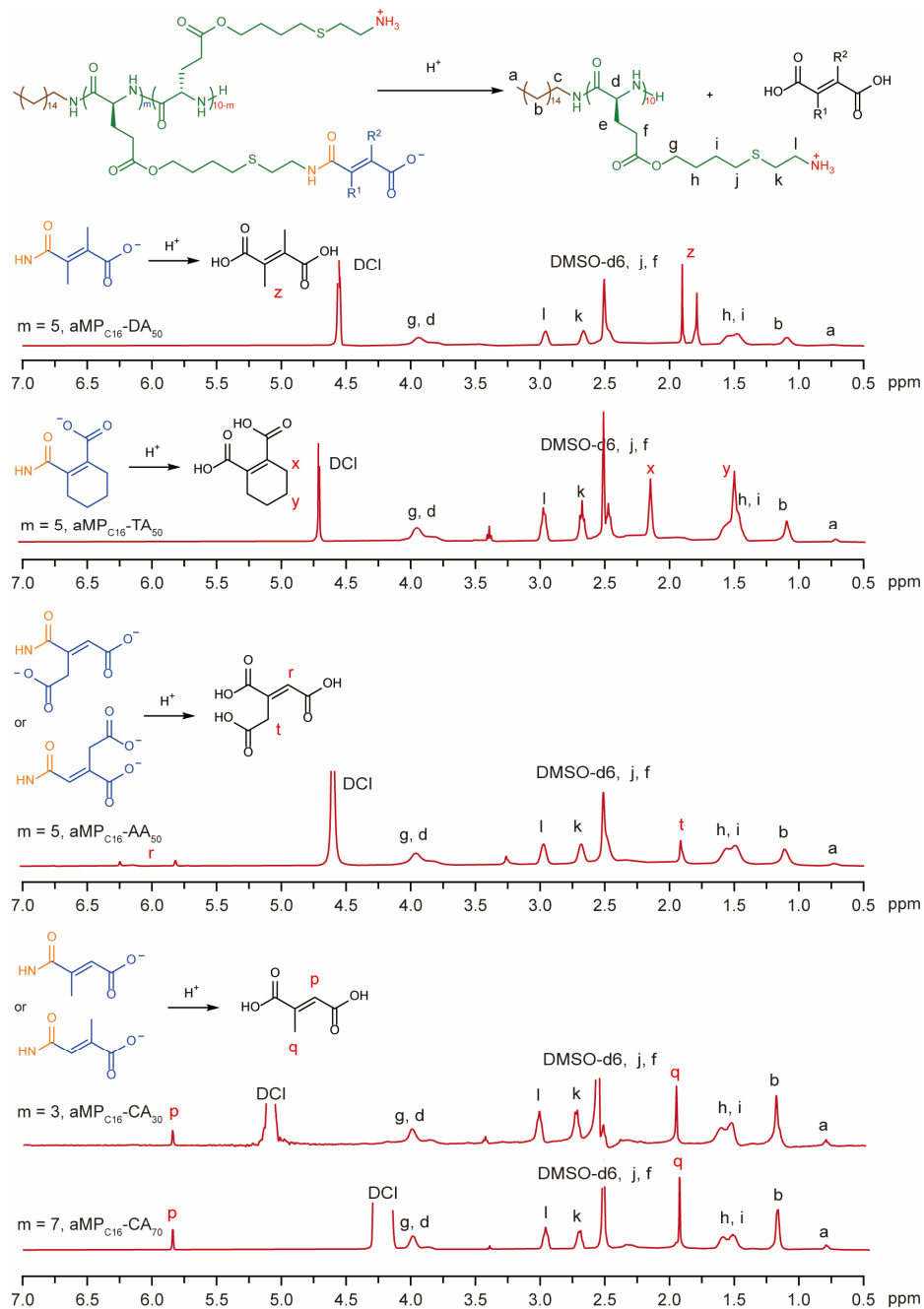
Experimental design. Mice were subcutaneously vaccinated with wildtype MC38 cells (MC38-sgNTC) or with cells deficient in *Mkl1* (MC38-sg*Mkl1*), *Gsdme* (MC38-sg*Gsdme*), or *Ninj1* (MC38-sg*Ninj1*), after treatment with aMP_{C16}-CA₅₀ (160 µg ml⁻¹) or freeze-thawing. **g**, Tumour growth curves following viable MC38 cell challenge in vaccinated mice. In **b–e**, data are mean ± s.d., *n* = 3 biological replicates. In **g**, data are mean ± s.e.m., *n* = 5 mice. Statistical significance was determined using one-way ANOVA comparing the indicated groups to the aMP_{C16}-CA₅₀-treatment group without inhibitors (**b–e**) and two-way ANOVA (**g**), each followed by Tukey's multiple-comparison test. Diagrams in **a,f** were created using BioRender, Xiong.M. <https://BioRender.com/p8xag3c> (**a**), <https://BioRender.com/ap2k7ea> (**f**).



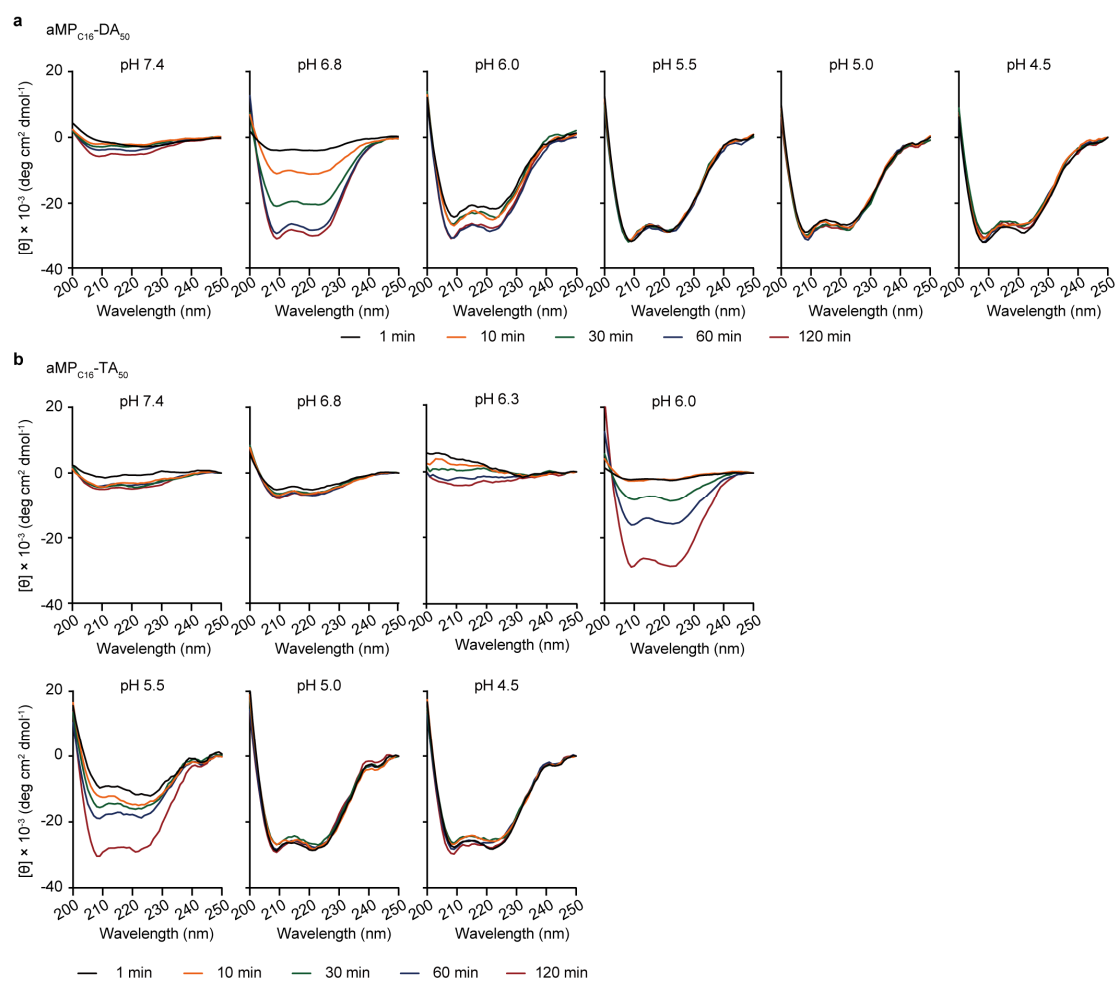
Supplementary Figure 18. Effect of aMP_{C16}-CA₅₀ concentration on modulating the LMR-PMR interval and enhancing the immunogenicity of lytic tumour cells. a–c, Time-lapse confocal imaging of MC38_{Gal3-GFP} cells incubated with aMP_{C16}-CA₅₀ at varying concentrations and PI (red) at pH 6.8. **a**, Representative images from two independent experiments. Scale bar, 5 μm. **b**, Percentage of MC38_{Gal3-GFP} cells exhibiting Gal3-GFP puncta. **c**, Time interval between Gal3-GFP puncta appearance and PI-positive staining in each cell. **d**, Flow cytometry analysis of the relative MFI of LysoTracker Green staining in aMP_{C16}-CA₅₀-treated MC38 cells compared to untreated cells. **e,f**, Analysis of CD8⁺ T cells activation. OT-I T cells were co-incubated with BMDCs that had been pre-incubated with aMP_{C16}-CA₅₀-treated MC38_{OVA} cells, following the treatment schedule in Fig. 3d. Flow cytometry analysis of CD8⁺ T-cell proliferation (**e**) and expression of IFN-γ, granzyme B, and perforin in CD8⁺ T cells (**f**). In **d–f**, data are mean ± s.d., *n* = 3 biological replicates. Statistical significance was determined using one-way ANOVA with Tukey's multiple-comparison test (**e,f**).



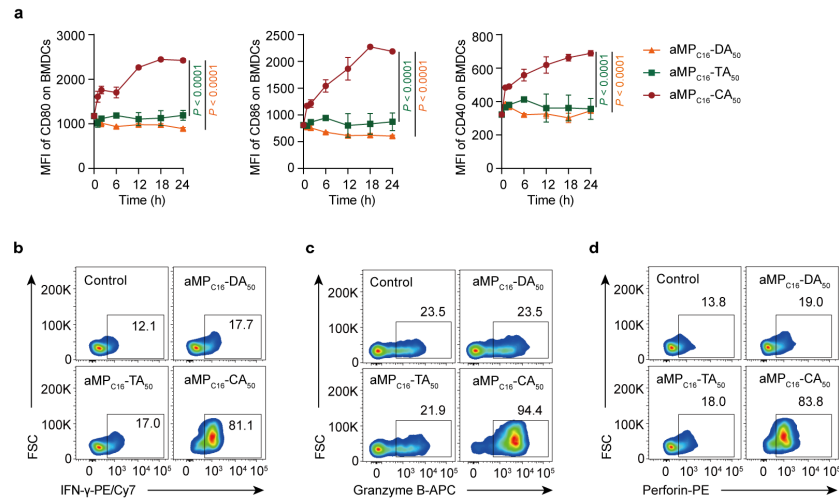
Supplementary Figure 19. Design and structural characterization of aMP_{C16}-A_x. **a**, Schematic illustration of the structures of aMP_{C16}-A_x with varying pH-responsive kinetics by altering types of side-chain modifying anhydrides and modification percentage of side-chain amines. 'A' represents the type of side-chain modifying anhydrides, including CA, TA, DA, and AA; and 'x' denotes the modification percentage of side-chain amines, including 30%, 50%, and 70%. **b**, ¹H-NMR spectra (in DMSO-d₆) of aMP_{C16}-A_x.



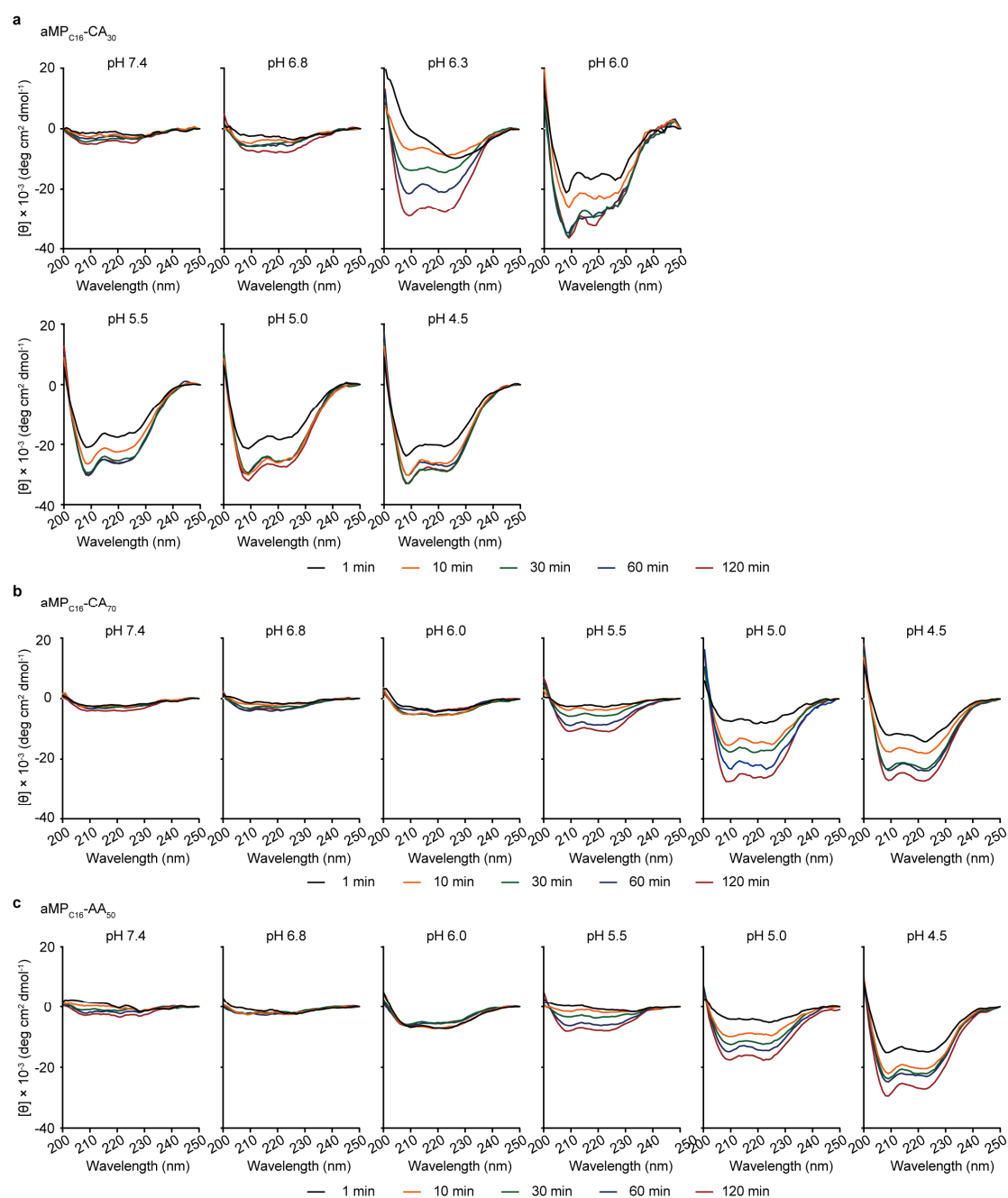
Supplementary Figure 20. ¹H-NMR spectra of aMP_{C16}-A_x upon incubation with DCI for 120 min. DMSO-d₆, D₂O, and DCI (400, 200, and 20 μl, respectively) were used.



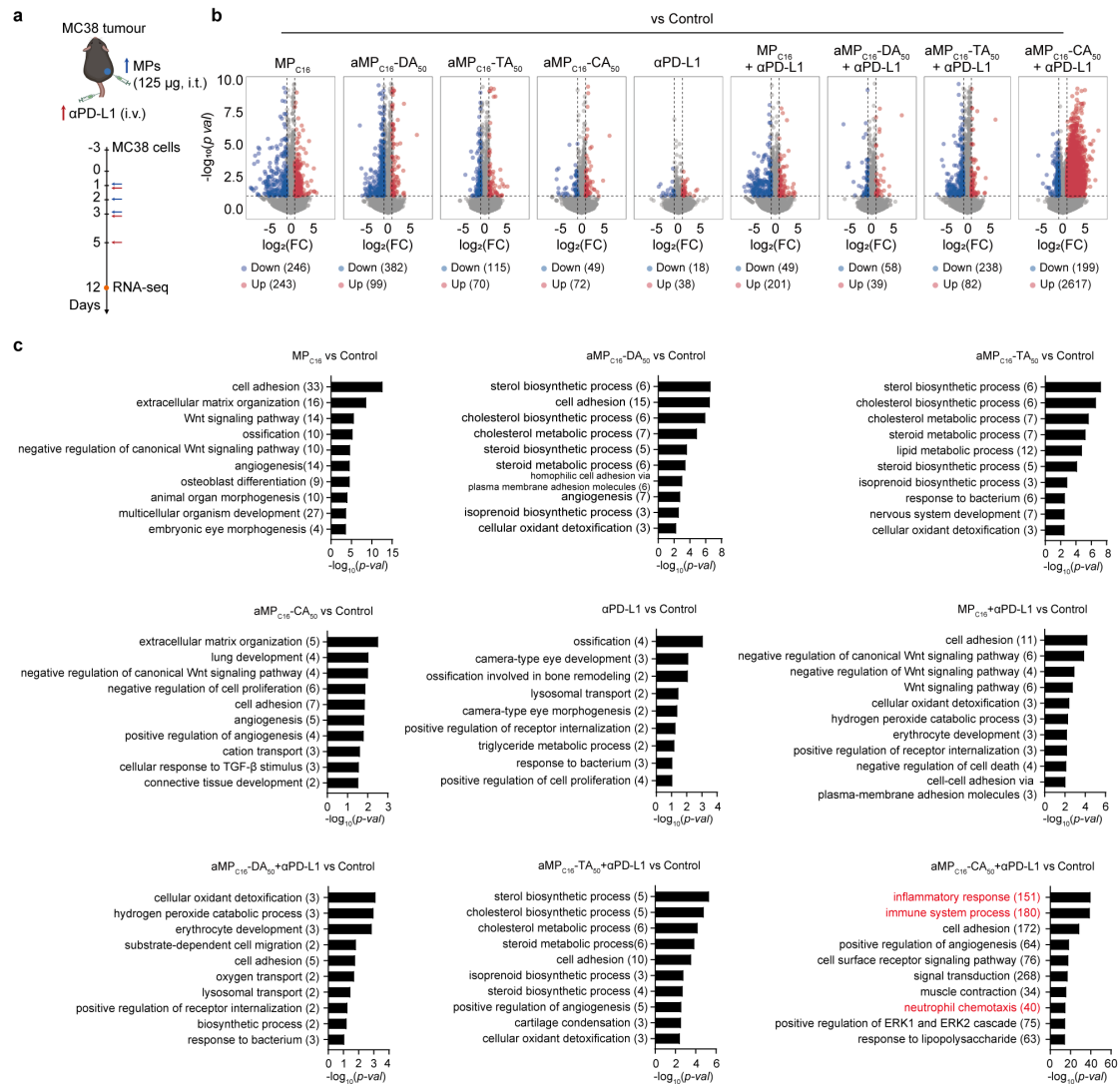
Supplementary Figure 21. Time-dependent CD spectra of $\text{aMP}_{\text{C16}}\text{-DA}_{50}$ (a) and $\text{aMP}_{\text{C16}}\text{-TA}_{50}$ (b) at different pH. Peptide concentration, $100 \mu\text{g ml}^{-1}$.



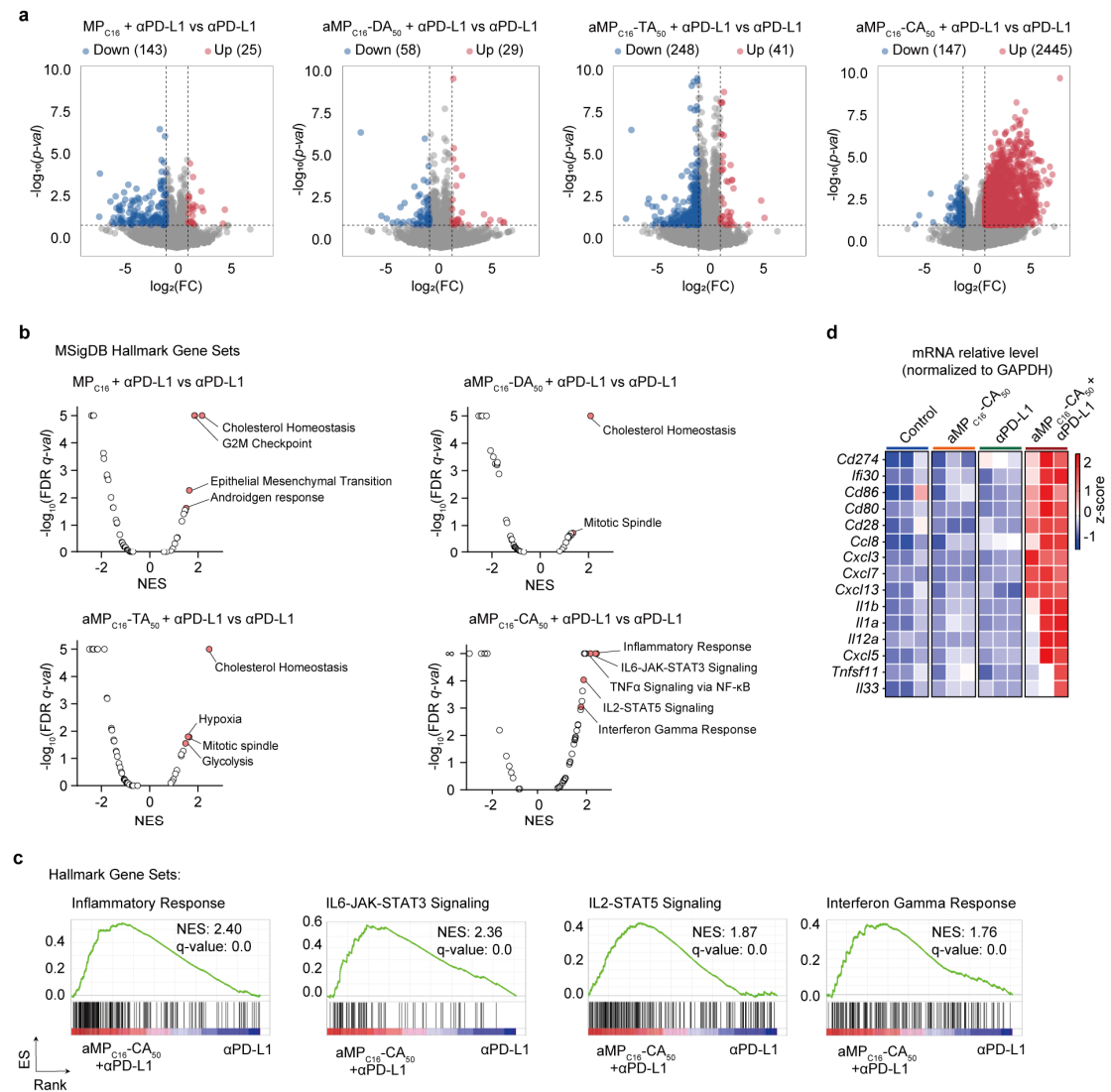
Supplementary Figure 22. pH-responsive kinetics of aMPs are crucial for enhancing the immunogenicity of lytic tumour cells. Following the experimental scheme depicted in Fig. 3d, MC38_{OVA} cells were treated with aMP_{C16}-CA₅₀, aMP_{C16}-TA₅₀, or aMP_{C16}-DA₅₀ (160 $\mu\text{g ml}^{-1}$) at pH 6.8 for varying durations. BMDCs were co-cubated with the pretreated MC38_{OVA} cells, and subsequent with OT-I T cells. **a**, Flow cytometry analysis of CD80, CD86, and CD40 expression on BMDCs. **b–d**, Representative flow cytometry plots showing IFN- γ (**b**), granzyme B (**c**), and perforin (**d**) expression in CD8⁺ T cells from groups treated with aMP_{C16}-CA₅₀, aMP_{C16}-TA₅₀, or aMP_{C16}-DA₅₀ (160 $\mu\text{g ml}^{-1}$) for 24 h. In **a**, data are mean $\pm$ s.d., $n = 3$ biological replicates, and statistical significance was determined using two-way ANOVA with Tukey's multiple-comparison test.



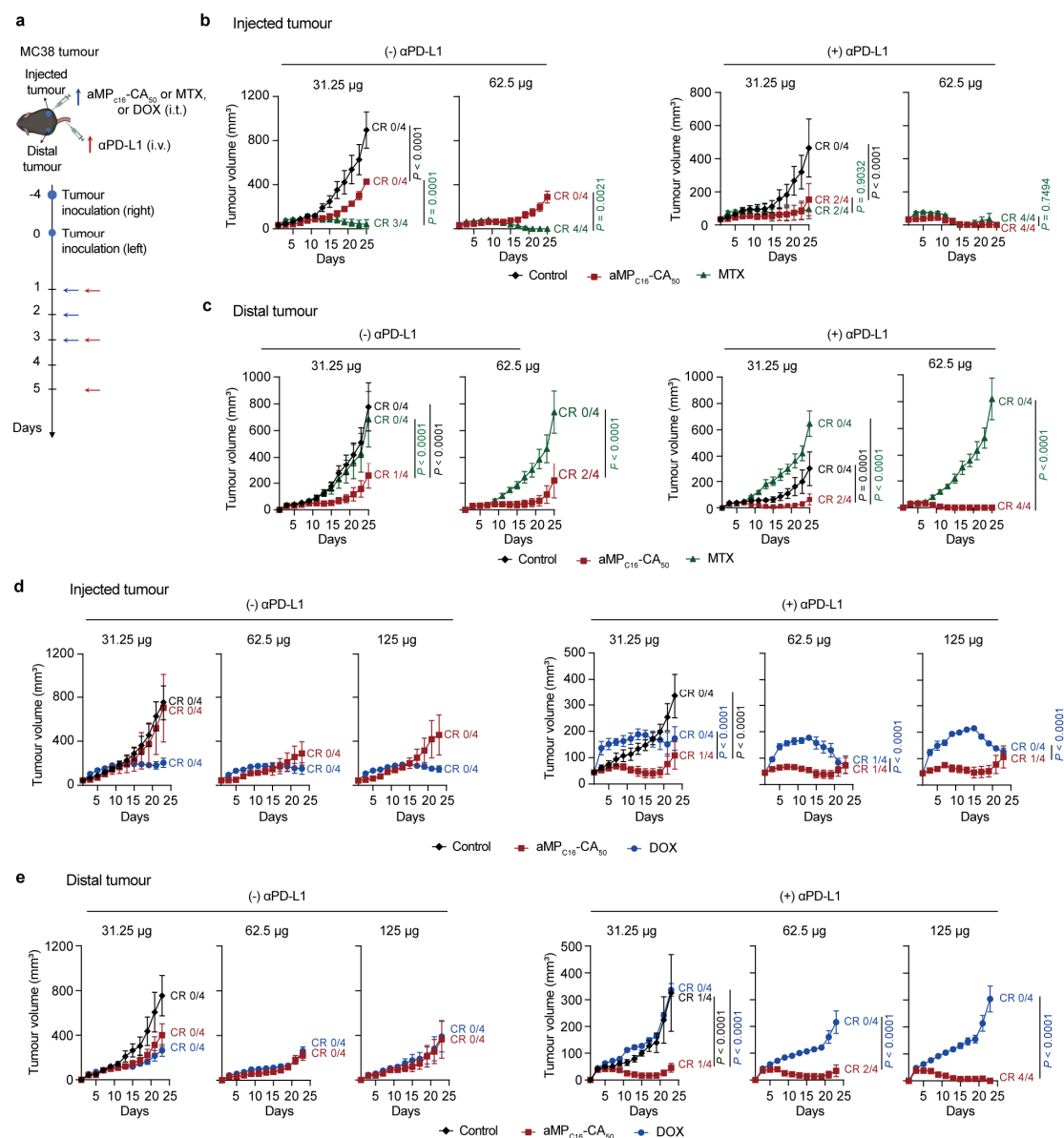
Supplementary Figure 23. Time-dependent CD spectra of aMP_{C16}-CA₃₀ (a), aMP_{C16}-CA₇₀ (b), and aMP_{C16}-AA₅₀ (c) at different pH. Peptide concentration, 100 $\mu\text{g ml}^{-1}$.



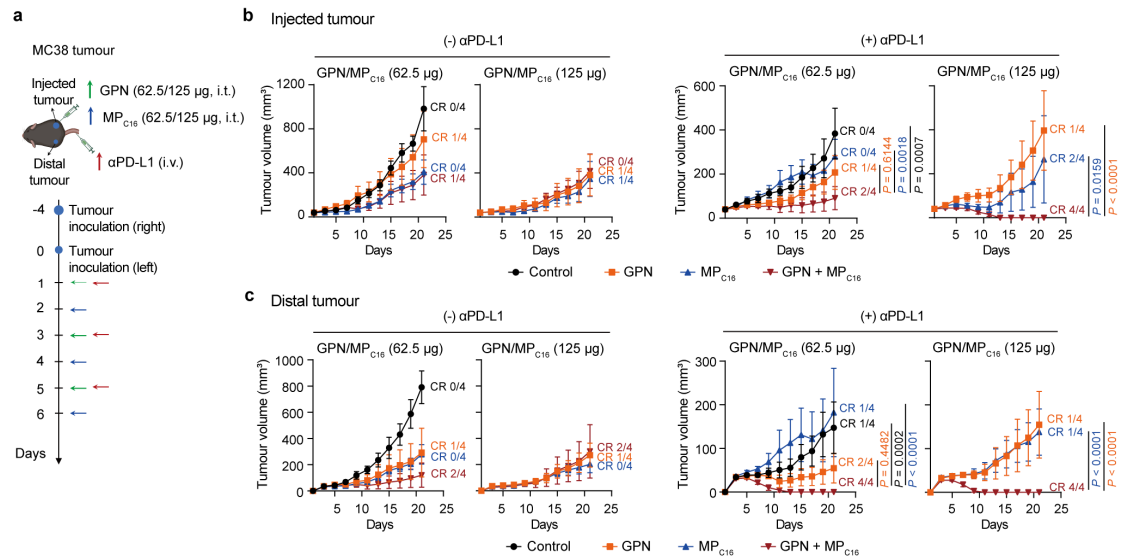
Supplementary Figure 25. Transcriptomics analysis of MC38 tumours by comparing the indicated treatment groups with the control group. **a**, Schematic representation of the schedule employed for tumour inoculation, treatments, and the isolation of MC38 tumour tissue samples for RNA-seq analysis. $n = 3$ mice. **b**, Volcano scatter plots comparing gene expression in the indicated treatment groups with the control group. Upregulated (fold change >2 , $P < 0.05$) and downregulated (fold change <0.5 , $P < 0.05$) genes are depicted as red and blue dots, respectively. **c**, GO analysis of the upregulated genes. The top 10 most significant enriched GO biological processes are shown, with immune response-related processes highlighted in red. Statistical significance was determined using DESeq2 (two-sided Wald tests, no multiple-comparison adjustment) (**b**), DAVID (one-sided Fisher's exact test, EASE, no multiple-comparison adjustment) (**c**). Diagram in **a** was created using BioRender, Xiong.M. <https://BioRender.com/ap2k7ea>.



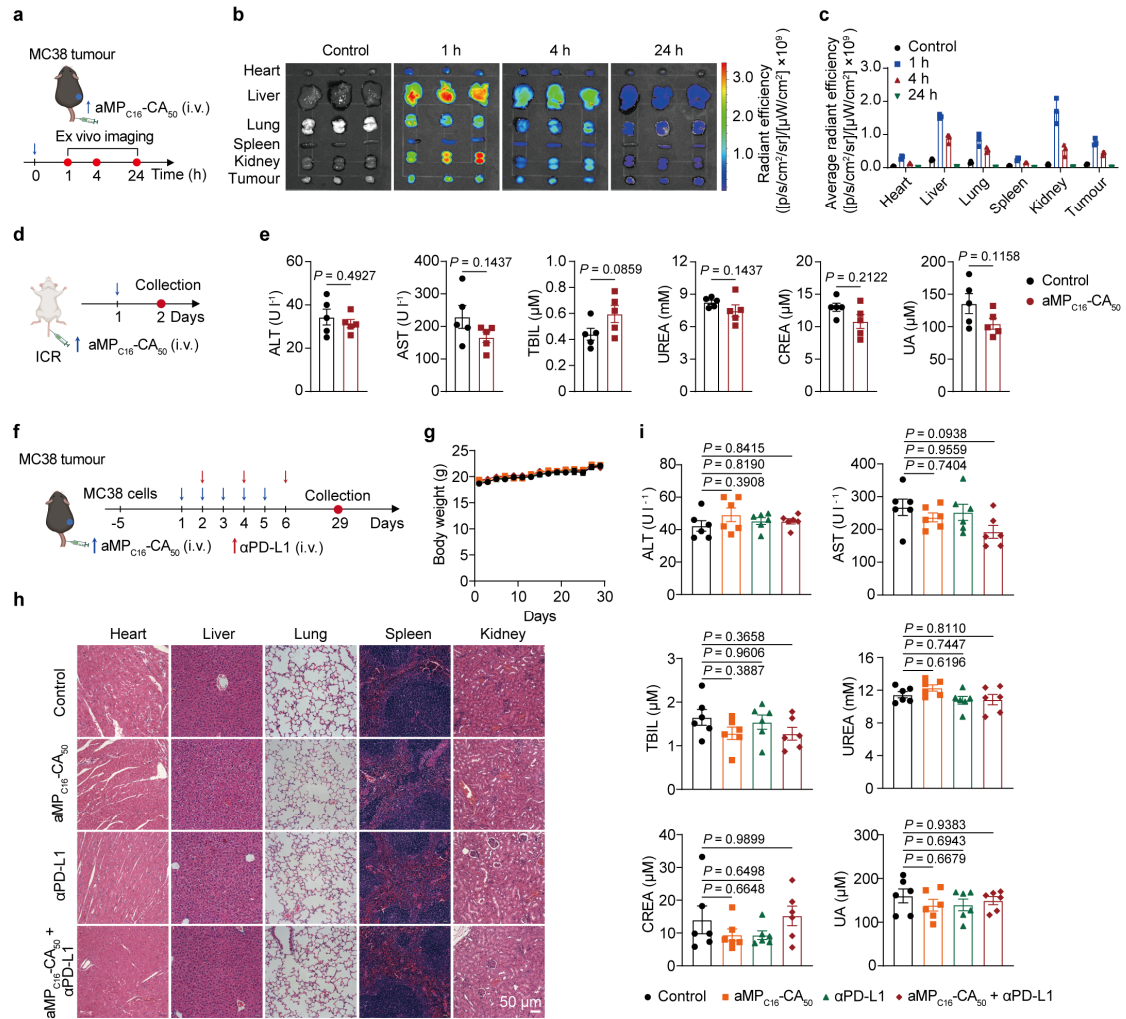
Supplementary Figure 26. Transcriptomics analysis of MC38 tumours by comparing the combined (MPs and α PD-L1) and single α PD-L1 treatment groups. **a**, Volcano scatter plots comparing gene expression levels. Upregulated (fold change >2 , $P < 0.05$) and downregulated (fold change <0.5 , $P < 0.05$) genes are depicted as red and blue dots, respectively. **b**, GSEA of transcriptional profiles using the hallmark gene sets from MSigDB. **c**, Enriched gene sets using GSEA. NES: normalized enrichment scores. FDR: false-discovery rate. **d**, qRT-PCR analysis of representative inflammatory response-related genes that were upregulated in the MC38 tumours of the combined (aMP_{C16}-CA₅₀ and α PD-L1) treatment groups. Statistical significance was determined using DESeq2 (two-sided Wald tests, no multiple-comparison adjustment) (**a**), and by permutation testing with FDR for GSEA (**b,c**).



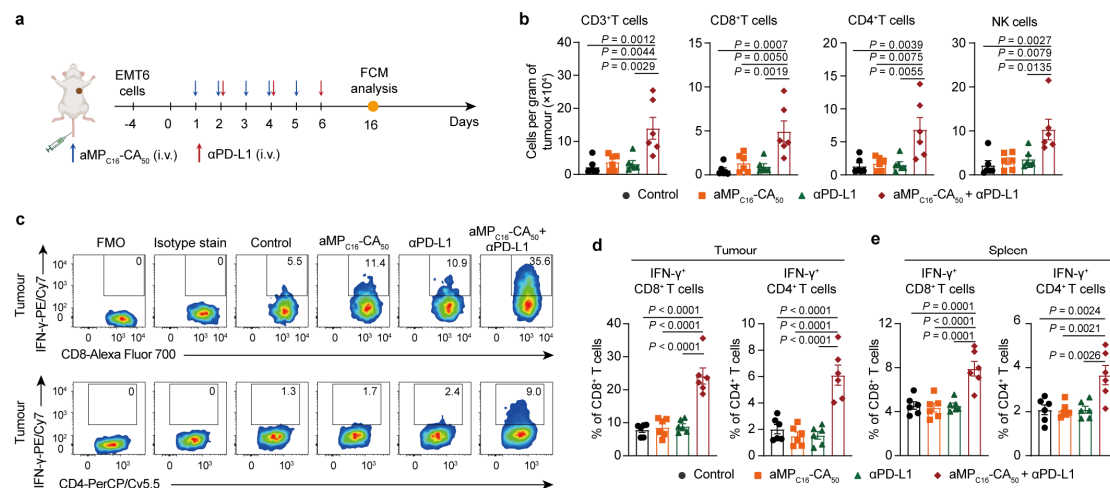
Supplementary Figure 27. Comparison of aMP_{C16}-CA₅₀ versus MTX/DOX on enhancing antitumour efficacy of α PD-L1 therapy. **a**, Experimental schedule for tumour inoculation and treatments. Mice bearing bilateral MC38 tumours received i.t. injections of either aMP_{C16}-CA₅₀, MTX or DOX into the right flank tumours, in combination with α PD-L1 (i.v., 0.75 mg kg⁻¹). **b–e**, Tumour growth curves of the injected (**b,d**) and distal (**c,e**) tumours. CR, complete response. In **b–e**, data are mean $\pm$ s.e.m., $n = 4$ mice, and statistical significance was determined using two-way ANOVA followed by Tukey's multiple-comparison test. Diagram in **a** was created using BioRender, Xiong.M. <https://BioRender.com/ap2k7ea>.



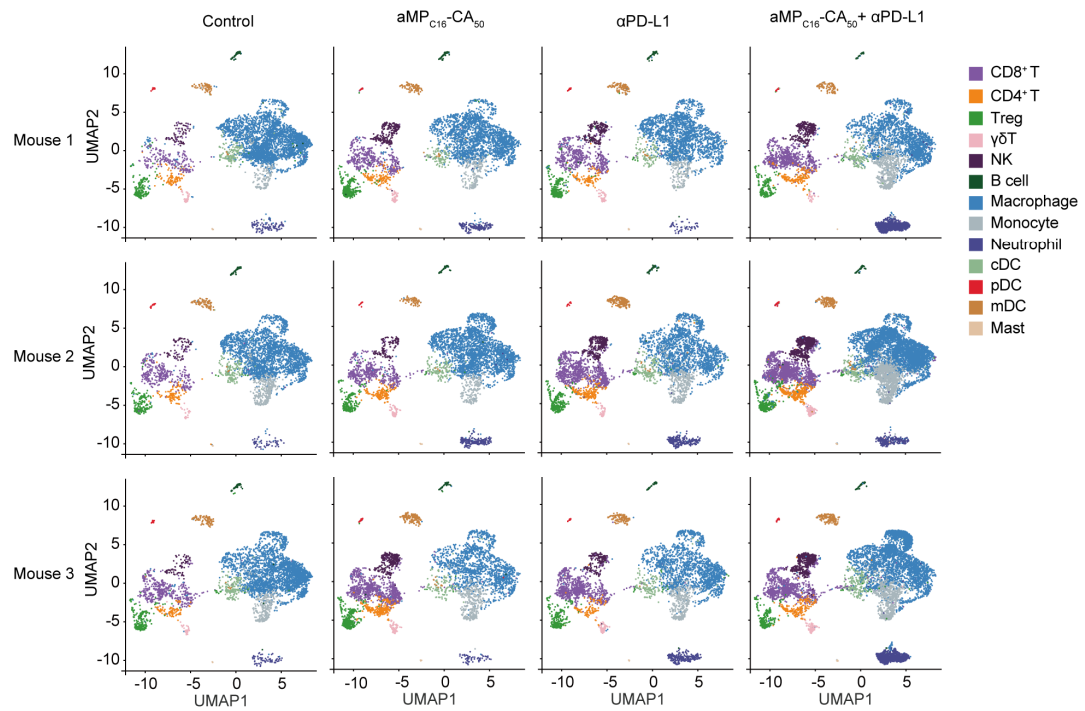
Supplementary Figure 28. Combination of GPN and MPC₁₆ markedly potentiates αPD-L1 therapy. **a**, The schedule for tumour inoculation and treatments of mice. Mice were inoculated with MC38 cells on both the right and left flanks to establish two tumours per mouse, followed by i.t. injections of GPN and/or MPC₁₆ into the right flank tumours and the i.v. administration of αPD-L1 (0.75 mg kg⁻¹). Antitumour efficacy was evaluated for both the right flank tumours (injected tumours) and the left flank tumours (distal tumours). **b,c**, Tumour growth curves of injected tumours (**b**) and distal tumours (**c**). CR, complete response. In **b,c**, data are mean ± s.e.m., *n* = 4 mice, and statistical significance was determined using two-way ANOVA followed by Tukey's multiple-comparison test. Diagram in **a** was created using BioRender, Xiong.M. <https://BioRender.com/ap2k7ea>.



Supplementary Figure 29. *In vivo* biodistribution and toxicity of aMP_{C16}-CA₅₀. **a–c**, Tumour accumulation and major organ distribution of aMP_{C16}-CA₅₀-Cy5 following intravenous injection. *n* = 3 mice. **a**, Experimental design. aMP_{C16}-CA₅₀-Cy5 (15 mg kg⁻¹) was intravenously administered to MC38 tumour-bearing mice at Day 0. **b,c**, Fluorescence images (**b**) and average radiant efficiency (**c**) of major organs and tumours. **d,e**, Evaluating the toxicity in ICR mice at 24 h following intravenous administration of aMP_{C16}-CA₅₀ at a dose of 15 mg kg⁻¹. *n* = 5 mice. **d**, Experimental design. **e**, Concentrations of alanine transaminase (ALT), aspartate aminotransferase (AST), total bilirubin (TBIL), urea (UREA), creatinine (CREA), and uric acid (UA) in serum samples. **f–i**, Evaluating the toxicity in MC38 tumour-bearing mice following intravenous administration of aMP_{C16}-CA₅₀ and αPD-L1 at a dose of 15 mg kg⁻¹ and 0.75 mg kg⁻¹, respectively. *n* = 6 mice. **f**, Experimental design. **g**, Mice weight change during and after treatments. **h**, H&E staining of heart, liver, lung, spleen, and kidney tissue sections. Scale bars, 50 μm. **i**, Concentrations of ALT, AST, TBIL, UREA, CREA, and UA in serum samples. Tissues and serum samples were collected at day 29. In **c,e,g,i**, data are mean ± s.e.m.. Statistical significance was determined using Student's *t*-tests (**e**) and one-way ANOVA with Tukey's multiple-comparison test (**i**). Representative images from three independent mice are shown in **h**. Diagrams in **a,d,f** were created using BioRender, Xiong.M. <https://BioRender.com/ap2k7ea>.



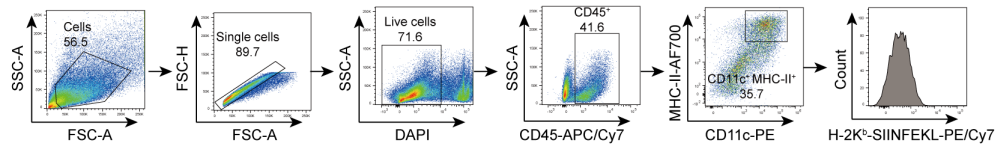
Supplementary Figure 30. Flow cytometry analysis of T cells in the tumour and spleen of EMT6 tumour-bearing mice. **a**, Schematic representation of tumour inoculation, treatments, and sample isolation for flow cytometry analyses. Mice bearing orthotopic EMT6 tumours were intravenously administered with aMP_{C16}-CA₅₀ and αPD-L1 at doses of 15 and 0.75 mg kg⁻¹, respectively. **b**, Counts of CD3⁺, CD8⁺, and CD4⁺ T cells as well as NK cells, per gram of tumour tissue. **c**, Representative flow cytometry plots of IFN-γ expression in CD8⁺ and CD4⁺ T cells from tumour tissues. FMO, fluorescence minus one; isotype stain, isotype control antibody staining. **d, e**, Percentage of IFN-γ-positive cells among CD8⁺ and CD4⁺ T cells in tumour (**d**) and spleen (**e**). In **b, d, e**, data are mean ± s.e.m., *n* = 6 mice, and statistical significance was determined using one-way ANOVA with Tukey's multiple-comparison test. Diagram in **a** was created using BioRender, Xiong.M. <https://BioRender.com/ap2k7ea>.



Supplementary Figure 31. scRNA-seq transcriptional profiling of CD45⁺ cells isolated from MC38 tumour. UMAP of all CD45⁺ cells analyzed denoting experimental replicates and cell types.

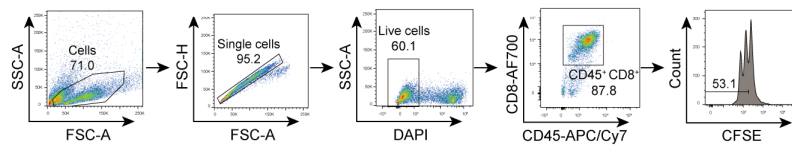
SI Fig. 32a

Fig. 3e,f, 4j,k



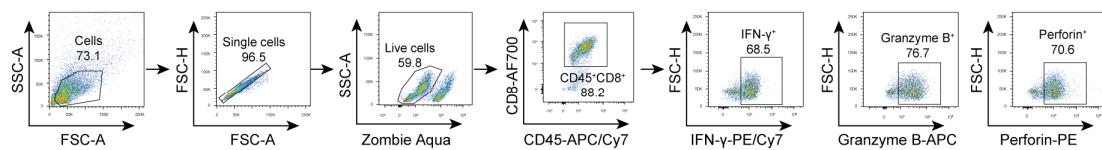
SI Fig. 32b

Fig. 3g,h, 4l,m, Extended data Fig 3l,t,w, 4g,i, 7k, Supplementary Fig. 18e



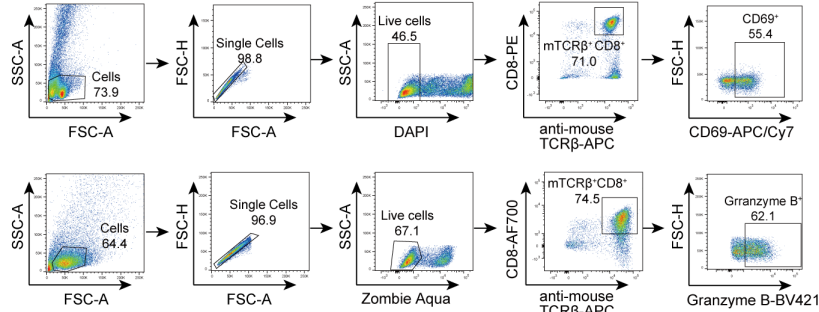
SI Fig. 32c

Fig. 3i, 4n, Extended data Fig. 2h,j, Supplementary Fig. 13c, 22b-d



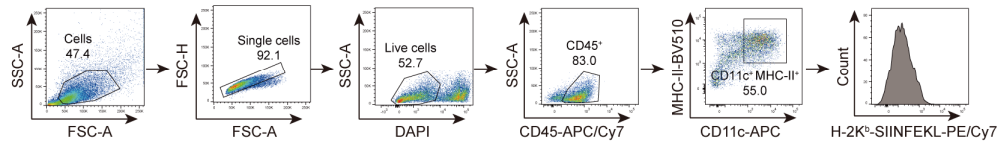
SI Fig. 32d

Fig. 3l, Supplementary Fig. 14



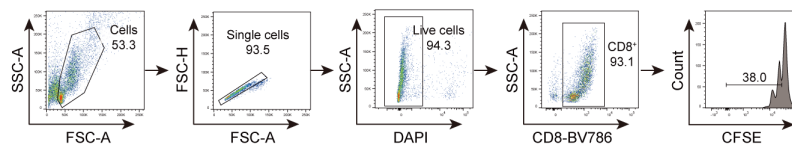
SI Fig. 32e

Fig. 3m,o



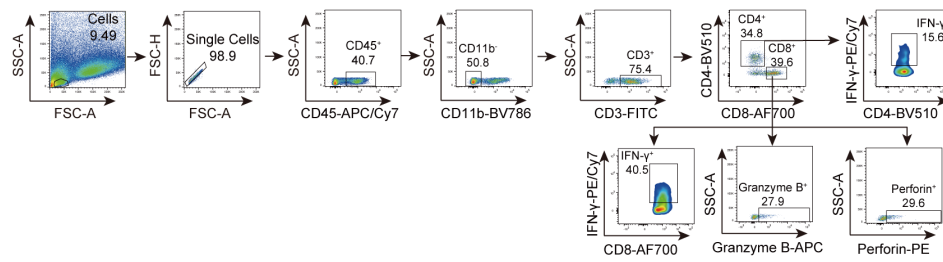
SI Fig. 32f

Fig. 3n, p, Supplementary Fig. 17b-e, 13g



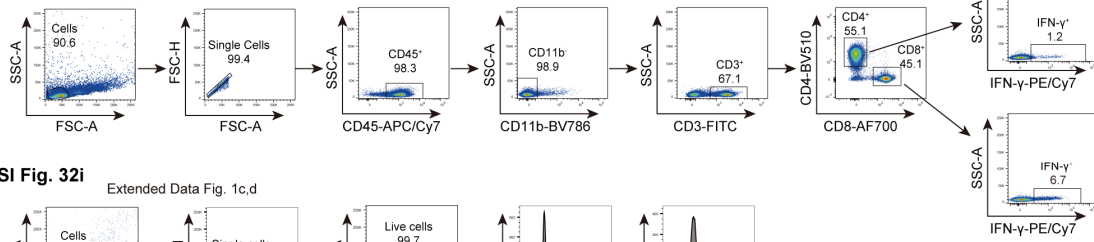
SI Fig. 32g

Fig. 5e-i



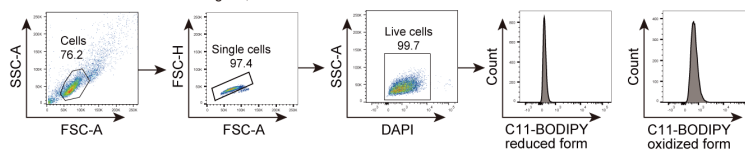
SI Fig. 32h

Fig. 5j-k



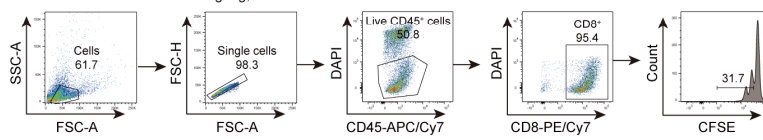
SI Fig. 32i

Extended Data Fig. 1c,d



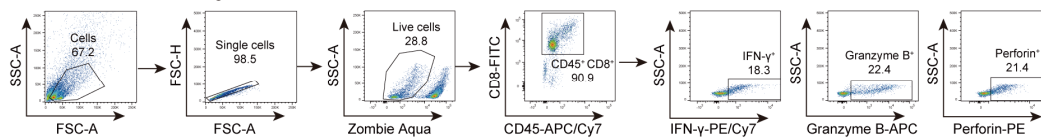
SI Fig. 32j

Extended Data Fig. 2g,i



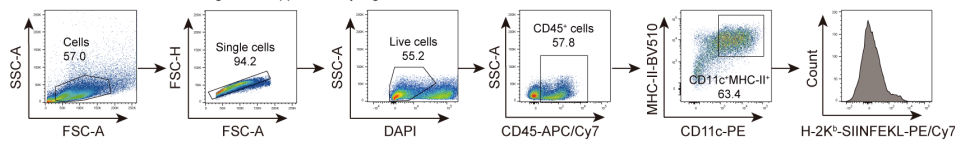
SI Fig. 32k

Extended Data Fig. 3m,u,x, 4h, 7l

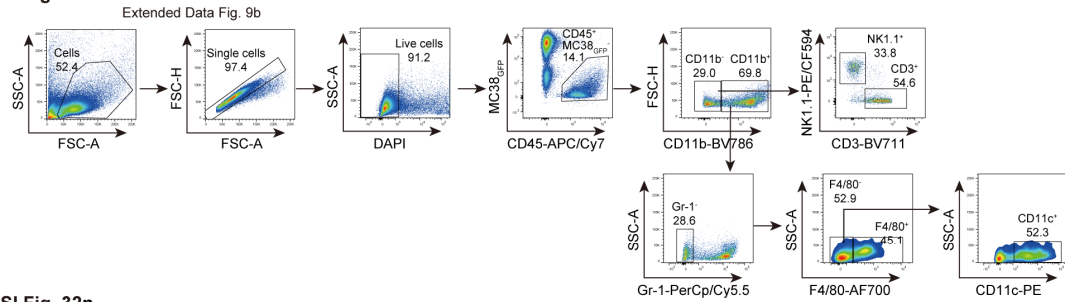


SI Fig. 32l

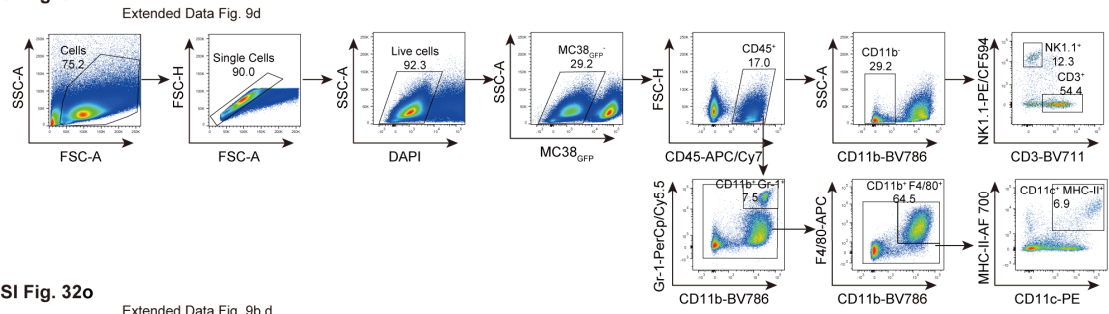
Extended Data Fig. 3s,v, Supplementary Fig. 13e



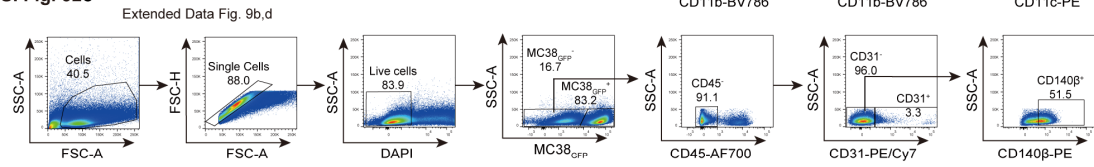
SI Fig. 32m



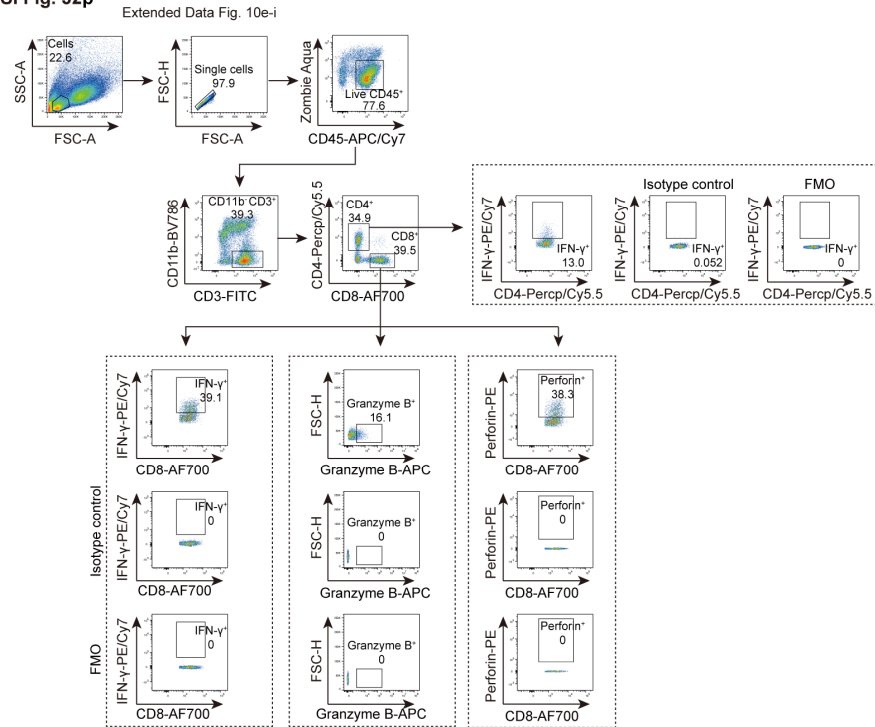
SI Fig. 32n



SI Fig. 32o

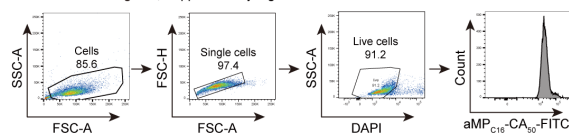


SI Fig. 32p



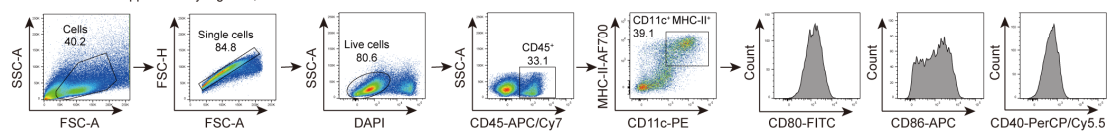
SI Fig. 32q

Fig. 1m, Supplementary Fig. 8a



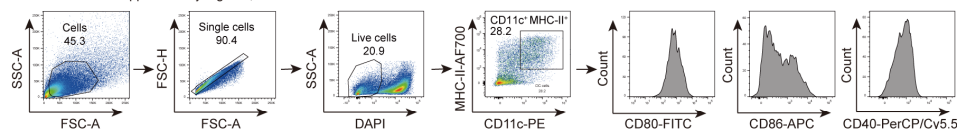
SI Fig. 32r

Supplementary Fig. 13a,b



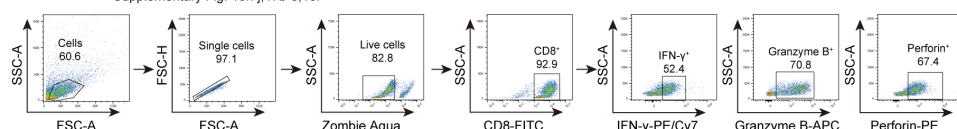
SI Fig. 32s

Supplementary Fig. 13f, 22a



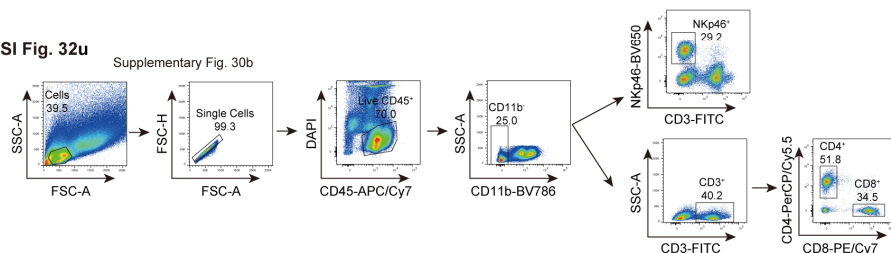
SI Fig. 32t

Supplementary Fig. 13h-j, 17b-e, 18f



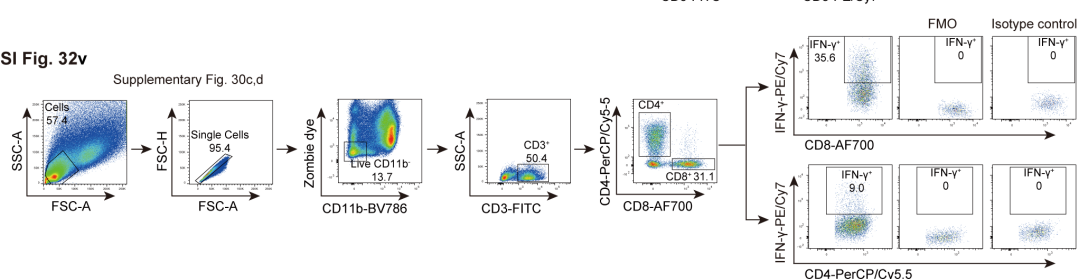
SI Fig. 32u

Supplementary Fig. 30b



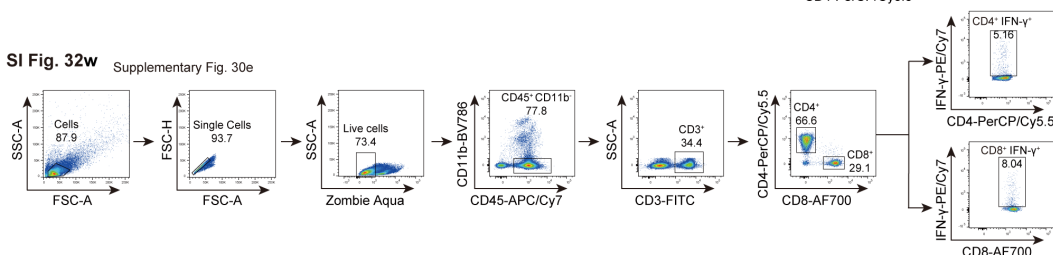
SI Fig. 32v

Supplementary Fig. 30c,d



SI Fig. 32w

Supplementary Fig. 30e



Supplementary Figure 32. Gating strategies for flow cytometry analysis. **a**, Gating strategy of Fig. 3e,f, 4j,k. **b**, Gating strategy of Fig. 3g,h, 4l,m, Extended Data Fig. 3l,t,w, 4g,i, 7k, and Supplementary Fig. 18e. **c**, Gating strategy of Fig. 3i, 4n, Extended Data Fig. 2h,j, and Supplementary Fig. 13c, 22b-d. **d**, Gating strategy of Fig. 3l and Supplementary Fig. 14. **e**, Gating strategy of Fig. 3m,o. **f**, Gating strategy of Fig. 3n,p, and Supplementary Fig. 17b-e, 13g. **g**, Gating strategy of Fig. 5e-i. **h**, Gating strategy of Fig. 5j-k. **i**, Gating strategy of Extended Data Fig. 1c,d. **j**, Gating strategy of

Extended Data Fig. 2g,i. **k**, Gating strategy of Extended Data Fig. 3m,u,x, 4h, 7l. **l**, Gating strategy of Extended Data Fig. 3s,v, and Supplementary Fig. 13e. **m**, Gating strategy of Extended Data Fig. 9b. **n**, Gating strategy of Extended Data Fig. 9d. **o**, Gating strategy of Extended Data Fig. 9b,d. **p**, Gating strategy of Extended Data Fig. 10e-i. **q**, Gating strategy of Fig. 1m and Supplementary Fig. 8a. **r**, Gating strategy of Supplementary Fig. 13a,b. **s**, Gating strategy of Supplementary Fig. 13f, 22a. **t**, Gating strategy of Supplementary Fig. 13h-j, 17b-e, 18f. **u**, Gating strategy of Supplementary Fig. 30b. **v**, Gating strategy of Supplementary Fig. 30c,d. **w**, Gating strategy of Supplementary Fig. 30e.

Supplementary Table S1. Hydrogen bond (HB) occupancies for MP_{C16} and aMP_{C16}-CA₅₀. Representative types of HBs along with their occupancies are listed for each peptide (B and S represent backbone and side-chain, respectively). A 10-ns MD simulation was performed for each peptide to evaluate the HB interactions. The simulation began using the elongated structure, which was derived using steered MD simulation, with the terminal C_α atoms being restrained by a force constant of 1,000 kJ mol⁻¹ nm⁻². The HBs between backbones and side chains as well as between the side chains were monitored for each frame of the simulation trajectory; the occupancy for each type of HB was calculated using the VMD software. In **Table S1**, N (B, residue 4) represents the nitrogen atom of the backbone amide group in residue 4, and OA (S, residue 10), the oxygen atom of the side-chain ester group in residue 10. N1 represents the nitrogen atom of the positively-charged amide group. OA (carbonyl oxygen) represents the oxygen atom in the ester group of the side chains. OM1 and OM2 represent the carbonyl oxygen in the negatively-charged carboxyl groups, while OD represents the carbonyl oxygen of amide groups in the side chains of aMP_{C16}-CA₅₀. S represents the sulfur atom in the side chain of either aMP_{C16}-CA₅₀ or MP_{C16}.

Peptide	Types of H-bonds	Donor	Acceptor	Occupancy
MP _{C16}	BB-SC	N (B, residue7)	OA (S, residue 10)	18.59%
		N (B, residue 2)	S (S, residue 4)	14.04%
		N1 (S, residue 4)	O (B, residue 2)	12.59%
		N (B, residue 7)	OA (S, residue 7)	5.71%
aMP _{C16} -CA ₅₀	BB-SC	N (B, residue 7)	OM1 (S, residue 7)	61.65%
		N1 (S, residue 7)	O (B, residue 10)	53.14%
		N (B, residue 6)	OM2 (S, residue 7)	36.81%
		N (B, residue 10)	OD (S, residue 8)	30.38%
		N (B, residue 9)	S (S, residue 9)	22.22%

Supplementary Table S2. Primer sequences used for real-time qPCR.

Gene Symbol	Forward (5' -> 3')	Reverse (5' -> 3')
<i>Cxcl1</i>	CTGGGATTACCTCAAGAACATC	CAGGGTCAAGGCAAGCCTC
<i>Cxcl2</i>	TCTCAAGGGCGGTCAAAAAGTT	TTCTCTTTGGTTCTTCCGTTGAG
<i>Cxcl10</i>	CCAAGTGCTGCCGTCATTTTC	GGCTCGCAGGGATGATTTCAA
<i>Csf2</i>	GGCCTTGGAAGCATGTAGAGG	GGAGAACTCGTTAGAGACGACTT
<i>Tnfsf9</i>	CGGCGCTCCTCAGAGATAC	ATCCCGAACATTAACCGCAGG
<i>Dnajb1</i>	TTCGACCGCTATGGAGAGGAA	CACCGAAGAACTCAGCAAACA
<i>Hspal1b</i>	GAGATCGACTCTCTGTTCGAGG	GCCCGTTGAAGAAGTCCTG
<i>Dnaj1</i>	ACCACTTACTACGATGTTTTGGG	GCCCTCTTTAATCGCCTGCT
<i>Dnajb4</i>	ACCGAAAGCAAGCCCTCAAAT	CCTTCTTCGCCAAACAGATCA
<i>Hsp90aa1</i>	AATTGCCCAGTTAATGTCCTTGA	CGTCCGATGAATTGGAGATGAG
<i>CD274</i>	GCTCCAAAGGACTTGTACGTG	TGATCTGAAGGGCAGCATTTC
<i>Ifi30</i>	CCTGGTCTCCGATCCTACCAT	TTGCAGGTGGTTGTGCCTT
<i>CD86</i>	CTGGACTCTACGACTTCACAATG	AGTTGGCGATCACTGACAGTT
<i>CD80</i>	ACCCCAACATAACTGAGTCT	TTCCAACCAAGAGAAGCGAGG
<i>CD28</i>	GTTCTTGGCTCTCAACTTCTTCT	TGAGGCTGACCTCGTTGCTAT
<i>Ccl8</i>	TCTACGCAGTGCTTCTTTGCC	AAGGGGGATCTTCAGCTTTAGTA
<i>Cxcl3</i>	GCACCCAGACAGAAGTCATA	AGACACATCCAGACACCGTT
<i>Cxcl7</i>	CTCAGACCTACATCGTCCTGC	AGCGCAACAAGGATCAGGC
<i>Cxcl13</i>	GGCCACGGTATTCTGGAAGC	GGGCGTAACTTGAATCCGATCTA
<i>Il1b</i>	GCAACTGTTCTGAACTCAACT	ATCTTTTGGGGTCCGTCAACT
<i>Il1a</i>	CGAAGACTACAGTTCTGCCATT	GACGTTTCAGAGGTTCTCAGAG
<i>Il12a</i>	CTGTGCCTTGGTAGCATCTATG	GCAGAGTCTCGCCATTATGATTC
<i>Cxcl5</i>	GTTCCATCTCGCCATTCATGC	GCGGCTATGACTGAGGAAGG
<i>Tnfsf11</i>	CAGCATCGCTCTGTTCCTGTA	CTGCGTTTTTCATGGAGTCTCA
<i>Il33</i>	ACTGCATGAGACTCCGTTCTG	CCTAGAATCCCGTGGATAGGC
<i>GAPDH</i>	AGGTCGGTGTGAACGGATTTG	TGTAGACCATGTAGTTGAGGTCA

Supplementary Videos

Supplementary Video 1. Video of MC38_{GFP/mCherry} cells when incubated with aMP_{C16-CA50} at a concentration of 160 $\mu\text{g ml}^{-1}$ in the presence of DRAQ7TM (0.6 μM) at pH 6.8.

Supplementary Video 2. Video of MC38_{Gal3-GFP} cells when incubated with aMP_{C16-CA50} at a concentration of 160 $\mu\text{g ml}^{-1}$ in the presence of PI (1 $\mu\text{g ml}^{-1}$) at pH 6.8.

Supplementary Video 3. Video of Panc02_{Gal3-GFP} cells when incubated with aMP_{C16-CA50} at a concentration of 160 $\mu\text{g ml}^{-1}$ in the presence of PI (1 $\mu\text{g ml}^{-1}$) at pH 6.8.

Supplementary Video 4. Video of MC38_{Gal3-GFP} cells when incubated with aMP_{C12-CA50} at a concentration of 160 $\mu\text{g ml}^{-1}$ in the presence of PI (1 $\mu\text{g ml}^{-1}$) at pH 6.8.

Supplementary Video 5. Video of MC38_{Gal3-GFP} cells when incubated with aMP_{C6-CA50} at a concentration of 160 $\mu\text{g ml}^{-1}$ in the presence of PI (1 $\mu\text{g ml}^{-1}$) at pH 6.8.

Supplementary Video 6. Video of MC38_{Gal3-GFP} cells when incubated with aMP_{C16-DA50} at a concentration of 160 $\mu\text{g ml}^{-1}$ in the presence of PI (1 $\mu\text{g ml}^{-1}$) at pH 6.8.

Supplementary Video 7. Video of MC38_{Gal3-GFP} cells when incubated with aMP_{C16-TA50} at a concentration of 160 $\mu\text{g ml}^{-1}$ in the presence of PI (1 $\mu\text{g ml}^{-1}$) at pH 6.8.

Supplementary Video 8. Video of MC38_{Gal3-GFP} cells when incubated with aMP_{C16-CA30} at a concentration of 160 $\mu\text{g ml}^{-1}$ in the presence of PI (1 $\mu\text{g ml}^{-1}$) at pH 6.8.

Supplementary Video 9. Video of MC38_{Gal3-GFP} cells when incubated with aMP_{C16-CA70} at a concentration of 160 $\mu\text{g ml}^{-1}$ in the presence of PI (1 $\mu\text{g ml}^{-1}$) at pH 6.8.

Supplementary Video 10. Video of MC38_{Gal3-GFP} cells when incubated with aMP_{C16-AA50} at a concentration of 160 $\mu\text{g ml}^{-1}$ in the presence of PI (1 $\mu\text{g ml}^{-1}$) at pH 6.8.

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