

Membranolytic peptide programs immunogenic cell death for cancer therapy

<https://doi.org/10.1038/s41586-026-10899-5>

Received: 19 March 2024

Accepted: 9 July 2026

Published online: 05 August 2026

 Check for updates

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A rational design for facilitating innovative cell death modes can substantially aid advances in antitumour therapy^{1,2}. Here we design and implement a unique mode of immunogenic membranolytic cell death (mLCD) in tumour cells, characterized by time-lagged rupture from the lysosomal to plasma membranes; this approach was found to robustly potentiate immune checkpoint blockade therapy. This mode of mLCD was induced by the synthetic-acid-responsive membranolytic peptide (aMP) aMP_{C16-CA50}, which exhibits hierarchical responsiveness to the decreasing pH associated with the tumour extracellular environment and lysosomes. aMP_{C16-CA50} activated an inflammatory transcriptional program in tumour cells, potentiating their ability to induce antigen presentation on class I major histocompatibility complex molecules on dendritic cells and the subsequent activation of T cells. The pH-responsive kinetics and membranolytic activity of the membranolytic peptides had a critical role in enhancing the immunogenicity of lytic tumour cells through the spatiotemporal regulation of the membrane-rupture processes. Furthermore, aMP_{C16-CA50} exhibited a considerable advantage in enhancing the antitumour efficacy of immune checkpoint blockade therapy through the promotion of antitumour immune response. Moreover, its systemic administration was well tolerated in mice. Overall, we successfully programmed a unique mode of immunogenic mLCD in tumour cells through the spatiotemporal regulation of membrane-rupture processes using a synthetic pH-responsive membranolytic peptide amenable to manipulation.

Cancer immunotherapies represented by immune checkpoint blockades have revolutionized cancer treatment paradigms^{3–5}. However, their current low clinical responsiveness has spurred intense investigation into the design of improved immunotherapies^{6–8}. The induction of regulated mLCD, including pyroptosis^{9,10}, necroptosis^{11,12} and necrosis^{13,14}, in tumour cells effectively enhances antitumour immune responses. Regulated mLCD is characterized by the activation of pore-forming cytolytic proteins through complex signalling pathways, followed by plasma membrane rupture (PMR) and the release of inflammatory mediators, including damage-associated molecular patterns and cytokines^{15–17}. This inflammatory process promotes the infiltration and activation of

tumour-infiltrating lymphocytes, offering great potential to potentiate immune checkpoint blockade immunotherapy^{17–20}. However, the pore-forming cytolytic proteins such as the gasdermin family of proteins and MLKL, which are the key executors of regulated mLCD, exhibit variability with respect to their types, expression levels and mutations^{21–23}. Moreover, their activation is orchestrated through diverse networks of complex signalling pathways and further influenced by variations in the expression and activation of executors such as caspases^{24–26}. The considerable heterogeneity and complexity of cytolytic proteins can result in different modes of cell death in response to the same stimuli, allowing for switching and cross-talk between regulated mLCD and the relatively

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immunologically silent process of apoptosis^{26–28}. This, in turn, leads to variations in the released intracellular contents and their immunogenicity. These complexities associated with cytolysins prompted us to investigate the possibility of manipulating an immunogenic mLCD mode that is independent of pore-forming cytolysins.

Membranolytic materials capable of effectively disrupting cell membrane structures through electrostatic and hydrophobic interactions have formed the research focus of various groups, including ours^{29–34}. Importantly, these materials exhibit the ability to execute mLCD independent of intracellular signalling pathways^{29,30,35,36}. Furthermore, through responsiveness design, the control over the activation of these materials can be achieved in specific microenvironments such as those with acidic pH or the presence of specific enzymes^{29,34,37–39}. We are intrigued by the potential of manipulating the responsiveness and activation of membranolytic materials to achieve spatiotemporal regulation of the membrane rupture processes, eliciting artificial mode of immunogenic mLCD independent of cytolysins.

Here we programmed a time-lagged process from lysosomal membrane rupture (LMR) to PMR by manipulating the response kinetics of aMPs, inducing immunogenic mLCD in tumour cells and robustly potentiating immune checkpoint blockade therapy. The aMPs were engineered to selectively accumulate in and be activated within lysosomes to potentially induce LMR of tumour cells under the slightly acidic tumour microenvironment (pH 6.8). Critically, their acid-responsive kinetics must be finely tuned to ensure that the activated membranolytic peptides (MPs) released from damaged lysosomes do not induce PMR in rapid succession after LMR, a regulation that relies on the dose-dependent yet saturable inhibition effect of anionic phospholipids. This kinetic transition from robust LMR to weak and delayed PMR created a sufficient time window, allowing leaked lysosomal contents to adequately elicit robust immunogenicity in tumour cells before the terminal execution of cell death. This time-lagged LMR-to-PMR process therefore induced an artificial mode of immunogenic mLCD in tumour cells, demonstrating a potent ability to activate antitumour CD8⁺ T cell responses. The engineered aMPs showed high potential for creating potent antitumour vaccines and potentiating immune checkpoint blockade therapy, and were well tolerated in mice after intravenous (i.v.) administration.

aMP_{C16}-CA₅₀ induces mLCD in tumour acidity

aMP_{C16}-CA₅₀ was synthesized by modifying half of the side-chain amines of a helical membranolytic peptide MP_{C16} (helicity of around 90.5%) with citraconic anhydride (Supplementary Figs. 2–5). aMP_{C16}-CA₅₀ contains the cationic amino (–NH₃⁺) and anionic carboxyl (–COO[–]) groups as well as the acid-labile maleic acid amide bonds on the side chains (Fig. 1a). aMP_{C16}-CA₅₀ displayed a non-helical structure (helicity, 16.9%) (Fig. 1b) in which the maleic acid amide bonds serve as off/on switches that cleave to trigger its conversion to MP_{C16} with rapid helicity recovery (Supplementary Fig. 6a,b).

All-atom molecular dynamics simulations revealed that, after forced denaturation into an elongated random coil structure, aMP_{C16}-CA₅₀ remained a disordered structure with an average root-mean-square deviation (r.m.s.d.) value of 3.60 ± 0.11 Å, while MP_{C16} spontaneously folded into an α -helical conformation with an average r.m.s.d. value of 1.23 ± 0.22 Å (Fig. 1c,d). Moreover, aMP_{C16}-CA₅₀ exhibited an uneven angular distribution between the amino and carboxyl groups, indicating strong ionic interactions between these two groups (Fig. 1e and Supplementary Fig. 7a). By contrast, the amino groups distributed uniformly in MP_{C16} (Fig. 1e and Supplementary Fig. 7a). Further simulations revealed a higher occupancy of hydrogen bonds (H-bonds) between the backbone amide groups and the side-chain amide bonds or carboxyl/amino groups in aMP_{C16}-CA₅₀ compared with MP_{C16} (Fig. 1f, Supplementary Table 1 and Supplementary Fig. 7b–f). These results indicate that aMP_{C16}-CA₅₀ exhibits ionic interactions and hydrogen

bonds among the backbone and side chains, preventing its folding into an α -helical conformation.

aMP_{C16}-CA₅₀ exhibited hierarchical responses as pH dropped from physiological pH (7.4) to the slightly acidic conditions characteristic of the tumour microenvironment (pH 6.8)^{40,41} and then to the more acidic microenvironment within the endosomal/lysosomal lumens⁴² (pH 6.0–4.5). At pH 7.4, aMP_{C16}-CA₅₀ exhibited a non-helical structure and self-assembled into negatively charged nanoparticles with a size of about 136.6 nm and a zeta potential of around –17.7 mV (Fig. 1a,g–j). At pH 6.8, the nanoparticles became electroneutral (around –0.6 mV) with increased size (about 308.5 nm; Fig. 1h–j). Notably, aMP_{C16}-CA₅₀ showed a transition pH (pH_T) of around 5.5 for helicity recovery, a threshold enabling over 80% recovery within 2 h, whereas it exhibited very slow responsive kinetics at pH ≥ 6.0 (Fig. 1g and Supplementary Fig. 6c). At pH 5.5, aMP_{C16}-CA₅₀ transformed into positively charged nanoparticles (around 20.9 mV) with a decreased size (around 41.2 nm) (Fig. 1h–j). The membranolytic activity of aMP_{C16}-CA₅₀ activated after preincubation at pH ≤ 5.5 , evidenced by dye leakage from giant unilamellar vesicles and liposomes (Fig. 1k,l). As a control, iMP, which was synthesized by modifying MP_{C16} with maleic anhydride and is not acid-activatable, remained non-helical structure with low membranolytic activity and cytotoxicity at all tested pH (Fig. 1k,l and Supplementary Fig. 6d–f).

aMP_{C16}-CA₅₀ exhibited highly selective cellular uptake and cytotoxicity against various tumour cells at pH 6.8 in contrast to at pH 7.4 (Fig. 1m,n and Supplementary Fig. 8a,b). In colon adenocarcinoma MC38 cells at pH 6.8, aMP_{C16}-CA₅₀ induced extensive leakage of intracellular proteins after 18 h, including the large protein lactate dehydrogenase (LDH) and HMGB1 (Fig. 1o–q). Time-lapse imaging captured aMP_{C16}-CA₅₀-induced leakage of mCherry from MC38^{GFP/mCherry} cells, with MyrPalm–GFP on the membranes and mCherry in the cytoplasm (Fig. 1r and Supplementary Video 1). Analysis using transmission electron microscopy (TEM) confirmed lysis of membranes, including the plasma membrane and nuclear envelope (Supplementary Fig. 8c). These results demonstrate that aMP_{C16}-CA₅₀ selectively induces mLCD in tumour cells under tumour acidity.

Notably, aMP_{C16}-CA₅₀ activates multiple regulated mLCD pathways, but these modalities do not contribute to its cytotoxic effects (Extended Data Fig. 1). In MC38 tumour cells at pH 6.8, aMP_{C16}-CA₅₀ upregulated key markers of regulated mLCD: phosphorylated RIPK3 and phosphorylated MLKL for necroptosis, GSDME cleavage for pyroptosis and increased lipid peroxidation (C11-BODIPY assay) for ferroptosis (Extended Data Fig. 1a–d). However, inhibition of necroptosis, pyroptosis or ferroptosis did not obviously inhibit aMP_{C16}-CA₅₀-induced cytotoxicity (Extended Data Fig. 1f–h). Moreover, knockout of *Mkl1*, *Gsdme* or incomplete knockout of *Nin1* did not reduce aMP_{C16}-CA₅₀-induced cytotoxicity or the release of LDH and HMGB1 in MC38 cells (Extended Data Fig. 1i–p), indicating that these pore-forming cytolysins are dispensable for these effects.

This mLCD shows time-lagged LMR to PMR

We next investigated the mechanism of aMP_{C16}-CA₅₀-induced selective mLCD in tumour cells. The membranolytic activity of aMP_{C16}-CA₅₀ was activated at lysosomal pH (≤ 5.5) but not at the acidity of the tumour microenvironment (pH 6.8; Fig. 1k,l), suggesting a dependency of the endolysosomal pathway. aMP_{C16}-CA₅₀-induced cytotoxicity on MC38 cells at pH 6.8 was inhibited by low temperature (4 °C) or endocytosis inhibitors, indicating an endocytosis dependency (Fig. 2a,b). Consistently, aMP_{C16}-CA₅₀ showed substantially increased lysosomal accumulation at pH 6.8 compared with at pH 7.4, evidenced by colocalization (yellow regions) of FITC-labelled aMP_{C16}-CA₅₀ (aMP_{C16}-CA₅₀–FITC) with LAMP1–mCherry-marked lysosomes (Fig. 2c). Critically, aMP_{C16}-CA₅₀ selectively induced LMR in tumour cells at pH 6.8. A galectin puncta assay⁴³ was performed on MC38^{Gal3–GFP} cells expressing GFP-tagged galectin-3 (Gal3–GFP) in the cytoplasm. After LMR

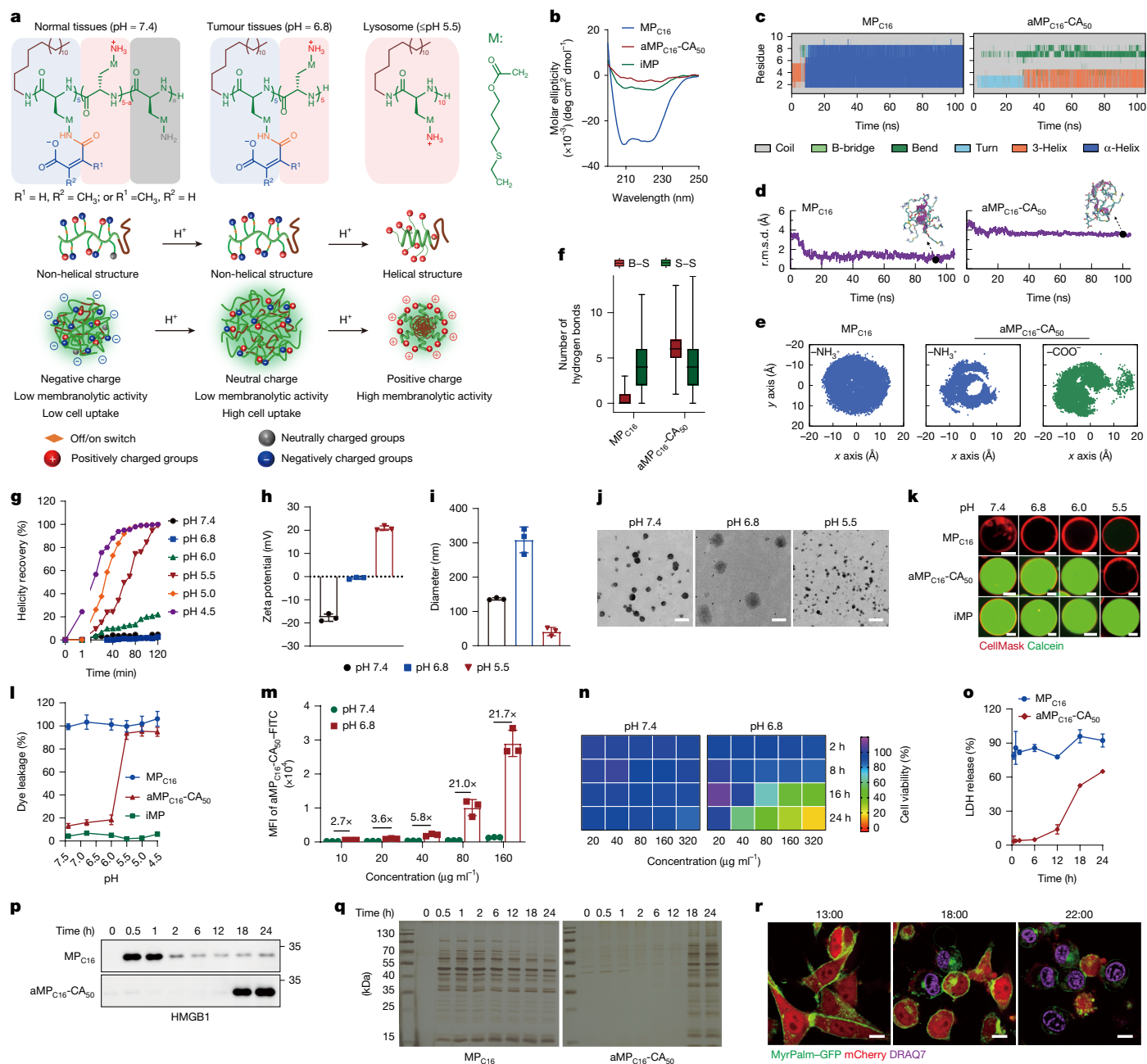


Fig. 1 | Lysosomal-acidity-activable aMP_{C16}-CA₅₀ selectively induces mLCD in tumour cells under a slightly acidic tumour microenvironment. **a**, Schematic of the hierarchical responsiveness of aMP_{C16}-CA₅₀ under acidic tumour and lysosomal conditions. **b**, Circular dichroism spectra at pH 7.4. **c–f**, Molecular dynamics simulations. **c**, Time-course assessment of secondary structure formation. **d**, The time evolution of r.m.s.d. for the backbone with respect to an ideal α -helical form. **e**, The side-chain termini ($-NH_3^+$ or $-COO^-$) projected onto a plane perpendicular to the helix axis. **f**, Quantitative representation of hydrogen bonds between backbones and side chains (B–S) as well as between side chains (S–S). Data are mean $\pm$ s.d. $n = 4,100$ and 2,500 molecular dynamics frames for MP_{C16} and aMP_{C16}-CA₅₀, respectively. **g**, Time-dependent helicity recovery of aMP_{C16}-CA₅₀ under different pH conditions. **h–j**, The zeta potential (**h**), size (**i**) and TEM images (**j**) of aMP_{C16}-CA₅₀ under different pH conditions. **k, l**, Dye leakage from giant unilamellar vesicles (**k**) and

liposomes (**l**) after incubation with the indicated peptides preincubated under different pH conditions. **m**, pH- and concentration-dependent uptake of aMP_{C16}-CA₅₀-FITC by MC38 cells at 12 h. MFI, mean fluorescence intensity. **n**, Concentration- and time-dependent cytotoxicity of aMP_{C16}-CA₅₀ against MC38 cells at pH 7.4 and 6.8. **o–q**, Time-dependent release of LDH (**o**), HMGB1 (**p**) and total proteins (**q**) from MC38 cells treated with MP_{C16} or aMP_{C16}-CA₅₀ at pH 6.8. **r**, Time-lapse confocal imaging of MC38_{GFP/mCherry} cells treated with aMP_{C16}-CA₅₀ at pH 6.8. Peptide concentrations were as follows: 100 μ g ml⁻¹ (**b** and **g**), 80 μ g ml⁻¹ (**h–j**), 20 μ g ml⁻¹ (**k**) and 160 μ g ml⁻¹ (**l** and **o–r**). For **h, i, l, m** and **o**, data are mean $\pm$ s.d. of $n = 3$ biological replicates. For **j, k** and **p–r**, representative images are shown from at least two independent experiments. Scale bars, 200 nm (**j**), 5 μ m (**k**) and 10 μ m (**r**). Uncropped immunoblot and gel images are shown in Supplementary Fig. 1.

induction, Gal3–GFP enters permeabilized lysosomes and binds to *N*-acetylglucosamine-containing glycans, forming puncta⁴³. aMP_{C16}-CA₅₀ treatment at pH 6.8 (but not at pH 7.4) induced Gal3–GFP puncta colocalized with lysosomes marked by LAMP1–mCherry (Fig. 2d).

Furthermore, MC38 cells were incubated with ferrocene-labelled aMP_{C16}-CA₅₀ (aMP_{C16}-CA₅₀-Fe) for TEM imaging. At pH 7.4, lysosomes remained intact without noticeable aMP_{C16}-CA₅₀-Fe (Fig. 2e). By contrast, at pH 6.8, aMP_{C16}-CA₅₀-Fe accumulated in and severely damaged

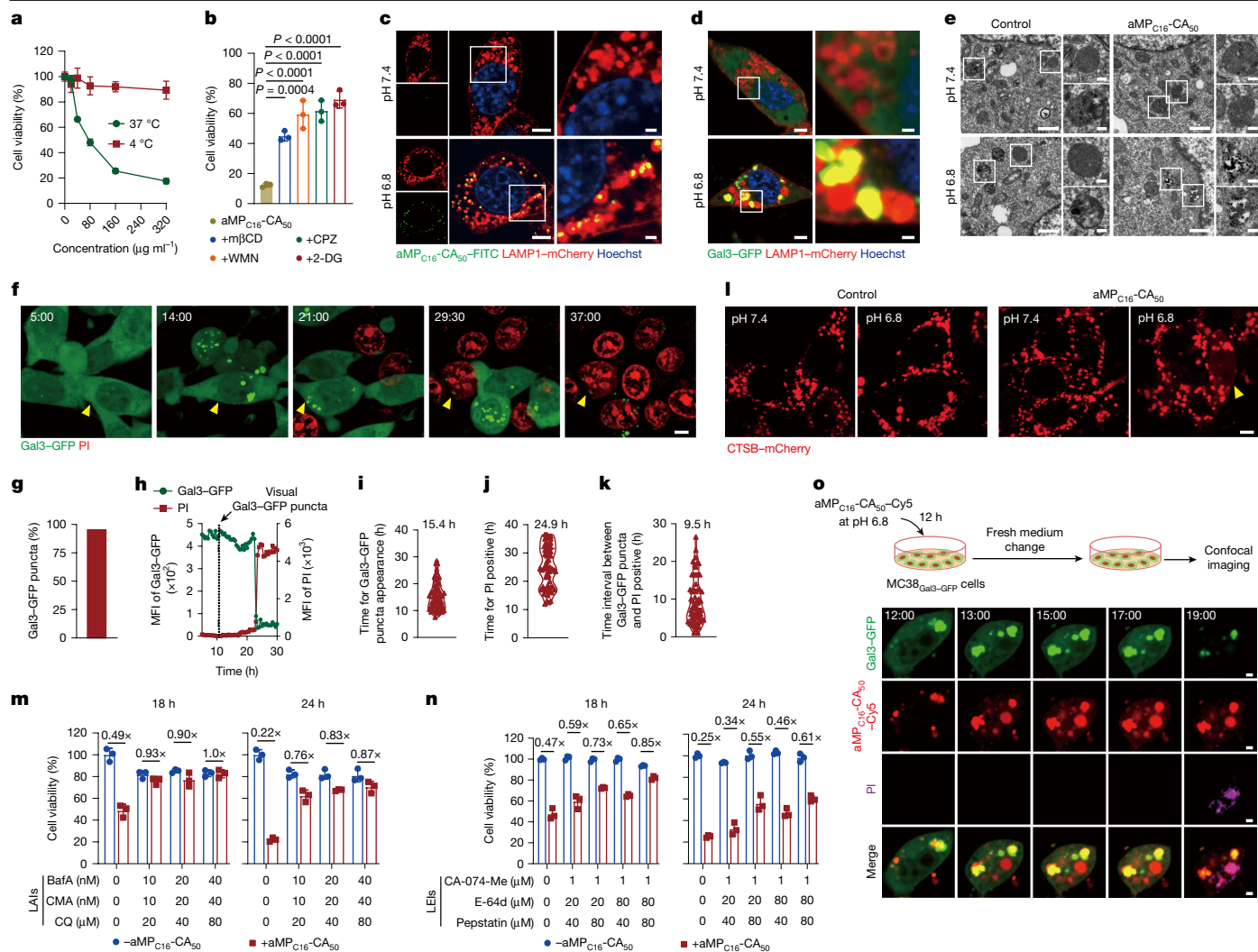


Fig. 2 | aMP_{C16}-CA₅₀ induces time-lagged rupture from lysosomal to plasma membranes. a, b, aMP_{C16}-CA₅₀-induced cytotoxicity in MC38 cells after 24 h at pH 6.8. **a**, The effect of temperature. **b**, The effect of endocytosis inhibitors: methyl-β-cyclodextrin (mβCD, 5 μM), wortmannin (WMN, 10 nM), chlorpromazine (CPZ, 6 μM) and 2-deoxy-glucose (2-DG, 20 mM). **c**, Confocal imaging of MC38_{LAMP1-mCherry} cells treated with aMP_{C16}-CA₅₀-FITC for 1 h. **d**, Confocal imaging of MC38_{LAMP1-mCherry} cells treated with aMP_{C16}-CA₅₀ for 12 h. **e**, TEM imaging of MC38 cells treated with aMP_{C16}-CA₅₀-Fe for 16 h. **f–k**, Time-lapse confocal imaging of MC38_{Gal3-GFP} cells incubated with aMP_{C16}-CA₅₀ and PI at pH 6.8. **f**, Representative images. **g**, The percentage of cells exhibiting Gal3-GFP puncta. **h**, The time-dependent MFI of Gal3-GFP and PI for the cell indicated by arrowheads in f. **i, j**, The time for Gal3-GFP puncta appearance (i) and PI-positive staining (j) in each cell. **k**, The time interval between Gal3-GFP puncta appearance and PI-positive staining in each cell. **l**, Confocal imaging

analysis of MC38_{CTSB-mCherry} cells treated with aMP_{C16}-CA₅₀ for 12 h at pH 7.4 or 6.8. **m, n**, The effect of LAIs (**m**) or LEIs (**n**) on aMP_{C16}-CA₅₀-induced cytotoxicity in MC38 cells. **o**, Time-lapse confocal imaging of MC38_{Gal3-GFP} cells treated with aMP_{C16}-CA₅₀-Cy5. Peptide concentration was as follows: 160 $\mu\text{g ml}^{-1}$ (**b–d** and **f–o**) and 320 $\mu\text{g ml}^{-1}$ (**e**). For **a, b, m** and **n**, data are mean $\pm$ s.d. of $n = 3$ biological replicates. Statistical significance was determined using one-way analysis of variance (ANOVA) with Tukey's multiple-comparison test (**b**). In **c–f, l** and **o**, representative images are shown from at least two independent experiments. Scale bars, 5 μm (**c** and **d** (lower magnification), **f** and **l**), 1 μm (**c** and **d** (higher magnification) and **e** (lower magnification)), 200 nm (**e** (higher magnification)) and 2 μm (**o**). The diagram in **o** was created using BioRender; Xiong, M. <https://biorender.com/p8xag3c> (2026). BafA, bafilomycin A1; CA-074-Me, cathepsin B inhibitor CA-074 methyl ester; CMA, concanamycin A; CQ, chloroquine; E-64d, broad-spectrum cysteine protease inhibitor afoxistatin.

lysosomes (Fig. 2e). aMP_{C16}-CA₅₀-induced selective LMR was also observed in Panc02 cells (Supplementary Fig. 9). Thus, aMP_{C16}-CA₅₀ selectively accumulates in lysosomes and induces LMR in cancer cells under the acidic tumour microenvironment.

Next, we evaluated the temporal relationship between LMR and mLCD in each cell by measuring the time interval between Gal3-GFP punctum formation (LMR) and positivity for propidium iodide (PI) staining (PMR, the terminal execution event of mLCD). Sequential LMR-to-PMR processes were observed in 96% of MC38_{Gal3-GFP} cells (73 out of 76) treated with aMP_{C16}-CA₅₀ (160 $\mu\text{g ml}^{-1}$) at pH 6.8 (Fig. 2f, g and Supplementary Video 2). Notably, a time interval was observed between LMR and PMR (Fig. 2f, h–k). On average, Gal3-GFP puncta

appeared at approximately 15.4 h, followed by PI-positivity at about 24.9 h, with Gal3 puncta preceding PI positivity by about 9.5 h (Fig. 2h–k). During this interval between LMR and PMR, lysosomal enzymes accumulated within the cytoplasm in a dispersed pattern (Fig. 2l). Thus, aMP_{C16}-CA₅₀ induces a distinct mLCD mode characterized by time-lagged rupture from lysosomal to plasma membranes, allowing leaked lysosomal contents to accumulate in the cytoplasm before mLCD.

We next demonstrated that the cytotoxicity of aMP_{C16}-CA₅₀ is LMR dependent. Lysosomal acidification inhibitors (LAIs), combining chloroquine, concanamycin A and bafilomycin A1, suppressed the majority of aMP_{C16}-CA₅₀-induced Gal3-GFP punctum formation and

cytotoxicity in MC38 cells (Fig. 2m and Supplementary Fig. 10a,b). This observation aligns with the requirement for lysosomal acidity to activate aMP_{C16}-CA₅₀. Moreover, lysosomal enzymes contribute to aMP_{C16}-CA₅₀-induced cytotoxicity (Fig. 2n). A combination of three different types of lysosomal enzyme inhibitors (LEIs), including the cathepsin B inhibitor CA-074 methyl ester, the broad-spectrum cysteine protease inhibitor aloxistatin and the aspartyl protease inhibitor pepstatin A, effectively inhibited aMP_{C16}-CA₅₀-induced cytotoxicity while not obviously inhibiting Gal3-GFP punctum formation at pH 6.8 (Fig. 2n and Supplementary Fig. 10c,d). After aMP_{C16}-CA₅₀ was activated into MP_{C16} to induce LMR, time-lapse confocal imaging revealed that a substantial fraction remained lysosome associated, while an increasing amount dispersed throughout the cells before PMR (Fig. 2o). Thus, the cytotoxicity of aMP_{C16}-CA₅₀ results from the combined effects of the released lysosomal contents and MP_{C16} from ruptured lysosomes.

The LMR-inducing aMP_{C16}-CA₅₀ induced autophagy, which partially contributed to its cytotoxicity (Supplementary Fig. 11). In MC38 cells at pH 6.8, aMP_{C16}-CA₅₀ treatment increased LC3-II levels while decreasing p62 levels, indicating autophagy induction and efficient autophagy flux (Supplementary Fig. 11a). This elevated autophagic flux was further confirmed using MC38 cells expressing tandem-tagged GFP-mCherry-LC3 probe (MC38_{GFP-mCherry-LC3}), whereby aMP_{C16}-CA₅₀ treatment led to an increase in red-only (mCherry) puncta, signifying the presence of functional autolysosomes (Supplementary Fig. 11b–d). Furthermore, autophagy inhibitors partially reduced the cytotoxicity of aMP_{C16}-CA₅₀ (Supplementary Fig. 11e,f).

This mLCD is highly immunogenic

Subsequently, we investigated how aMP_{C16}-CA₅₀ reprograms tumour cells by inducing time-lagged LMR to PMR. RNA-sequencing (RNA-seq) analysis revealed progressive upregulation of immunogenic signatures in aMP_{C16}-CA₅₀-treated MC38 cells after 12 h (Fig. 3a–c and Supplementary Fig. 12a–d). At 24 h, dying/dead MC38 cells exhibited significant upregulation (fold change > 2) of 334 genes (Supplementary Fig. 12a). Gene Ontology (GO) enrichment analysis of these genes revealed that the top 10 biological processes were related to immune and stress responses (ranked by *P* value; Supplementary Fig. 12b). Gene set enrichment analysis (GSEA) identified IL-6–JAK–STAT3 signalling, inflammatory response, IFN γ response and TNF–NF- κ B signalling among the top enriched sets (Fig. 3b and Supplementary Fig. 12c,d). By contrast, MP_{C16}-treated dying/dead MC38 cells scarcely exhibited immune-related enrichment among the upregulated genes (Supplementary Fig. 12e–g). Thus, aMP_{C16}-CA₅₀ elicits immune and stress responses in tumour cells.

Compared with MP_{C16}, aMP_{C16}-CA₅₀-treated MC38 cells stimulated greater antigen presentation by dendritic cells (DCs) and subsequent CD8⁺ T cell activation (Fig. 3d–i). MC38 cells expressing chicken ovalbumin (OVA; MC38_{OVA}) were treated with aMP_{C16}-CA₅₀ or MP_{C16} at pH 6.8 for varying durations, then co-cultured with bone-marrow-derived DCs (BMDCs) from naive mice (Fig. 3d). aMP_{C16}-CA₅₀-treated MC38_{OVA} cells stimulated significantly higher presentation of the OVA-derived peptide SIINFEKL (OVA_{257–264}) on MHC-I H-2K^b molecules of BMDCs and triggered greater BMDC activation compared with MP_{C16}-treated MC38_{OVA} cells (Fig. 3e,f and Supplementary Fig. 13a,b). BMDCs co-cultured with aMP_{C16}-CA₅₀-treated MC38_{OVA} cells induced stronger activation of CD8⁺ T cells from OT-I mice, evidenced by increased proliferation and elevated expression of IFN γ , granzyme B and perforin (Fig. 3g–i and Supplementary Fig. 13c). However, lysing MC38_{OVA} cells before aMP_{C16}-CA₅₀ treatment abolished these differences in antigen presentation and CD8⁺ T cell activation (Supplementary Fig. 13d–h), underscoring the need for live tumour cell interaction. Moreover, in contrast to MP_{C16}, aMP_{C16}-CA₅₀-treated MC38 cells acted as an effective vaccine, providing complete protection against challenge with live MC38 cells, further indicating enhanced immunogenicity (Fig. 3j).

A similar time-lagged LMR to PMR, along with enhanced immunogenic effects induced by aMP_{C16}-CA₅₀, was observed across various cell lines, including Panc02, EO771 and A549 cells (Extended Data Fig. 2 and Supplementary Video 3). Particularly, after aMP_{C16}-CA₅₀ treatment, A549_{NY-ESO-1} cells (a human lung adenocarcinoma line genetically modified to express NY-ESO-1 antigen) induced stronger activation of NY-ESO-1 TCR-T cells (Fig. 3k,l and Supplementary Fig. 14).

LMR activity of aMP_{C16}-CA₅₀ is critical for enhancing the immunogenicity of tumour cells. Both LAIs and LEIs significantly suppressed the ability of aMP_{C16}-CA₅₀-treated MC38 cells to activate antigen-specific CD8⁺ T cells, highlighting the crucial role of LMR and lysosomal contents (Fig. 3m–p and Supplementary Fig. 13i,j). We further synthesized two additional lysosome-targeting variants with varying hydrophobic terminal groups, aMP_{C12}-CA₅₀ and aMP_{C6}-CA₅₀, which exhibited similar pH-responsive kinetics to aMP_{C16}-CA₅₀, but displayed distinct LMR activities (Extended Data Fig. 3a–k, Supplementary Figs. 15, 16 and Supplementary Videos 4 and 5). Like aMP_{C16}-CA₅₀, aMP_{C12}-CA₅₀ induced robust LMR followed by delayed PMR and potently enhanced the ability of MC38_{OVA} cells to activate OT-I CD8⁺ T cells (Extended Data Fig. 3f–m). By contrast, aMP_{C6}-CA₅₀, inducing low LMR, did not enhance the immunogenicity of tumour cells (Extended Data Fig. 3f–m). Moreover, sequential treatment with Gly-Phe- β -naphthylamide (GPN, a LMR inducer)⁴⁴, followed by MP_{C16}, produced similar effects to aMP_{C16}-CA₅₀ on antigen presentation and T cell activation (Extended Data Fig. 3n–x), contrasting with the marked difference between MP_{C16} and aMP_{C16}-CA₅₀.

Inhibiting necroptosis, pyroptosis, ferroptosis and autophagy partially reduced aMP_{C16}-CA₅₀-induced T cell activation (Supplementary Fig. 17a–e), consistent with the reported interplay between LMR and various cell death pathways⁴⁵. Yet, after *Mkl1* or *Gsdme* knockout, or incomplete *Nin1* knockout, aMP_{C16}-CA₅₀-treated MC38 cells retained potent vaccine efficacy, conferring complete protection against viable MC38 cells rechallenge (Supplementary Fig. 17f,g). Thus, none of these individual pore-forming cytolysins that execute regulated mLCD modalities are essential for aMP_{C16}-CA₅₀-induced immunogenic cell death.

The time interval between LMR and PMR critically influences the immunogenic effects of aMP_{C16}-CA₅₀. Using MP_{C16} treatment to temporally control PMR, we demonstrated that aMP_{C16}-CA₅₀-induced immunogenicity of tumour cells decreased as the time interval between LMR and PMR shortened (Extended Data Fig. 4). A strong positive correlation was observed, with shorter time interval corresponding to a reduced ability of MC38_{OVA} cells to stimulate OT-I CD8⁺ T cell proliferation (Extended Data Fig. 4j). Higher concentrations of aMP_{C16}-CA₅₀ induced more extensive LMR and achieved comparable OT-I CD8⁺ T cell activation within a shorter LMR to PMR interval (Extended Data Fig. 4g–i and Supplementary Fig. 18), suggesting that this interval–immunogenicity correlation is influenced by LMR extent.

Responsive kinetics of aMPs is crucial

To ensure a prolonged time interval between LMR and PMR, it is necessary for aMPs to be activated into a sufficient amount of MP_{C16} within lysosomes to effectively induce LMR, while MP_{C16} released from damaged lysosomes must not rapidly induce PMR. The critical switch resides in the dose-dependent inhibition of MP_{C16} after interaction with anionic phospholipids, such as phosphatidylserine (PS) and phosphatidylinositol (Pdl)—key targets of membranolytic materials^{29,46} (Extended Data Fig. 5). MP_{C16} exhibited high affinity to both PS and Pdl (Extended Data Fig. 5a). During titration of PS- or Pdl-containing liposomes with MP_{C16}, particle hydrodynamic diameter gradually increased from about 110 nm to the micrometre scale, culminating in macroscopic precipitation (Extended Data Fig. 5b). Notably, the cytotoxic effects of MP_{C16}, in terms of both kinetics and intensity, were substantially reduced with increasing amounts of co-treated PS or Pdl (Extended Data Fig. 5c,d). These results suggest that, after activation of aMP_{C16}-CA₅₀ into MP_{C16},

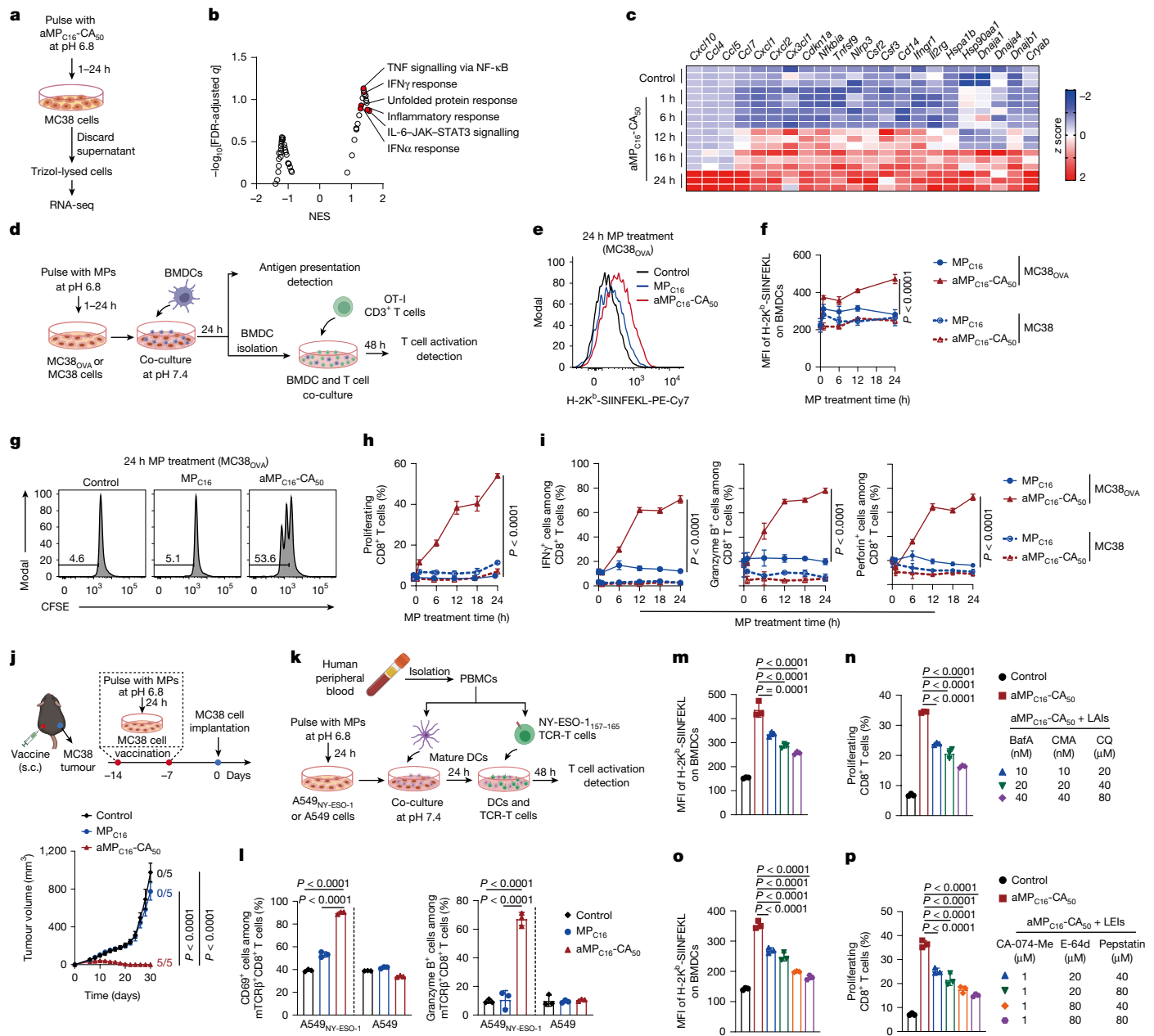


Fig. 3 | aMP_{C16}-CA₅₀-induced mLCD is highly immunogenic. a–c, RNA-seq analysis of MC38 cells after aMP_{C16}-CA₅₀ treatment at pH 6.8. *n* = 3 biological replicates. **a**, Schematic of sample preparation. **b**, GSEA of transcriptional profiles using the MSigDB Hallmark gene sets, comparing MC38 cells treated with aMP_{C16}-CA₅₀ for 24 h with the control. FDR, false-discovery rate; NES, normalized enrichment score. **c**, The expression of representative inflammatory and stress-response genes upregulated after aMP_{C16}-CA₅₀ treatment. **d–i**, aMP_{C16}-CA₅₀ enhances the ability of MC38_{OVA} cells to stimulate antigen cross-presentation and T cell activation. **d**, Experimental design. **e, f**, Flow cytometry histogram (**e**) and quantification (**f**) of H-2K^b-SIINFEKL expression on BMDCs. **g–i**, Flow cytometry analysis of CD8⁺ T cell proliferation (histogram (**g**) and quantification (**h**)) and expression of IFNγ, granzyme B and perforin (**i**). **e, g**, Representative histograms of the 24 h MP treatment groups. **j**, MC38 tumour growth curves in mice that were pre-vaccinated with MP-treated MC38

cells according to the schematic. s.c., subcutaneous. **k, l**, aMP_{C16}-CA₅₀ potentiates A549_{NY-ESO-1} cells to activate NY-ESO-1-specific TCR-T cells. **k**, Experimental design. **l**, Flow cytometry analyses of CD69 and granzyme B expression in NY-ESO-1 TCR-T cells. **m–p**, The effect of LAIs (**m, n**) and LEIs (**o, p**) on the ability of aMP_{C16}-CA₅₀-treated MC38_{OVA} cells to stimulate antigen presentation (**m, o**) and OT-I CD8⁺ T cell proliferation (**n, p**). Peptide concentrations were 160 μg ml⁻¹ (**a–p**). For **f, h, i** and **l–p**, data are mean ± s.d. of *n* = 3 biological replicates. For **j**, data are mean ± s.e.m. of *n* = 5 mice. Statistical significance was determined using two-way ANOVA (**f, h–j** and **l**) and one-way ANOVA (**m–p**) with Tukey's multiple-comparisons test; and permutation testing with FDR correction for GSEA (**b**). The diagrams in **a, d, j** and **k** were created using BioRender; Xiong, M. <https://biorender.com/p8xag3c> (**a** and **d**), <https://biorender.com/ap2k7ea> (**j**), <https://biorender.com/hgu6rgj> (**k**) (2026).

its activity would progressively diminish as it is restricted by increasing amounts of anionic phospholipids during outward diffusion through cellular compartments. This inhibitory effect could create an activity switch for MP_{C16}, enabling a kinetic transition from robust LMR to weak/slow PMR. However, this inhibitory effect is saturable; once the activated MP_{C16} concentration exceeds a critical threshold, it overcomes

anionic phospholipid-mediated suppression, resulting in rapid lysis of tumour cells within minutes (Extended Data Fig. 5e).

We subsequently investigated the critical roles of the pH-responsive kinetics of aMPs in modulating membrane rupture processes and enhancing tumour cell immunogenicity. We expanded the set of aMPs, named aMP_{C16}-A_x, with varying pH-responsive kinetics by altering two

structural parameters: (1) the type of side-chain modifying anhydrides (A); and (2) the modification percentage of side-chain amines ($x\%$) (Supplementary Figs. 19 and 20). These aMP_{C16}-A_x series exhibit different p*H*_i values.

aMP_{C16}-DA₅₀, which is rapidly activated in the acidic tumour extracellular environment (p*H* 6.8), quickly induces PMR in tumour cells (Fig. 4a–d, Extended Data Fig. 6a–h and Supplementary Video 6). With a p*H*_i of around 6.8, it restored over 90% α -helical conformation within 1 h and activated membranolytic activity at p*H* $\leq$ 6.8 (Fig. 4b–d and Supplementary Fig. 21a). In MC38 cells at p*H* 6.8, aMP_{C16}-DA₅₀ localized to the plasma membrane, followed by PMR (Extended Data Fig. 6g,h and Supplementary Video 6). It induced Gal3–GFP punctum formation in a small proportion (13.3%, 4 out of 30) of MC38 cells before PMR (Fig. 4e,f). In this subset of cells, Gal3–GFP puncta and PI-positive staining occurred in rapid succession (Fig. 4e,g,h).

aMP_{C16}-TA₅₀, which converts into MP_{C16} more rapidly and efficiently under lysosomal acidic conditions than aMP_{C16}-CA₅₀, induces sequential LMR and PMR with a short time interval in tumour cells at p*H* 6.8 (Fig. 4a–h and Supplementary Video 7). With a p*H*_i of around 6.0, it restored over 90% α -helical conformation within 2 h at p*H* $\leq$ 6.0 and 1 min at p*H* $\leq$ 5.0, and activated membranolytic activity at p*H* $\leq$ 6.0 (Fig. 4b–d and Supplementary Fig. 21b). Like aMP_{C16}-CA₅₀, it colocalized with lysosomes and showed endocytosis-dependent cytotoxicity (Extended Data Fig. 6a–g). However, compared with aMP_{C16}-CA₅₀, aMP_{C16}-TA₅₀ induced faster and more extensive lysosomal disruption before cell death, as shown by LysoTracker staining (Extended Data Fig. 6i). A high percentage (69.4%, 25 out of 36) of aMP_{C16}-TA₅₀-treated MC38_{Gal3-GFP} cells exhibited Gal3–GFP puncta before PMR (Fig. 4e,f). The time interval between LMR and PMR was short, with Gal3–GFP puncta appearing on average around 0.4 h before PI-positive staining (Fig. 4e,g,h). After LMR, the peptide dispersed throughout tumour cells, rapidly followed by PMR (Extended Data Fig. 6j). Notably, LEIs minimally affected the cytotoxicity of aMP_{C16}-TA₅₀, indicating less reliance on released lysosomal contents than aMP_{C16}-CA₅₀ (Extended Data Fig. 6k).

Compared with aMP_{C16}-CA₅₀, aMP_{C16}-DA₅₀ and aMP_{C16}-TA₅₀ minimally enhanced the immunogenicity of MC38 cells. The upregulation of inflammatory or stress-response-related genes induced by aMP_{C16}-CA₅₀ was less pronounced in aMP_{C16}-DA₅₀- or aMP_{C16}-TA₅₀-treated MC38 cells (Fig. 4i and Supplementary Table 2). aMP_{C16}-TA₅₀- or aMP_{C16}-DA₅₀-treated MC38_{OVA} cells induced significantly lower SIINFEKL presentation on MHC-I H-2K^b molecules of BMDCs (Fig. 4j,k) and lower BMDC activation (Supplementary Fig. 22a). Subsequently, BMDCs co-cultured with aMP_{C16}-TA₅₀- or aMP_{C16}-DA₅₀-treated MC38_{OVA} cells activated CD8⁺ T cells to a significantly lower extent, as shown by decreased proliferation and reduced expression of IFN γ , granzyme B and perforin (Fig. 4l–n and Supplementary Fig. 22b–d). Moreover, in contrast to aMP_{C16}-CA₅₀, neither aMP_{C16}-TA₅₀- nor aMP_{C16}-DA₅₀-treated MC38 cells could function as an effective vaccine (Fig. 4o).

To further validate the critical role of pH-responsive kinetics, we evaluated aMP_{C16}-CA₃₀ (p*H*_i $\approx$ 6.3), aMP_{C16}-CA₇₀ (p*H*_i $\approx$ 5.0) and aMP_{C16}-AA₅₀ (p*H*_i $\approx$ 4.5) with distinct pH-responsive profiles (Extended Data Fig. 7, Supplementary Fig. 23 and Supplementary Videos 8–10). aMP_{C16}-CA₇₀ (p*H*_i $\approx$ 5.0), similar to aMP_{C16}-CA₅₀ (p*H*_i $\approx$ 5.5), induced sequential LMR and PMR with a long-time interval (on average, around 12.3 h), and potentially enhanced the ability of MC38_{OVA} cells to activate OT-1CD8⁺ T cells (Extended Data Fig. 7). By contrast, aMP_{C16}-CA₃₀ (p*H*_i $\approx$ 6.3), which primarily induced PMR, and aMP_{C16}-AA₅₀ (p*H*_i $\approx$ 4.5), which exhibited lysosomal localization but induced weak Gal3–GFP punctum formation and cytotoxicity, did not induce such immunogenic effects (Extended Data Fig. 7).

Kinetically programmed aMPs boost anti-PD-L1

The advantage of kinetically programmed aMPs for potentiating immune checkpoint blockade therapy was investigated. Bilateral tumour models were established with one tumour receiving intratumoural (i.t.)

injection of MPs, in combination with i.v.-administered anti-PD-L1 antibodies (Fig. 5a). Synergistic antitumour effects with anti-PD-L1 were observed for aMP_{C16}-CA₅₀, aMP_{C16}-CA₇₀ and aMP_{C12}-CA₅₀, all of which induced a time-lagged LMR to PMR process (Fig. 5a–c, Extended Data Fig. 8 and Supplementary Fig. 24). Specifically, aMP_{C16}-CA₅₀ (125 μ g per mouse) combined with anti-PD-L1 achieved durable efficacy against both aMP_{C16}-CA₅₀-injected and non-injected distal tumours, resulting in complete eradication of at least 66% ($\geq$ 4 out of 6) and 100% (6 out of 6) of distal tumours in subcutaneous MC38 colon adenocarcinoma and orthotopic EMT6 triple-negative breast cancer mouse models, respectively (Fig. 5a–c and Supplementary Fig. 24a–f). Marked synergy persisted even after the substantial reduction in aMP_{C16}-CA₅₀ dosage (12.5 μ g per mouse; Supplementary Fig. 24g–i). Synergy with anti-PD-L1 did not correlate directly with the *in vitro* cytotoxicity of MPs against tumour cells. Neither the highly cytotoxic MP_{C16}, aMP_{C16}-DA₅₀, aMP_{C16}-TA₅₀ and aMP_{C16}-CA₃₀, nor the weakly cytotoxic aMP_{C6}-CA₅₀ and aMP_{C16}-AA₅₀ achieved strong synergistic antitumour effects with anti-PD-L1 therapy (Fig. 5a–c, Extended Data Fig. 8 and Supplementary Fig. 24).

In contrast to MP_{C16}, i.t. administration of aMP_{C16}-CA₅₀ did not significantly reduce the overall viable cell counts of MC38 tumour cells or various non-tumour cell types per unit tumour weight (Extended Data Fig. 9a–d). Staining with haematoxylin and eosin (H&E) showed that aMP_{C16}-CA₅₀ induced localized necrosis characterized by karyorrhexis, karyolysis, cytoplasmic leakage and eosinophilic structureless regions (Extended Data Fig. 9e). Notably, consecutive tumour sections displayed the presence of CD31⁺ blood vessels and CD3⁺ T cells within these necrotic zones (Extended Data Fig. 9e–g). By contrast, MP_{C16} caused more extensive and severe necrosis, with substantially fewer CD31⁺ blood vessels and CD3⁺ T cells in necrotic areas (Extended Data Fig. 9e–g). Importantly, alternating administration with MP_{C16} did not compromise the antitumour efficacy of aMP_{C16}-CA₅₀, ruling out that its weaker cytotoxicity towards non-tumour cells in tumour tissues accounts for the therapeutic advantage (Extended Data Fig. 9h–j).

Combining aMP_{C16}-CA₅₀ with anti-PD-L1 conferred a marked advantage in enhancing antitumour immunity. Bulk RNA-seq analysis of whole-tumour tissues 7 days after treatment showed significant upregulation of 2,445 genes (fold change > 2 , $P < 0.05$) in the aMP_{C16}-CA₅₀ + anti-PD-L1 combination group versus anti-PD-L1 alone, compared with each of the MP_{C16}, aMP_{C16}-DA₅₀ or aMP_{C16}-TA₅₀ combinations showing ≤ 41 upregulated genes (Supplementary Figs. 25 and 26). GSEA revealed prominent enrichment of immune-related sets with the aMP_{C16}-CA₅₀ combination, including inflammatory responses, IFN γ responses, IL-6–JAK–STAT3, TNF–NF- κ B and IL-2–STAT5 signalling (Supplementary Fig. 26b,c).

Flow cytometry analysis indicated that combination of aMP_{C16}-CA₅₀ and anti-PD-L1 outperformed other MPs combinations, including MP_{C16}, aMP_{C16}-DA₅₀ or aMP_{C16}-TA₅₀, in promoting T cell activation (Fig. 5d–k). This combination significantly increased IFN γ expression in CD8⁺ and CD4⁺ T cells in both tumours and tumour-draining lymph nodes (tdLNs), as well as granzyme B and perforin expression in tumour-infiltrating CD8⁺ T cells (Fig. 5d–k). Depletion of CD8⁺ or CD4⁺ T cells markedly reduced the antitumour efficacy of combining aMP_{C16}-CA₅₀ and anti-PD-L1 against both injected and distal tumours, confirming their essential roles (Fig. 5l,m). The antitumour effect on distal tumours was specific to homologous tumours: i.t. administration of aMP_{C16}-CA₅₀ in EO771 tumours combined with anti-PD-L1 did not protect against distal MC38 tumours (Fig. 5n,o).

Notably, when administered i.t. and combined with anti-PD-L1, aMP_{C16}-CA₅₀ showed superior antitumour efficacy against distal tumours compared with the chemotherapeutic drugs mitoxantrone and doxorubicin that induce immunogenic cell death⁴⁷ (Supplementary Fig. 27). Furthermore, alternating i.t. administration of GPN and MP_{C16}, in combination with anti-PD-L1, showed potent antitumour efficacy, suggesting the high therapeutic potential of rationally combining an LMR-inducer with membranolytic strategies (Supplementary Fig. 28).

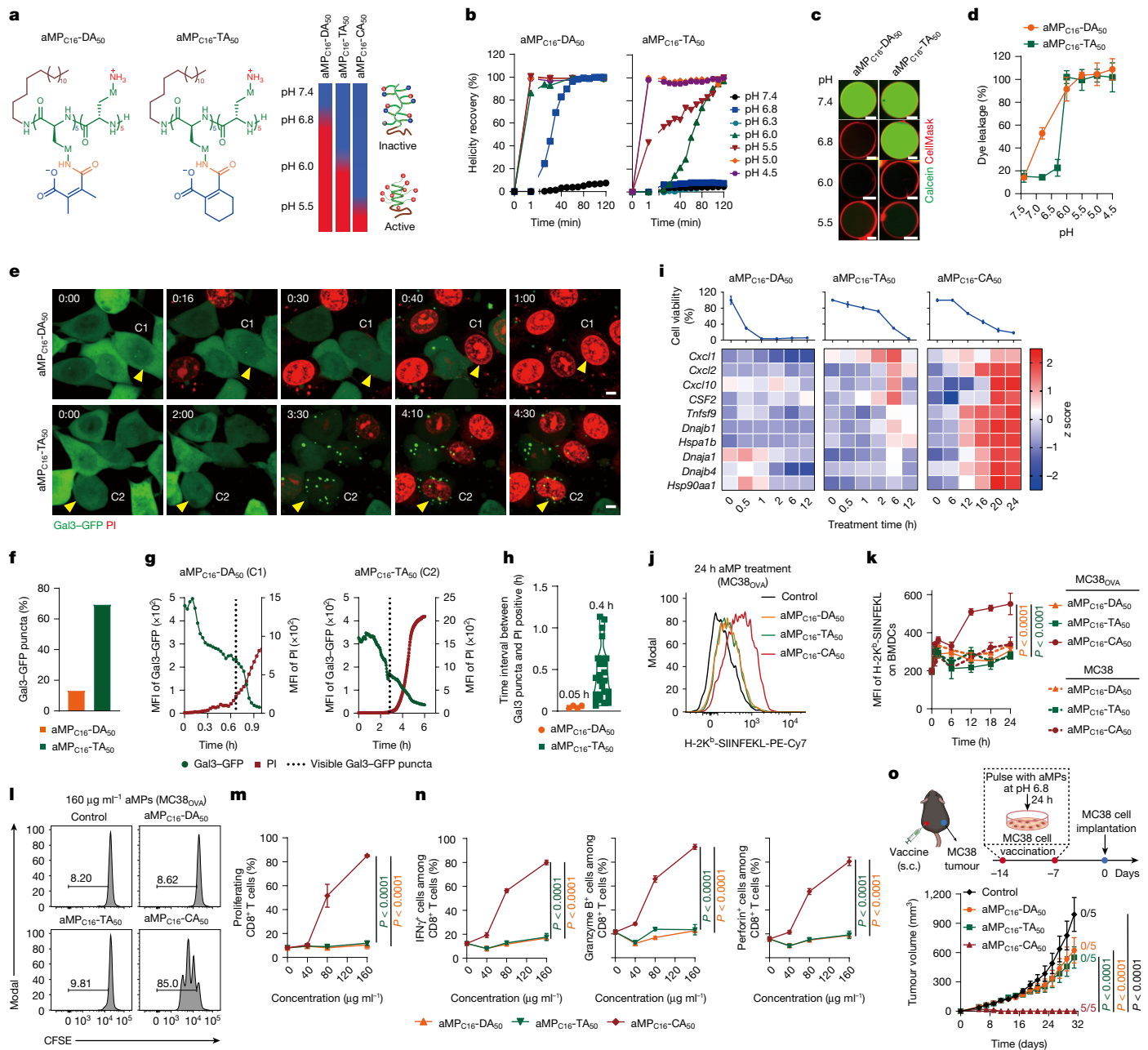


Fig. 4 | The pH-responsive kinetics of aMPs are crucial. **a**, Schematic of aMP_{C16}-DA₅₀ and aMP_{C16}-TA₅₀. **b**, Time-dependent helicity recovery of the indicated aMPs under different pH conditions. **c**, **d**, Dye leakage from giant unilamellar vesicles (**c**) and liposomes (**d**) after incubation with the indicated aMPs preincubated under different pH conditions. **e**–**h**, Time-lapse confocal imaging of MC38_{Gal3-GFP} cells incubated with the indicated aMPs and PI at pH 6.8. **e**, Representative images. **f**, The percentage of cells exhibiting Gal3-GFP puncta. **g**, The time-dependent MFI of Gal3-GFP and PI for the cells indicated by arrowheads in **e**. **h**, The time interval between Gal3-GFP puncta appearance and PI-positive staining in each cell. **i**, Quantitative PCR with reverse transcription (RT-qPCR) analysis of representative inflammatory and stress-response genes in aMP-treated MC38 cells at pH 6.8 (bottom). *n* = 3 biological replicates. Top, time-dependent cell viability. **j**–**n**, Following the experimental design described in Fig. 3d, H-2K^b-SIINFEKL expression on BMDCs (histogram (**j**) and

quantification (**k**)) was analysed after stimulation with aMP-treated MC38_{OVA} along with the subsequent effects on OT-1 CD8⁺ T cells, including proliferation (histograms (**l**) and quantification (**m**)) and expression of IFN γ , granzyme B and perforin (**n**). In **j**–**n**, MC38_{OVA} cells were pretreated with aMPs for 24 h. **o**, MC38 tumour growth curves in mice that were pre-vaccinated with aMP-treated MC38 cells according to the schematic. aMP concentrations were as follows: 100 $\mu\text{g ml}^{-1}$ (**b**), 20 $\mu\text{g ml}^{-1}$ (**c**) and 160 $\mu\text{g ml}^{-1}$ (**d**–**l** and **o**). For **d**, **k**, **m**, **n** and **i** (top), data are mean $\pm$ s.d. of *n* = 3 biological replicates. For **o**, data are mean $\pm$ s.e.m. of *n* = 5 mice. Statistical significance was determined using two-way ANOVA with Tukey's multiple-comparison test (**k** and **m**–**o**). In **c** and **e**, representative images are shown from two independent experiments. Scale bars, 5 μm (**c** and **e**). The diagram in **o** was created using BioRender; Xiong, M. <https://biorender.com/ap2k7ea> (2026).

aMP_{C16}-CA₅₀ is systemically administrable

aMP_{C16}-CA₅₀ is a selective anti-cancer therapy suitable for i.v. administration. Systemically administered aMP_{C16}-CA₅₀ exhibited substantially reduced in vivo toxicity compared with MP_{C16}. The maximum tolerated

dose of aMP_{C16}-CA₅₀ exceeded 20 mg kg^{-1} , whereas that of MP_{C16} was 2.5 mg kg^{-1} (Extended Data Fig. 10a). Moreover, i.v. administration of aMP_{C16}-CA₅₀ (15 mg kg^{-1}), although primarily distributed in the liver and kidney like traditional nanoparticles^{48,49}, did not cause substantial hepatotoxicity or nephrotoxicity in mice, as evaluated by serum

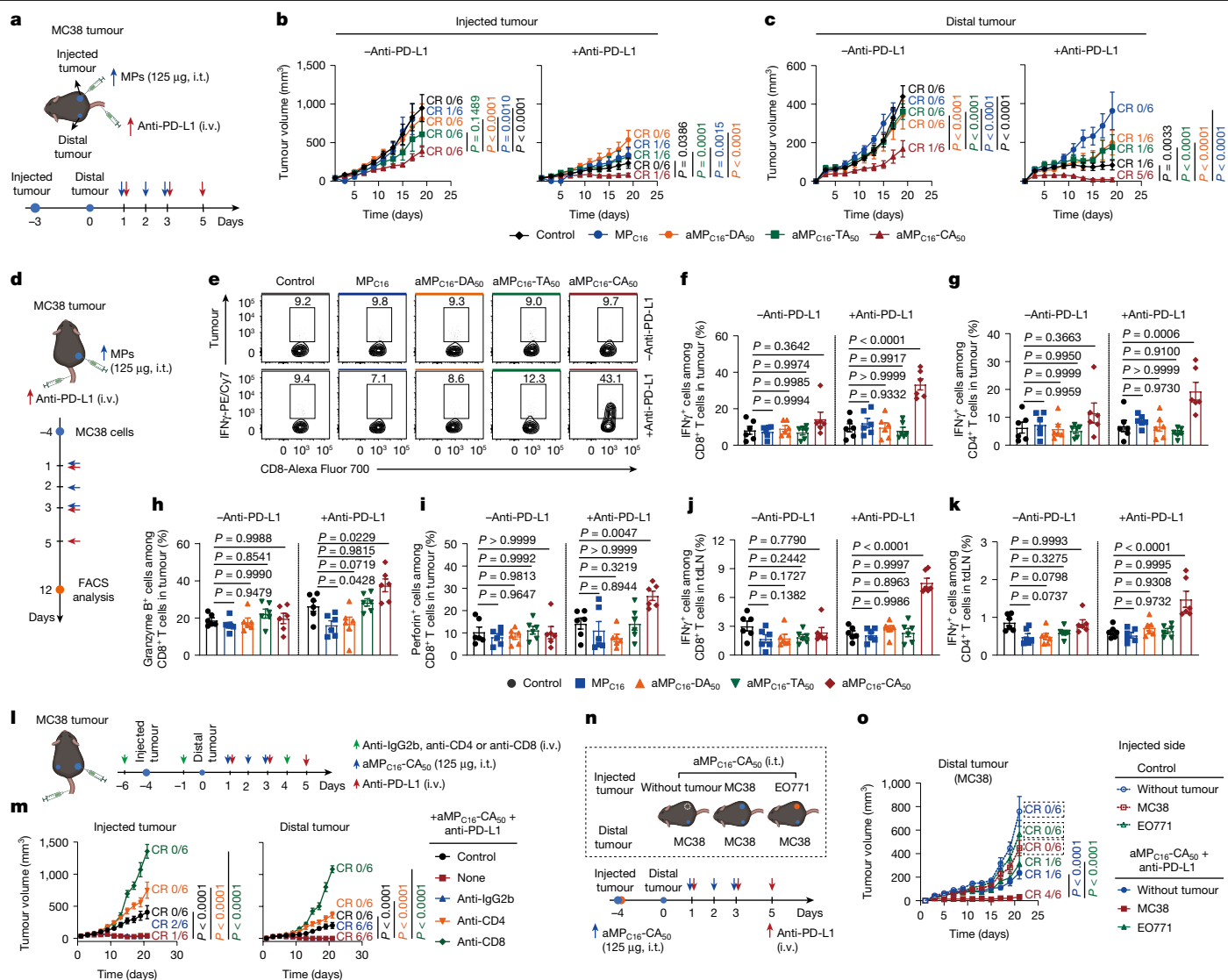


Fig. 5 | aMP_{C16}-CA₅₀ exhibited a substantial advantage in enhancing the efficacy of anti-PD-L1 therapy. Mice bearing bilateral or single tumours received i.t. injections of MPs at 125 µg per tumour per injection and i.v. administration of anti-PD-L1 at a dose of 0.75 mg kg⁻¹. **a–c**, Antitumour efficacy in a bilateral subcutaneous MC38 tumour model. **a**, The experimental schedule for tumour inoculation and treatments. **b, c**, Tumour growth curves of the right flank tumours injected with MPs (injected tumour) (**b**) and the left flank tumours that were not subjected to direct MP administration (distal tumour) (**c**). **d–k**, Flow cytometry analysis of T cells in MC38 tumours and tLNs following the schedule shown in **d**. **e**, Representative plots showing IFN γ expression in CD8⁺ T cells within the tumours. **f, g**, The percentage of IFN γ ⁺ cells among CD8⁺ (**f**) and CD4⁺ (**g**) T cells in tumours. **h, i**, The percentage of granzyme B⁺ (**h**) and

perforin⁺ (**i**) cells among CD8⁺ T cells in tumours. **j, k**, The percentage of IFN γ ⁺ cells among CD8⁺ (**j**) and CD4⁺ (**k**) T cells in tLNs. **l, m**, The effect of anti-CD4 and anti-CD8 antibodies on the antitumour efficacy of combined treatment (aMP_{C16}-CA₅₀ and anti-PD-L1) in the MC38 bilateral tumour model following the schedule in **l**. Anti-IgG2b was used as the isotype control antibody. **n, o**, Growth curves of distal MC38 tumours after combined treatment of injected MC38 or EO771 tumours with aMP_{C16}-CA₅₀ and anti-PD-L1 (**o**) following the schedule in **n**. CR, complete response. For **b, c, f–k, m** and **o**, data are mean $\pm$ s.e.m. of $n = 6$ mice; statistical significance was determined using two-way ANOVA followed by Tukey's multiple-comparison test. The diagrams in **a, d, l** and **n** were created using BioRender; Xiong, M. <https://biorender.com/ap2k7ea> (2026).

biochemical indicators and H&E-stained tissue sections (Extended Data Fig. 10b and Supplementary Fig. 29a–e).

i.v. aMP_{C16}-CA₅₀ combined with anti-PD-L1 demonstrated potent antitumour activity in both localized and metastatic tumour models. It achieved complete eradication of 50% (3 out of 6) of subcutaneous MC38 tumours (Extended Data Fig. 10c, d) without causing notable toxicity in major organs (Supplementary Fig. 29f–i). The combination significantly enhanced the activation of CD8⁺ T and CD4⁺ T cells within tumour tissues, as well as CD8⁺ T cell activation in the tLNs, spleen and peripheral blood (Extended Data Fig. 10e–i). In a peritoneal metastasis model of MC38, this combined therapy substantially prolonged mouse survival, with 2 out of 6 mice surviving beyond 150 days after treatment (Extended Data Fig. 10j, k). Furthermore, the combination exhibited

robust therapeutic effects in orthotopic EMT6 triple-negative breast cancer (around 57% complete eradication) and the less immunogenic Panc02 pancreatic cancer model (Extended Data Fig. 10l, m and Supplementary Fig. 30).

Single-cell RNA-seq (scRNA-seq) analysis of CD45⁺ immune cells from MC38 tumours revealed that the combination of aMP_{C16}-CA₅₀ plus anti-PD-L1 activates major immune cell populations, including CD8⁺ T cells, CD4⁺ T cells, natural killer cells, DCs, macrophages, monocytes and B cells (Extended Data Fig. 11 and Supplementary Fig. 31). Gene set variation analysis of Hallmark gene sets revealed robust enrichment of inflammatory and immune-related programs, including inflammatory response, IL-6–JAK–STAT3 signalling, IFN α/γ response and TNF signalling, across diverse immune cell populations

(Extended Data Fig. 11d). The combination also elevated activity scores of interferon-stimulated genes⁵⁰ in major immune cell populations (Extended Data Fig. 11e), along with upregulation of type I interferon (IFN-I)-associated genes (such as *Irf1*, *Isg15* and *Irf7*) and pro-inflammatory cytokines (for example, *Cxcl9*, *Cxcl10* and *Il15*) (Extended Data Fig. 11f). Antigen-presenting cells (APCs), including DCs, macrophages and B cells, exhibited elevated activity scores of antigen processing–cross presentation (Reactome: R-MMU-1236975) and increased expression of antigen presentation-related genes (such as *B2m*, *H2-K1* and *Psmb9*) (Extended Data Fig. 11g,h). CD8⁺ T cells showed elevated activity scores of T cell-mediated cytotoxicity (GO: 0001913) and upregulation of effector/cytotoxicity markers (such as *Gzmb*, *Prf1* and *Ifng*) (Extended Data Fig. 11i,j). Collectively, these results indicate that aMP_{C16}-CA₅₀, together with anti-PD-L1, promotes an immunostimulatory tumour microenvironment and potentiates the antitumour responses of CD8⁺ T cells.

Discussion

The rationally designed pH-responsive MPs were found to induce an artificial mode of immunogenic mLCD through spatiotemporal manipulation of membrane-rupture processes, robustly potentiating immune checkpoint blockade therapy. The simplicity of the design, induction of immunogenic mLCD and synergy with immune checkpoint blockade therapy render this strategy an attractive antitumour therapeutic option. The pH-responsive design used here can be easily applied to many membranolytic materials. Moreover, we predict that a strategic manipulation of the responsiveness and targeting of MPs would allow the selective programming of various mLCD modes in tumour cells, augmenting the synergy with different antitumour therapies.

This study demonstrates that the spatiotemporal regulation of membrane rupture processes is critical for membranolytic peptide-programmed immunogenic mLCD. We observed that MPs including MP_{C16}, aMP_{C16}-DA₅₀, aMP_{C16}-TA₅₀ and aMP_{C16}-CA₅₀ exhibited limited efficacy in activating antitumour CD8⁺ T cell responses, despite their ability to potentially induce PMR and release constitutive damage-associated molecular patterns such as HMGB1. These findings suggest that the mere release of constitutive intracellular contents from tumour cells is insufficient to effectively activate antitumour T cell responses. By contrast, aMP_{C16}-CA₅₀ enhances the immunogenicity of lytic tumour cells by sequentially inducing LMR followed by PMR, with the time interval between these events being critical. aMP_{C16}-CA₅₀ induces robust LMR and the subsequent release of lysosomal contents while simultaneously kinetically restraining the onset of PMR and terminal execution of cell death. This regulation provides a sufficient time window before cell death, allowing the released lysosomal contents to adequately elicit robust immunogenicity in tumour cells. Subsequently, the terminal PMR ensures the abundant release of endogenous antigens and adjuvants from lytic tumour cells, facilitating effective immune recognition.

We demonstrate that the LMR activity of aMP_{C16}-CA₅₀ is critical for enhancing the immunogenicity of tumour cells. LMR has been associated with multiple regulated cell death pathways, acting as an initiator, an amplifier or simply as a phenomenon accompanying the final execution phase⁴⁵. It is linked to traditionally immunogenic forms such as necroptosis and pyroptosis, as well as to apoptosis, which has generally been considered more immune silent^{51–53}. The intricate association with various regulated cell death pathways complicates the determination of a causal relationship and the key determinants between LMR and the immunogenicity of cell death. This study emphasizes the critical importance of temporally coordinated disruption of lysosomal and plasma membranes by aMPs in inducing the immunogenicity of tumour cells. We show that aMP_{C16}-CA₅₀ activated multiple regulated cell death pathways in tumour cells and, although each had a role in mediating its immunogenic effects, none was individually

dominant or sufficient. Notably, after LMR, aMP_{C16}-CA₅₀ treatment triggers substantial cellular stress and inflammatory responses in cancer cells, which were reported to elicit tumour-targeting immune responses^{47,54,55}. Stress responses appear to be particularly efficient at increasing both the adjuvanticity and antigenicity of tumour cells undergoing immunogenic cell death⁵⁴. The mechanisms underlying LMR-induced immunogenicity are intriguing and complex: given the broad diversity of lysosomal cargo, the consequences should be multifaceted, arising from the concerted action of numerous pathways and effectors. Unravelling this complexity requires further systematic studies.

Online content

Any methods, additional references, Nature Portfolio reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at <https://doi.org/10.1038/s41586-026-10899-5>.

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Methods

Synthesis of aMPs and iMP

MP_{C16}, MP_{C12} and MP_{C6} were synthesized according to previously reported procedures^{56,57}. aMP_{C16}-CA₅₀ was synthesized by the successive addition of citraconic anhydride and triethylamine (5- and 10-fold equivalent relative to MP_{C16}, respectively) to the solution of MP_{C16} in dimethyl sulfoxide (DMSO). After stirring at room temperature for 12 h, aMP_{C16}-CA₅₀ was obtained by dialysing (molecular mass cut-off of 1 kDa) against distilled water for 24 h followed by lyophilization. aMP_{C16}-CA₃₀ and aMP_{C16}-CA₇₀ were similarly synthesized by tuning the feed ratio of citraconic anhydride (3- and 7-fold equivalent relative to MP_{C16}, respectively). aMP_{C12}-CA₅₀ and aMP_{C6}-CA₅₀ were synthesized from MP_{C12} and MP_{C6}, respectively, using 5 equivalents of citraconic anhydride (relative to the peptide). aMP_{C16}-TA₅₀, aMP_{C16}-DA₅₀, aMP_{C16}-AA₅₀ and iMP were similarly synthesized using 3,4,5,6-tetrahydrophthalic anhydride, 2,3-dimethylmaleic anhydride, *cis*-aconitic anhydride and maleic anhydride as the starting materials, respectively.

Conformational transition of aMPs under different pH conditions

The restoration of the α -helical conformation of the peptides under different pH conditions was evaluated by dissolving the aMPs in methanol (1.0 ml) to obtain a concentration of 1 mg ml⁻¹; this was mixed with phosphate buffer solutions (1 mM, 9.0 ml) of different pH ranging from 7.4 to 4.5. The solutions were incubated at room temperature and analysed using circular dichroism at predetermined timepoints. The helicity recovery rate was calculated using the following formula: helicity recovery (%) = $[(H - H_{7.4}) / (H_{1.0} - H_{7.4})] \times 100\%$, where H , $H_{7.4}$ and $H_{1.0}$ represent the helicity of the peptide incubated at a specific pH for a specified time period, maintained at pH 7.4 and incubated at pH 1.0 for 2 h, respectively.

Confocal microscopy

Confocal imaging was performed on the Olympus IXplore SpinSR system, comprising an IX83 inverted microscope integrated with a Yokogawa CSU-W1 spinning-disk and a Prime BSI sCMOS camera. Imaging was performed using a $\times 60/1.49$ NA oil-immersion objective. Excitation was provided by solid-state lasers at 405 nm, 488 nm, 561 nm and 640 nm. Emission filters (Chroma Technology) were as follows: Hoechst (447/60 nm), FITC/GFP (525/50 nm), mCherry/PI (617/73 nm) and Cy5 (685/40 nm). Image acquisition was performed by CellSens software (v.2.2, Olympus), and the integrated z-drift compensation system was engaged for axial drift correction during time-lapse-imaging experiments. The microscope was equipped with a temperature-controlled incubator, and imaging for live-cell experiments was performed at 37 °C.

Analysis of colocalization of aMPs with lysosomes

MC38_{LAMP1-mCherry} or Panc02_{LAMP1-mCherry} cells were incubated with FITC-labelled aMPs (160 μ g ml⁻¹) at pH 7.4 or 6.8. Cells were incubated with aMPs for 10 min for those with faster pH-responsive kinetics (aMP_{C16}-DA₅₀-FITC and aMP_{C16}-CA₃₀-FITC) and for 1 h for all other labelled aMPs. The cells were then washed with PBS and the nuclei were stained with Hoechst 33342 (5 μ g ml⁻¹) for 15 min at 37 °C. Cells were imaged using confocal microscopy to assess the colocalization of aMPs with lysosomes.

Galectin puncta imaging

The galectin puncta assay was performed as previously described⁴³. MC38_{LAMP1-mCherry+Gal3-GFP} and Panc02_{LAMP1-mCherry+Gal3-GFP} cells were cultured overnight at 37 °C in glass-bottom cell culture dishes, followed by treatment with aMPs (160 μ g ml⁻¹) at pH 7.4 or 6.8 for 12 h. The cells were subsequently stained with Hoechst 33342 (5 μ g ml⁻¹) for 15 min at 37 °C. The colocalization of Gal3-GFP puncta with LAMP1-mCherry was assessed by confocal microscopy.

To measure the time interval between galectin puncta and PI-positive staining, the MC38_{Gal3-GFP}, Panc02_{Gal3-GFP}, EO771_{Gal3-GFP} and A549_{Gal3-GFP} cells were incubated with medium containing aMPs and PI (1 μ g ml⁻¹) at pH 6.8. Imaging was performed after aMP addition and before observing Gal3-GFP punctum formation in cells, with the x , y imaging positions randomly selected. Images were acquired every 2, 5, 15 or 30 min for 2 h (short-term time-lapse imaging) or 12–48 h (for overnight and longer-term time-lapse imaging). Multiple z-slices (approximately 9–15 slices with a 1 μ m step size) were imaged for each channel, ensuring that the entirety of the cell was captured. Maximum-intensity projections of all z-slices were generated using CellSens software (v.2.2, Olympus), and the resulting two-dimensional projections were used for fluorescence signal quantification and visualization. The changes in fluorescence intensity corresponding to GFP and PI within each cell was quantified within the regions of interest using CellSens software. Lysosomal membrane damage was inferred from the occurrence of visible Gal3-GFP puncta. A change in MFI of over 100 relative to the initial value for each cell was considered to be PI-positive staining.

In vitro stimulation of BMDCs

MC38_{OVA} or MC38 were seeded onto the wells of 24-well plates and treated with MPs (40, 80, 160, 320 or 640 μ g ml⁻¹) for varying durations (1, 2, 4, 6, 12, 18 or 24 h). Subsequently, BMDCs were added to the plates and co-incubated with MC38_{OVA} or MC38 cells (pretreated with MPs) for 24 h. To investigate the effect of time interval between lysosomal rupture and plasma membrane disruption, we sequentially added aMP_{C16}-CA₅₀ and MP_{C16} into MC38_{OVA} cells, gradually increasing the time lag between their additions. To investigate the effect of LAIs or LEIs, the MC38_{OVA} cells were co-incubated with aMP_{C16}-CA₅₀ and LEIs (CA-074-Me, E-64d and pepstatin) or LAIs (chloroquine, concanamycin A and bafilomycin A1) for 24 h, followed by co-culture with BMDCs for another 24 h. To investigate the effect of GPN and its combination with MP_{C16}, MC38_{OVA} or MC38 cells were treated with GPN (320 μ g ml⁻¹) for 24 h or with GPN for 23 h followed by MP_{C16} treatment (160 μ g ml⁻¹) for 1 h, followed by co-culture with BMDCs for another 24 h.

The cells were subsequently collected, blocked with anti-mouse CD16/32 antibodies (BioLegend, 93, 101301, 1:200), and stained with the following antibodies for 40 min on ice: anti-CD11c-PE (BioLegend, N418, 117308, 1:200), anti-CD11c-APC (BioLegend, N418, 117310, 1:200), anti-I-A/I-E-AF700 (BioLegend, M5/114.15.2, 107622, 1:200), anti-I-A/I-E-BV510 (BioLegend, M5/114.15.2, 107636, 1:200), anti-CD45.2-APC/Cy7 (BioLegend, 104, 109824, 1:200), anti-CD80-FITC (BioLegend, 16-10A1, 104706, 1:200), anti-CD86-APC (BioLegend, GL-1, 105011, 1:200), anti-CD40-PerCP/Cy5.5 (BioLegend, 3/23, 124624, 1:200) or anti-SIINFEL-H2K^b-PE/Cy7 (Thermo Fisher Scientific, 25-D1.16, 25-5743-82, USA, 1:200). Finally, flow cytometry analysis was performed using BD FACSCanto II or BD LSRFortessa flow cytometry systems and BD FACSDiva software (BD Biosciences), and the results were analysed using FlowJo v.10 (FlowJo). The gating strategies are shown in Supplementary Fig. 32.

In vitro T cell activation assay

The pretreated BMDCs as described above were isolated using MojoSort Mouse CD11c Nanobeads (BioLegend, 480078) and co-cultured with freshly isolated CD3⁺ T cells from OT-I mice at a ratio of 1:10 in T cell culture medium for 48 h.

Cell proliferation was assessed using the CellTrace CFSE Cell Proliferation Kit (Thermo Fisher Scientific, C34570). In brief, OT-I CD3⁺ T cells were labelled with 2.5 μ M carboxyfluorescein succinimidyl ester (CFSE) according to the manufacturer's protocol, neutralized with serum and then co-cultured with the BMDCs described above. CFSE-labelled T cells were first blocked with anti-mouse CD16/32 antibody (BioLegend, 93, 101301, 1:200) on ice for 20 min. Subsequently, the cells were stained with anti-CD45-APC/Cy7 (BioLegend, 104, 109824, 1:200) and

anti-CD8-PE/Cy7 (BioLegend, 53-6.7, 100722, 1:200) for 45 min. Fluorescence dilution was measured using flow cytometry to track cell division. To assess T cell activation, the pretreated OT-I T cells were first stained using the Zombie Aqua Fixable Viability Kit (BioLegend, 423102) for 25 min, followed by blocking with anti-mouse CD16/32 antibodies (BioLegend, 93, 101301, 1:200) on ice for 20 min. The cells were then stained with anti-CD45-APC/Cy7 (BioLegend, 104, 109824, 1:200), anti-CD8-Alexa Fluor 700 (BioLegend, 53-6.7, 100730, 1:200) or anti-CD8-FITC (53-6.7, BioLegend, 100706, 1:200) on ice for 30 min. After staining for cell surface markers, the cells were washed, fixed with fixation and permeabilization solution (BD Biosciences, BDB554722) in the dark for 20 min on ice and washed twice with perm/wash buffer (BD Biosciences, BDB554723). The cells were then stained with anti-IFN γ -PE/Cy7 (XMGI.2, BioLegend, 505826, 1:200), anti-perforin-PE (S16009B, BioLegend, 154406, 1:200) and anti-granzyme B-APC (QA16A02, BioLegend, 372204, 1:200) on ice overnight. Isotype control antibodies used included: rat IgG1k isotype control-PE/Cy7 (BioLegend, RTK2071, 400416, 1:200), rat IgG2ak isotype control-PE (BioLegend, RTK2758, 400508, 1:200) and Mouse IgG1k isotype control-APC (BioLegend, MOPC-21, 400142, 1:200). Flow cytometry analysis was performed to assess the expression of IFN γ , perforin and granzyme B in the CD8⁺ T cells. Gating strategies are shown in Supplementary Fig. 32.

Activation of NY-ESO-1-specific TCR-T Cells

Peripheral blood was collected from HLA-A*02:01-positive healthy donors. Peripheral blood mononuclear cells (PBMCs) were isolated by Lymphoprep gradient (StemCell, 18060). The PBMC layer was carefully collected and washed twice with PBS. For DC generation, adherent PBMCs were seeded in T25 flasks and cultured in SuperCulture L100 Serum-Free Medium (DAKWE, 6911011) supplemented with Dendritic Cell Culture Factors (2 U ml⁻¹, Novoprotein, GMP-1805). After 48 h, DC maturation was induced by adding 2 U ml⁻¹ of DC maturation factors (Novoprotein, GMP-1806) for an additional 48 h.

NY-ESO-1-specific TCR-T cells were prepared by Y. Zhang as previously described with modifications^{58,59}. In brief, non-adherent PBMCs were resuspended in RPMI 1640 medium supplemented with 10% FBS and 100 IU ml⁻¹ recombinant human IL-2 (Quanqi) (Quangang Pharmaceutical; national drug approval no. S20020004), then seeded into 24-well plates precoated overnight with 5 μ g ml⁻¹ anti-human CD3 (Novoprotein, GMP-A018) and anti-human CD28 (Novoprotein, GMP-A063) antibodies. After 24 h, cells were transduced with lentiviral particles carrying NY-ESO-1₁₅₇₋₁₆₅-TCR genes at a multiplicity of infection of 5 in the presence of 10 μ g ml⁻¹ polybrene. The transduced T cells were expanded in complete medium supplemented with IL-2 and collected on day 7.

A549_{NY-ESO-1} or wild-type A549 cells (5 $\times$ 10⁵ cells per well) were seeded in 24-well plates and treated with either MP_{C16} or aMP_{C16}-CA₅₀ (160 μ g ml⁻¹) for 24 h. The pretreated tumour cells (2 $\times$ 10⁴ cells per well) were co-cultured with the mature DCs plated in U-bottom 96-well plates (2 $\times$ 10⁴ cells per well) for 24 h. After removing the medium containing tumour cell debris, fresh medium containing NY-ESO-1₁₅₇₋₁₆₅ TCR-T cells (2 $\times$ 10⁵ cells per well) was added to the DCs. The co-culture was then incubated for an additional 48 h before assessing T cell activation.

The activation of NY-ESO-1₁₅₇₋₁₆₅ TCR-T cell was evaluated using flow cytometry. For assessing CD69 expression, cells were blocked with Human TruStain FcX (BioLegend, 422301, 1:100) for 20 min, and stained with anti-CD69-APC/Cy7 (BioLegend, FN50, 310913, 1:200), anti-CD8-PE (BioLegend, HIT8a, 300907, 1:200) and anti-TCR β -APC (BioLegend, H57-597, 109211, 1:200) for 30 min. DAPI (1 μ g ml⁻¹) was added to the samples before flow cytometry analysis to distinguish between live and dead cells. For assessing granzyme B expression, cells were stained with Zombie Aqua Fixable Viability Kit (BioLegend, 423102) for 25 min, followed by blocking with human TruStain FcX (BioLegend, 422301) on ice for 20 min. Cells were stained with anti-TCR β -APC (BioLegend,

H57-597, 109211, 1:200) and anti-CD8-Alexa Fluor 700 (BioLegend, HIT8a, 300920, 1:200) for surface marker detection. After staining, cells were fixed with fixation and permeabilization solution (BD Biosciences, BDB554722) on ice for 20 min and washed twice with perm/wash buffer (BD Biosciences, BDB554723). Cells were then stained with anti-granzyme B Brilliant Violet 421 (BioLegend, QA18A28, 396414, 1:200) overnight and analysed with flow cytometry. Gating strategies are shown in Supplementary Fig. 32.

Animals

Female BALB/c (aged 6–8 weeks), C57BL/6J (aged 6–8 weeks) and Institute of Cancer Research (ICR, aged 6–8 weeks) mice were purchased from Hunan SJA Laboratory Animal (China) or GemPharmatech (China). OT-I (C57BL/6-Tg(TcrTcrb)1100Mjb/J) TCR transgenic mice (originally purchased from The Jackson Laboratory) were obtained from the laboratory of T.-M. Sun. All animals received care in compliance with the guidelines outlined in the Guide for the Care and Use of Laboratory Animals. All mice were housed under specific-pathogen-free conditions in a room with regulated temperature (–22 °C) and humidity (45–60%) under 12 h–12 h light–dark cycle.

For tumour experiments, a maximal tumour volume of 2,000 mm³ was defined as the humane end point. Tumour volumes were measured using callipers and calculated using the following formula: volume = ($L \times W^2$)/2, where L and W represent two perpendicular diameters, namely the longest and shortest diameters, respectively⁶⁰. For tumours that appeared nearly spherical, as confirmed by measurements using callipers, a single-diameter was used as both L and W in the same formula⁶⁰. Subcutaneous tumours and orthotopic breast tumours that were non-palpable were recorded as 0 mm³.

Statistics and reproducibility

Sample sizes for animal experiments were determined on the basis of initial pilot studies, previous experience with experimental models or literature precedents to adequately show the difference in outcome between different groups. No statistical methods were used to predetermine sample size. For subcutaneous tumour and orthotopic breast tumour studies, tumour-bearing mice were randomly assigned to match similar tumour sizes among groups. For the MC38 peritoneal metastasis study and other in vivo studies, mice were randomly allocated into experimental groups. The investigators were not blinded to group allocation during data collection and data analysis. Quantitative data with error bars are presented as the mean $\pm$ s.d. for in vitro experiments and as the mean $\pm$ s.e.m. for animal experiments, where applicable. GraphPad Prism software (v.8.0, v.10.6) was used to perform statistical analyses. Statistical significance was determined using two-tailed unpaired Student's t -tests or Mann–Whitney U -tests, one-way ANOVA with Tukey's or Dunnett's multiple-comparison test or two-way ANOVA with Tukey's multiple-comparison test. Survival differences were analysed using the log-rank Mantel–Cox test; $P < 0.05$ was considered statistically significant. The number of replicates performed is indicated in each figure legend, where applicable. Pearson's r was used to calculate correlations.

Ethics statement

All experimental mouse procedures were approved by the Institutional Animal Care and Use Committee at South China University of Technology (approval no. 2023032, 2025003 and AE-2025041). Human blood from healthy donors was obtained and processed using a protocol approved by the Institutional Review Board of Sun Yat-Sen Memorial Hospital, Sun Yat-Sen University (approval no. SYSKY-2024-762-01). Written informed consent was obtained from the participants.

Reporting summary

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

Data availability

RNA-seq and scRNA-seq data generated in this study have been deposited in the NCBI Sequence Read Archive under the BioProject accession numbers PRJNA1091224, PRJNA1413508 and PRJNA1417626. Publicly available resources used for analysis include GRCm38 mouse genome assembly (https://www.ncbi.nlm.nih.gov/datasets/genome/GCF_000001635.26/), MSigDB for Hallmark, GO and Reactome gene sets (<https://www.gsea-msigdb.org/gsea/msigdb/mouse/genesets.jsp>) and the DAVID web server for functional annotation (<https://david.ncifcrf.gov>). Source data are provided with this paper.

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Acknowledgements We thank L. Tang (EPFL) for the discussion and X. Xia (Sun Yat-Sen University Cancer Center) for providing the MC38_{OVA} cells. Illustrations were created using BioRender.

Author contributions YY. contributed to the overall coordination and organization of the project, and to the design, data analysis and manuscript writing for studies on the membranolytic and mLCD mode, transcriptomic analyses and part of the animal experiments.

L.L. contributed to the design, data analysis and manuscript writing for studies of the immunological effects of MPs and most animal studies. J.L. contributed to the design, synthesis, structural characterization, data analysis and manuscript writing of the peptides. C.L., Z.W. and Yuhao Zhang assisted with polypeptide synthesis and characterization. F.W. performed western blot and RT-qPCR assays. K. Yan, L.Z., Z. Zhang, K. Yang and Z.L. assisted with scRNA-seq analysis. C.S. and H.Z. performed H&E and immunofluorescent staining and analysed the data. J. Chen assisted with confocal imaging and analysis. F.L. assisted with flow cytometry experiments. R.D. contributed to the generation of fluorescent-protein-expressing cell lines. Y. Zhou assisted with TEM experiments. X.X. assisted with the giant unilamellar vesicle study and cell cytotoxicity test. J.X. and S.H. constructed the CRISPR–Cas9 knockout cell lines. Yajing Zhang and P.Z. performed TCR-T studies. T.S. provided OT-I mice and technical support for the related experiments. Z. Zhao provided technical assistance on flow cytometry analyses. S.X. performed and analysed the simulation studies. J. Cheng contributed to the conception and design of the polypeptides and supervised the related experiments. Y.B. and M.X. contributed to the conception and design of the project, supervised all experiments and wrote the manuscript.

Funding This work was supported by the National Natural Science Foundation of China, 32522050 (Y.B.), 52233015 (J. Cheng), 32371389 (Y.B.), U22A20156 (T.S.), 22422306 (S.X.); National Key R&D Program of China, 2022YFB3804600 (M.X.); the GJYC Program of Guangzhou City 2024D03J0001 (M.X.); and the Science and Technology Planning Project of Guangdong Province, 2023B121060013 (Y.B.) and 2020B121030004 (Y.B.).

Competing interests YY., J.L., Y.B. and M.X. have submitted a patent application (202111564284.3) related to this study. The other authors declare no competing interests.

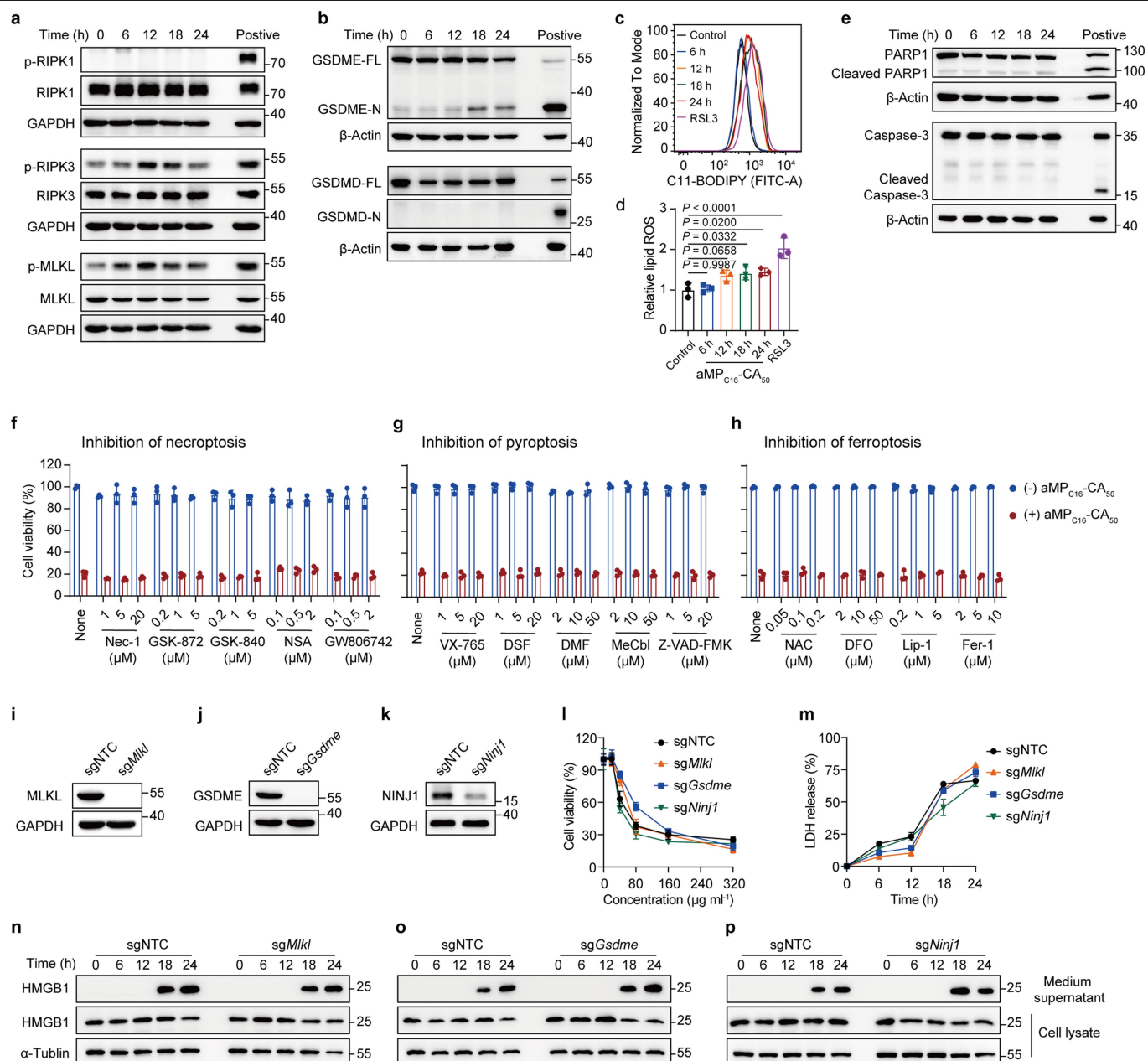
Additional information

Supplementary information The online version contains supplementary material available at <https://doi.org/10.1038/s41586-026-10899-5>.

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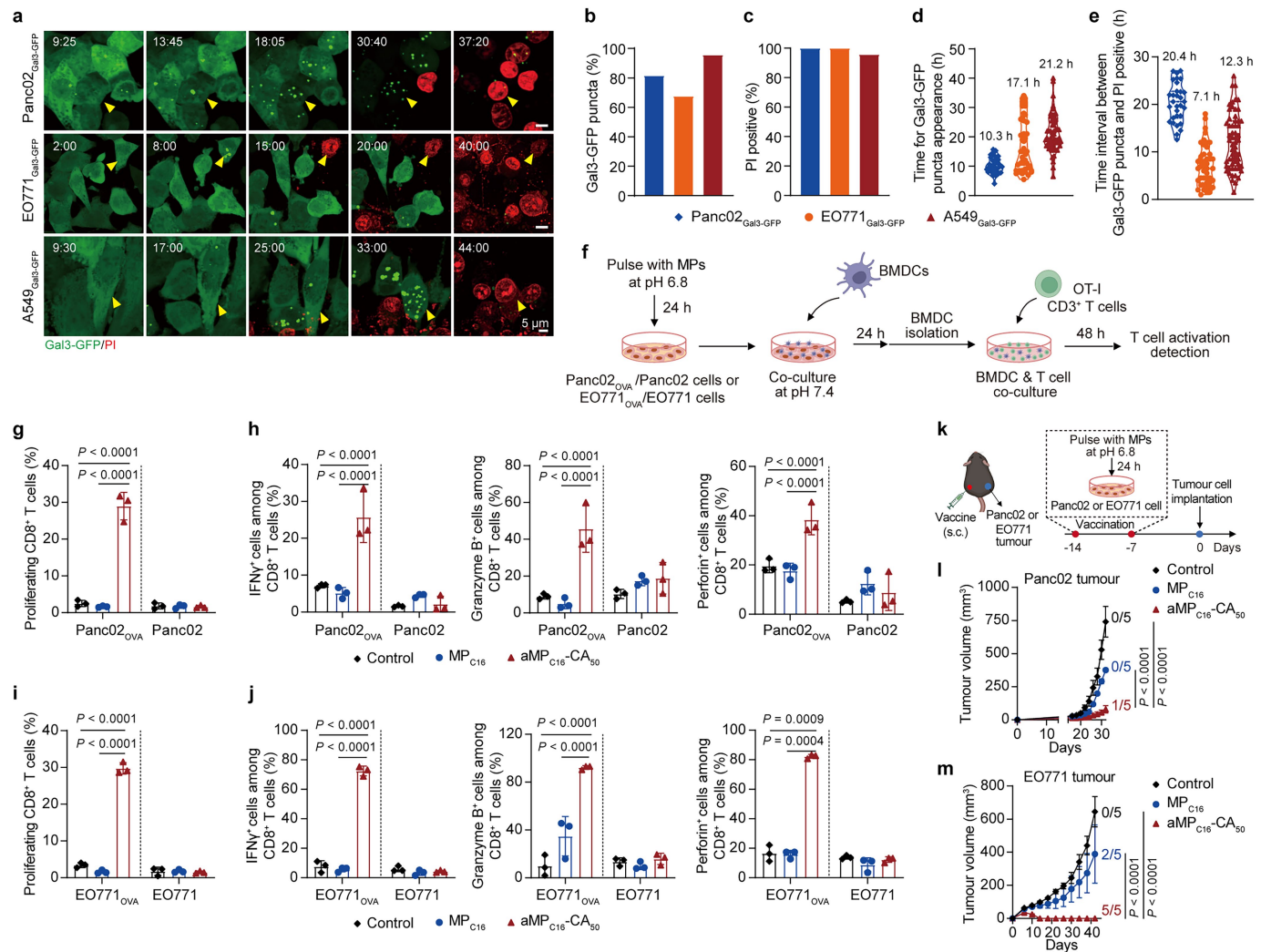
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Extended Data Fig. 1 | aMP_{C16}-CA₅₀ activates multiple regulated mLCD pathways without contributing to its cytotoxic effects. a–e, Activation of multiple regulated mLCD pathways in MC38 cells after aMP_{C16}-CA₅₀ (160 μg ml⁻¹) treatment for varying durations. **a**, Immunoblotting of p-RIPK1, RIPK1, p-RIPK3, RIPK3, p-MLKL, or MLKL. The combined treatment of TNF, cycloheximide (CHX), and Z-VAD-FMK serves as the positive control. **b**, Immunoblotting of GSDME-N and GSDMD-N. TNF plus CHX and doxorubicin treatment serves as the positive control for GSDME-N and GSDMD-N induction, respectively. **c,d**, Lipid peroxidation evaluated by C11-BODIPY 581/591 staining. RSL3 treatment serves as positive control. **c**, Representative flow cytometry plots of C11-BODIPY 581/591 in MC38 cells. **d**, Relative lipid ROS is expressed as the ratio of oxidized to reduced C11-BODIPY MFI. **e**, Immunoblotting of cleaved PARP1 or caspase-3. Doxorubicin treatment serves as positive control. **f–h**, Viability of MC38 cells after treatment with aMP_{C16}-CA₅₀ (160 μg ml⁻¹) for 24 h in the absence or presence of inhibitors. **f**, Necroptosis inhibitors: necrostatin-1 (Nec-1), GSK-872, GSK-840, necrosulfonamide (NSA), and

GW806742. **g**, Pyroptosis inhibitors: VX-765, disulfiram (DSF), dimethyl fumarate (DMF), methylcobalamin (MeCbl), and Z-VAD-FMK. **h**, Ferroptosis inhibitors: N-acetyl-L-cysteine (NAC), deferoxamine (DFO), liproxstatin-1 (Lip-1), and ferrostatin-1 (Fer-1). **i–k**, Immunoblotting of MLKL (**i**), GSDME (**j**), and NINJ1 (**k**) in MC38 cells deficient for *Mkl* (sgMkl), *Gsdme* (sgGsdme), and *Ninj1* (sgNinj1), respectively. NTC refers to the non-targeting control. **l,m**, Concentration-dependent cytotoxicity (**l**) and LDH release (**m**) induced by aMP_{C16}-CA₅₀ in MC38-sgNTC, MC38-sgMkl, MC38-sgGsdme, and MC38-sgNinj1 cells after incubation at pH 6.8 for 24 h. **n–p**, Immunoblotting of HMGB1 in medium supernatants and cell lysate of MC38-sgMkl (**n**), MC38-sgGsdme (**o**), and MC38-sgNinj1 (**p**) cells incubated with aMP_{C16}-CA₅₀ (160 μg ml⁻¹) at pH 6.8 for varying durations. MC38-sgNTC cells were included as controls. In **d,f–h,l,m**, data are mean ± s.d., $n = 3$ biological replicates. Statistical significance was determined using one-way ANOVA with Dunnett's multiple-comparison test (**d**). Uncropped immunoblot images are shown in Supplementary Fig. 1.



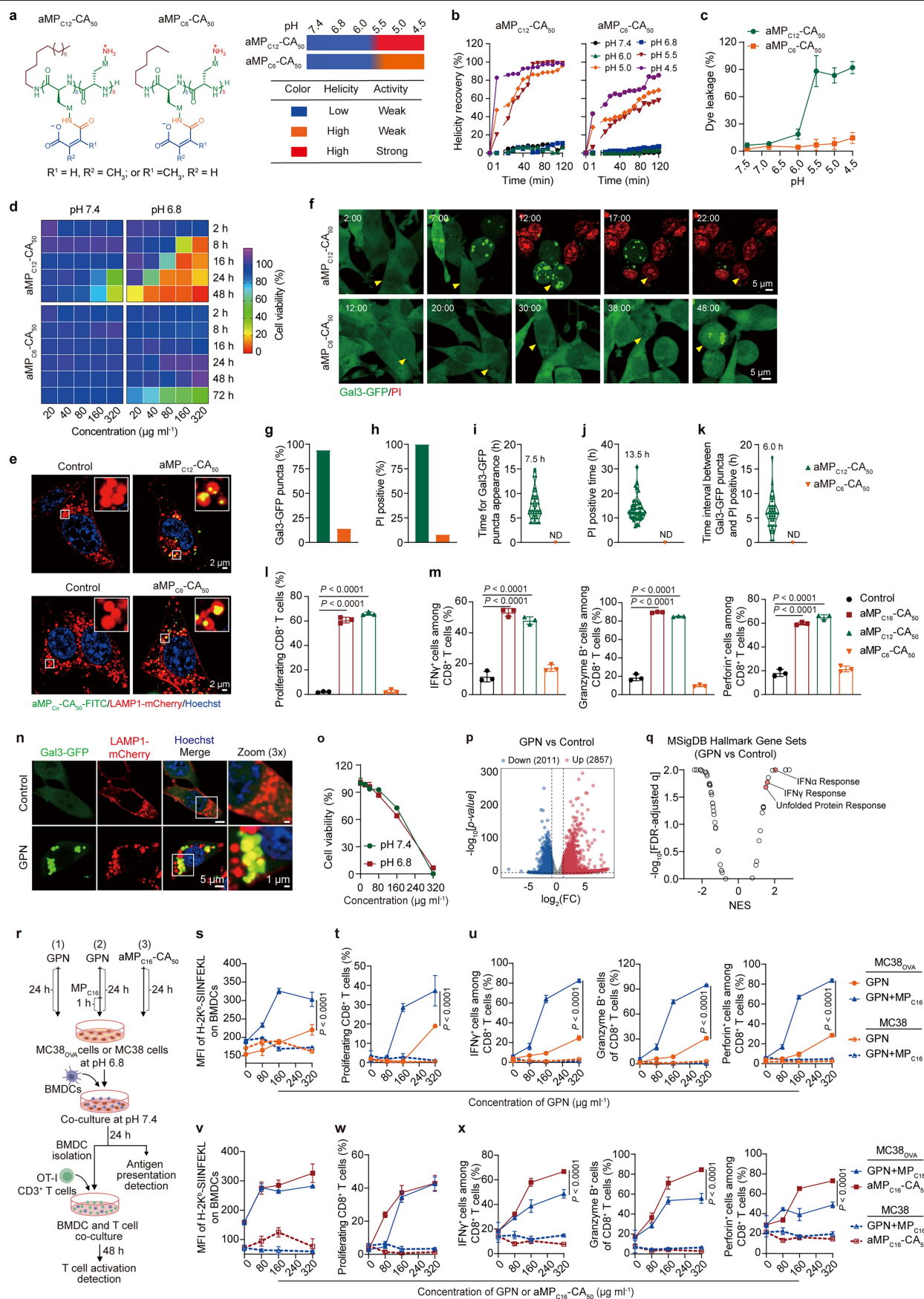
Extended Data Fig. 2 | aMP_{C16}-CA₅₀ induces time-lagged lysosomal-to-plasma membrane rupture and enhances the immunogenicity across.

a–e, Time-lapse confocal imaging of Panc02_{Gal3-GFP}, EO771_{Gal3-GFP} and A549_{Gal3-GFP} cells incubated with aMP_{C16}-CA₅₀ (160 μg ml⁻¹) and PI (red) at pH 6.8.

a, Representative images. Yellow arrowheads indicate the same cell at different time points. Scale bar, 5 μm. **b, c**, Percentage of cells exhibiting Gal3-GFP puncta (**b**) and PI-positive staining (**c**).

d, Appearance time of Gal3-GFP puncta in each cell. **e**, Time interval between Gal3-GFP puncta appearance and PI-positive staining in each cell. **f–j**, Analysis of CD8⁺ T cells activation. Peptides concentration, 160 μg ml⁻¹. **f**, Experimental design. OT-I T cells were co-incubated with BMDCs pre-incubated with MP_{C16}- or aMP_{C16}-CA₅₀-treated Panc02_{OVA} (**g, h**) or EO771_{OVA} (**i, j**) cells. Flow cytometry analysis of

CD8⁺ T-cell proliferation (**g, i**) and expression of IFN-γ, granzyme B, and perforin in CD8⁺ T cells (**h, j**). **k–m**, Prophylactic vaccination efficacy of aMP_{C16}-CA₅₀-treated tumour cells. **k**, Experimental design. Mice were vaccinated subcutaneously with Panc02 or EO771 cells that had been pretreated with 160 μg ml⁻¹ of either aMP_{C16}-CA₅₀ or MP_{C16} at pH 6.8 for 24 h. **l, m**, Tumour growth curves after challenge with viable, syngeneic tumour cells: Panc02 (**l**) and EO771 (**m**) cells, in the correspondingly vaccinated mice. In **g–j**, data are mean ± s.d., *n* = 3 biological replicates. In **l, m**, data are mean ± s.e.m., *n* = 5 mice. Statistical significance was determined using two-way ANOVA with Tukey's multiple-comparison test (**g–j, l, m**). In **a**, representative images from at least two independent experiments. Diagrams in **f, k** were created using BioRender; Xiong, M. <https://biorender.com/p8xag3c> (**f**), <https://biorender.com/ap2k7ea> (**k**).

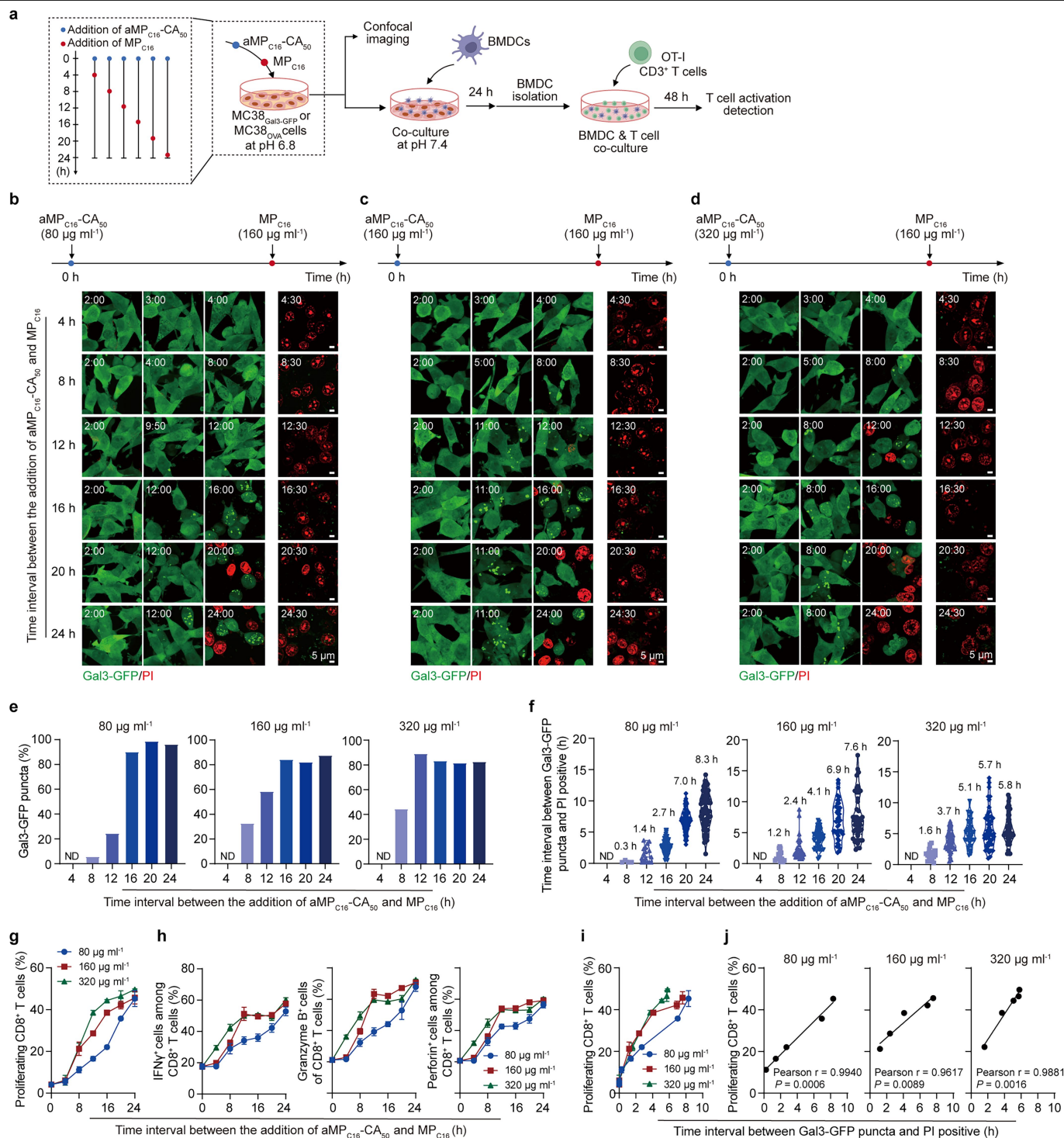


Extended Data Fig. 3 | See next page for caption.

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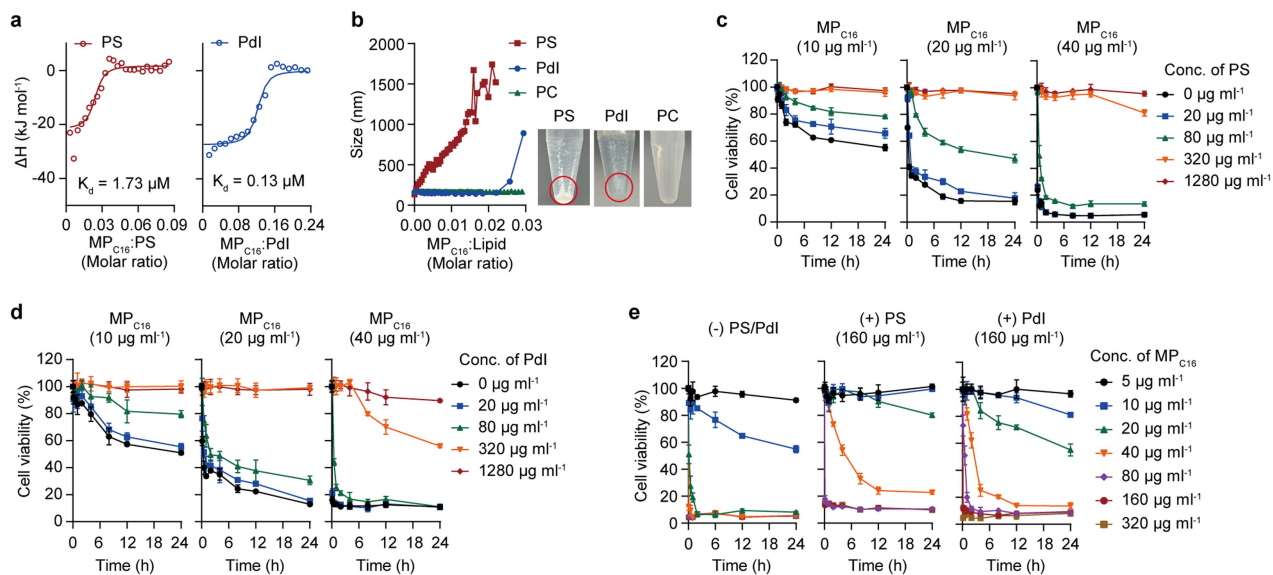
Extended Data Fig. 3 | Potent LMR activity of aMPs is crucial for enhancing the immunogenicity of lytic tumour cells. **a**, Schematic illustration of aMP_{C12}-CA₅₀ and aMP_{C6}-CA₅₀. **b**, Time-dependent helicity recovery of aMP_{Cn}-CA₅₀ (100 µg ml⁻¹) upon incubation in buffers of different pH. **c**, Dye leakage from liposomes upon incubation with aMP_{Cn}-CA₅₀ (160 µg ml⁻¹) following preincubation under different pH conditions overnight. **d**, Concentration- and time-dependent cytotoxicity of aMP_{Cn}-CA₅₀ against MC38 cells at pH 7.4 and 6.8 (*n* = 3 biological replicates). **e**, Confocal imaging of MC38_{LAMP1-mCherry} cells incubated with aMP_{Cn}-CA₅₀-FITC (160 µg ml⁻¹) at pH 6.8 for 1 h. Scale bars, 2 µm. **f–k**, Time-lapse confocal imaging of MC38_{Gal3-GFP} cells treated with aMP_{Cn}-CA₅₀ (160 µg ml⁻¹) and PI (red) at pH 6.8. **f**, Representative images. Yellow arrowheads indicate the same cell at different time points. Scale bar, 5 µm. **g, h**, Percentage of MC38_{Gal3-GFP} cells exhibiting Gal3-GFP puncta (**g**) and PI-positive staining (**h**). **i, j**, Time for Gal3-GFP puncta appearance (**i**) and PI-positive staining (**j**). **k**, Time interval between Gal3-GFP puncta appearance and PI-positive staining in each cell. ND refers to not detectable. **l, m**, Analysis of CD8⁺ T cells activation. OT-I T cells were co-cultured with BMDCs that had been pre-incubated with MC38_{OVA} cells treated with aMP_{Cn}-CA₅₀ (160 µg ml⁻¹) at pH 6.8 for 24 h. Flow cytometry analysis of CD8⁺ T-cell proliferation (**l**) and expression of IFN-γ, granzyme B, and perforin in CD8⁺ T cells (**m**). **n**, Confocal imaging of MC38_{LAMP1-mCherry&Gal3-GFP} cells treated with GPN (320 µg ml⁻¹) at pH 6.8 for 1 h. Scale bars, 5 and 1 µm for regular and magnified images, respectively. **o**, Concentration-dependent cytotoxic effects of GPN on MC38 cells upon

incubation at pH 7.4 or 6.8 for 24 h. **p, q**, RNA-seq analysis of MC38 cells treated with GPN (320 µg ml⁻¹) at pH 6.8 for 24 h versus untreated controls. **p**, 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. **q**, GSEA of transcriptional profiles using the hallmark gene set from MSigDB. NES: normalized enrichment scores. FDR: false-discovery rate. **r–x**, Combination of GPN and MP_{C16} effectively enhances the ability of MC38 cells to stimulate antigen presentation and T cell activation. **r**, Schematic illustration of sample preparation strategy. MC38_{OVA} or MC38 cells were treated with (1) GPN, (2) the combination of GPN and MP_{C16}, or (3) aMP_{C16}-CA₅₀ at varying concentrations for 24 h; MP_{C16} (160 µg ml⁻¹) was applied for 1 h. BMDCs were co-incubated with pretreated MC38_{OVA} or MC38 cells, and subsequent with OT-I T cells. Comparisons were made between the combination of MP_{C16} and GPN versus GPN treatment (**s–u**), and between the combination of MP_{C16} and GPN versus aMP_{C16}-CA₅₀ treatment (**v–x**). Flow cytometry analysis of H-2K^b-SIINFEKL expression on BMDCs (**s, v**), CD8⁺ T-cell proliferation (**t, w**), and expression of IFN-γ, granzyme B, and perforin in CD8⁺ T cells (**u, x**). In **c, l, m, o, s–x**, data are mean ± s.d., and *n* = 3 biological replicates. In **e, f, n**, representative images from at least two independent experiments. Statistical significance was determined using DESeq2 (two-sided Wald tests without adjustment) (**p**), permutation testing with FDR for GSEA (**q**), one-way ANOVA (**l, m**), and two-way ANOVA (**s–x**) with Tukey's multiple-comparison test. Diagrams in **r** were created using BioRender; Xiong, M. <https://biorender.com/p8xag3c>.



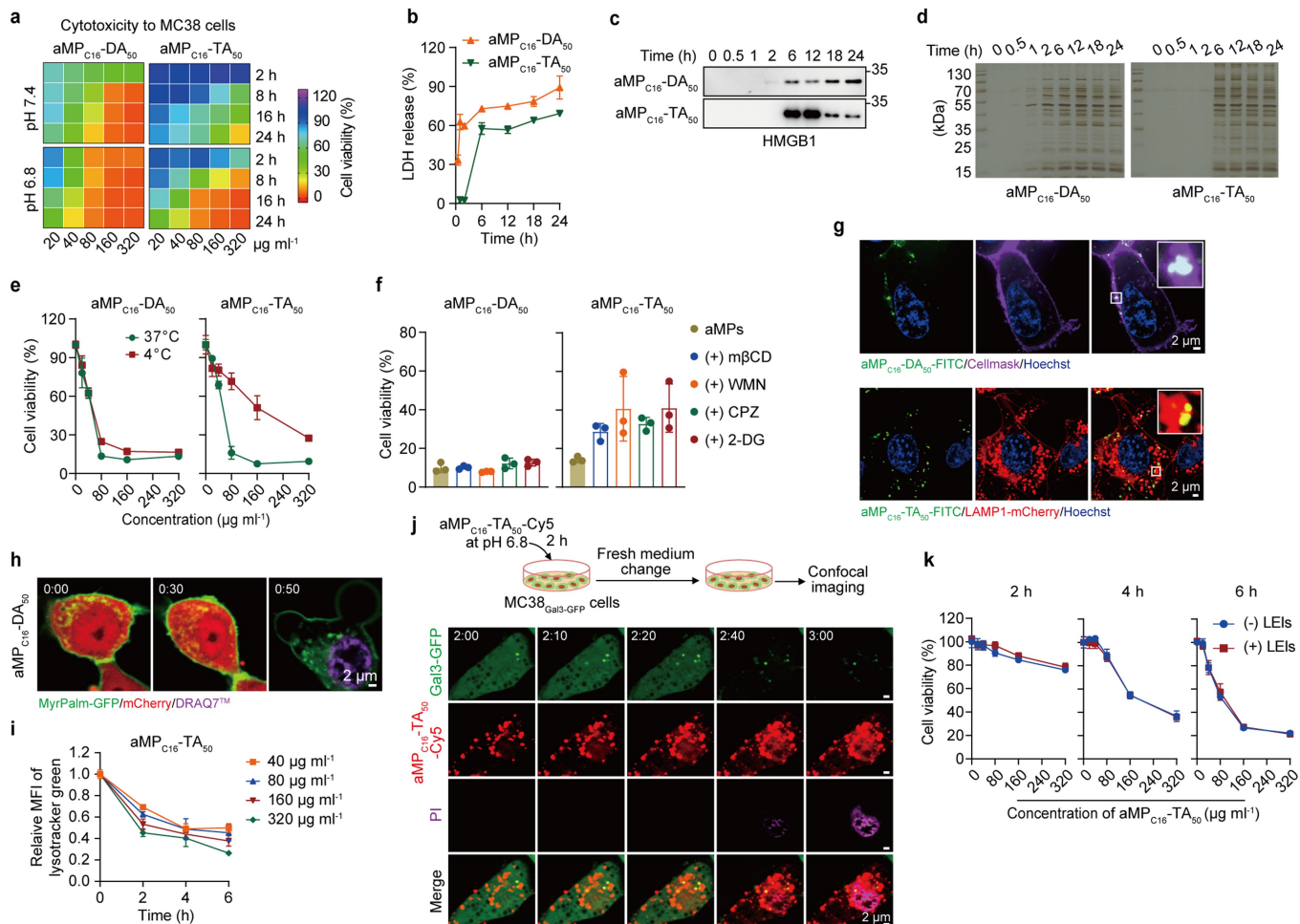
Extended Data Fig. 4 | Temporally controlled treatment with aMP_{C16}-CA₅₀ and MP_{C16} modulates the LMR-PMR interval and immunogenicity in lytic MC38 cells. **a, Experimental design. MC38_{Gal3-GFP} or MC38_{OVA} cells were sequentially treated with aMP_{C16}-CA₅₀ (80, 160, or 320 μg ml⁻¹) and MP_{C16} (160 μg ml⁻¹), while varying the time interval between their additions. **b–f**, Time-lapse confocal imaging of MC38_{Gal3-GFP} cells. **b–d**, Representative images from two independent experiments. Scale bar, 5 μm. The concentrations of aMP_{C16}-CA₅₀ in **b**, **c**, and **d** were 80, 160 and 320 μg ml⁻¹, respectively. **e**, Percentage of Gal3-GFP puncta-positive cells. **f**, Time interval between**

Gal3-GFP puncta appearance and PI-positive staining. **g, h**, Flow cytometry analysis of CD8⁺ T-cell proliferation (**g**) and expression of IFN-γ, granzyme B, and perforin in CD8⁺ T cells (**h**), following the stimulation schedule depicted in **a**. **i, j**, Correlation between CD8⁺ T-cell proliferation and the time interval between Gal3-GFP puncta appearance and PI-positive staining across treatments. Statistical significance was determined using two-sided Pearson correlation test. In **g–i**, data are mean ± s.d., and *n* = 3 biological replicates. Diagrams in **a** were created using BioRender; Xiong, M. <https://biorender.com/p8xag3c>.



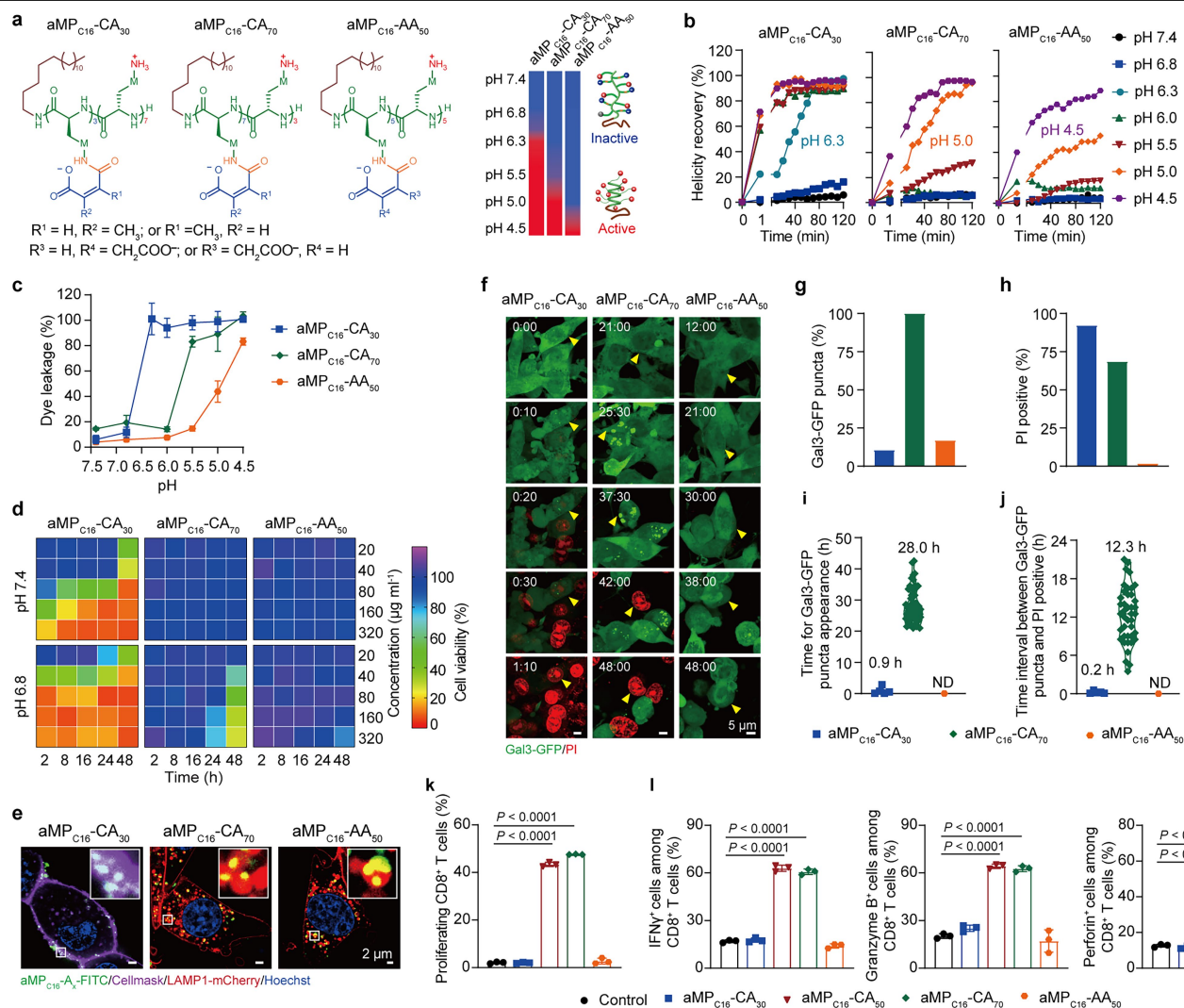
Extended Data Fig. 5 | Cytotoxicity of MP_{C16} is inhibited in a dose-dependent manner upon its interaction with anionic phospholipids. **a**, ITC-determined integrated heat release and equilibrium dissociation constant (K_d) of the interaction between MP_{C16} and anionic phospholipids (PS and Pdl). ΔH , enthalpy change. **b**, Size change of lipid vehicles when titrated with MP_{C16}. Images of the mixture of MP_{C16} and phospholipid are shown. **c,d**, Concentration-

and time-dependent cytotoxicity of MP_{C16} against MC38 cells in the absence or presence of PS (**c**) or Pdl (**d**) at varying concentrations. **e**, Concentration- and time-dependent cytotoxicity of MP_{C16} against MC38 cells in the absence or presence of PS or Pdl (160 μ g ml⁻¹). In **c-e**, data are mean $\pm$ s.d., and $n = 3$ biological replicates.



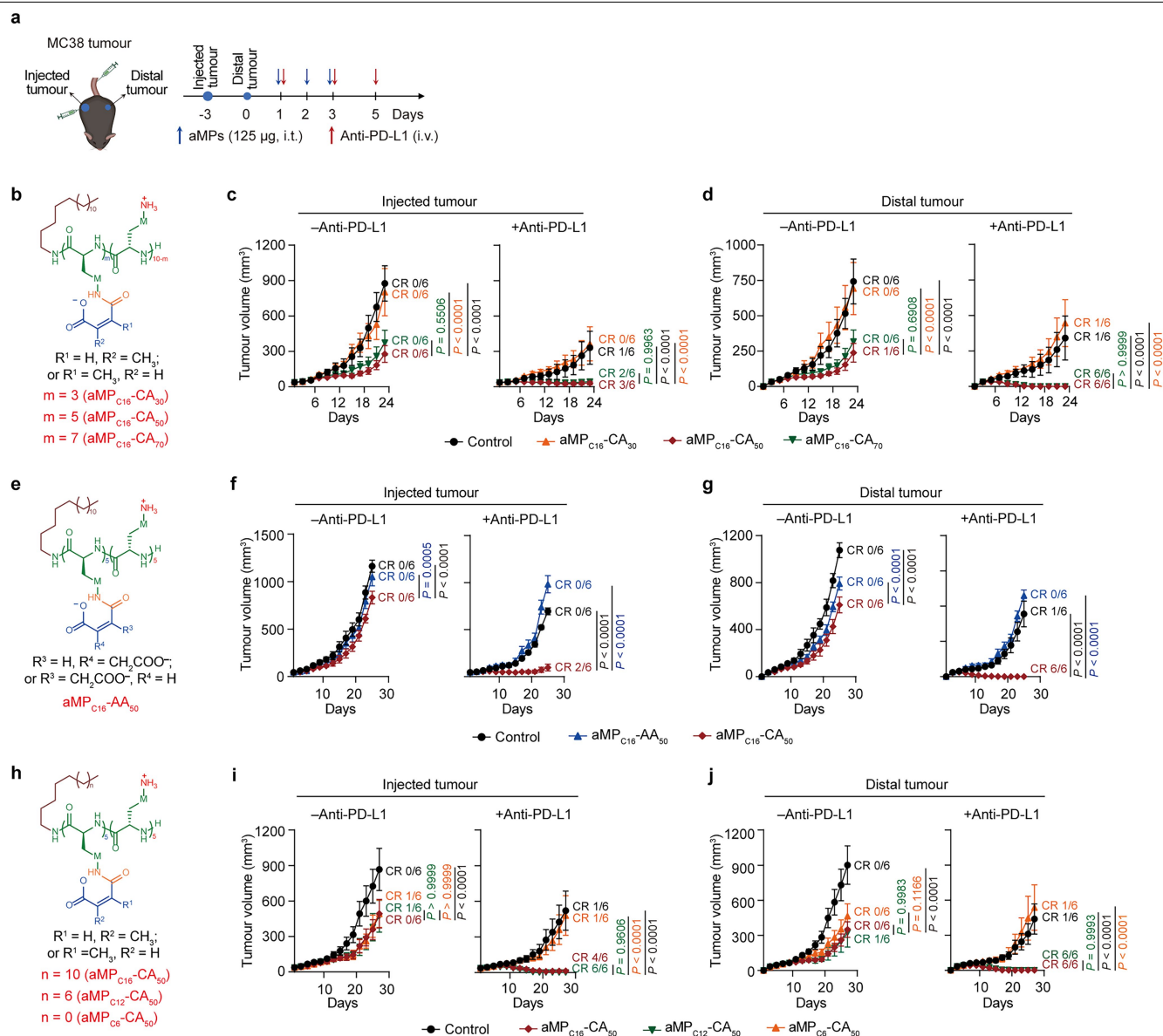
Extended Data Fig. 6 | pH dependent cytotoxic effects of aMP_{C16}-DA₅₀ and aMP_{C16}-TA₅₀. **a**, Concentration- and time-dependent cytotoxicity of aMP_{C16}-DA₅₀ and aMP_{C16}-TA₅₀ against MC38 cells at pH 7.4 or 6.8. $n = 3$ biological replicates. **b–d**, Time-dependent release of cellular contents of MC38 cells incubated with aMP_{C16}-DA₅₀ or aMP_{C16}-TA₅₀ (160 μg ml⁻¹) at pH 6.8 for varying durations. Levels of LDH (**b**), HMGB1 (**c**), and total proteins (**d**) in the supernatant were tested. **e**, Concentration- and temperature-dependent cytotoxicity of aMP_{C16}-DA₅₀ and aMP_{C16}-TA₅₀ against MC38 cells at pH 6.8 after 2 and 5 h incubation, respectively. **f**, Cytotoxicity of aMP_{C16}-DA₅₀ and aMP_{C16}-TA₅₀ (160 μg ml⁻¹) against MC38 cells at pH 6.8 without or with the presence of endocytosis inhibitors after 2 and 5 h incubation, respectively. **g**, Confocal imaging of MC38 cells incubated with aMP_{C16}-DA₅₀-FITC or aMP_{C16}-TA₅₀-FITC (160 μg ml⁻¹, in green) at pH 6.8 for 10 min and 1 h, respectively. Scale bars,

2 μm. **h**, Time-lapse confocal imaging of MC38_{Gal3-GFP}/mCherry cells incubated with aMP_{C16}-DA₅₀ (160 μg ml⁻¹) at pH 6.8. Scale bars, 2 μm. **i**, Flow cytometry analysis of relative MFI of LysoTracker Green in the aMP_{C16}-TA₅₀-treated MC38 cells compared to untreated cells. **j**, Time-lapse confocal imaging of MC38_{Gal3-GFP} cells upon incubation with aMP_{C16}-TA₅₀-Cy5 (160 μg ml⁻¹, in red) at pH 6.8. After incubating MC38_{Gal3-GFP} cells with aMP_{C16}-TA₅₀-Cy5 for 2 h, free aMP_{C16}-TA₅₀-Cy5 in the medium was washed out prior to time-lapse monitoring. **k**, Effect of LEIs on the viability of MC38 cells after treatment with aMP_{C16}-TA₅₀ (160 μg ml⁻¹). The LEIs included CA-074-Me (1 μM), E-64d (80 μM), and pepstatin (80 μM). In **b**, **e**, **f**, **i**, **k**, data are mean ± s.d., and $n = 3$ biological replicates. In **c**, **d**, **g**, **h**, **j**, representative images from at least two independent experiments. Uncropped immunoblot and gel images are shown in Supplementary Fig. 1. Diagram in **j** was created using BioRender, Xiong, M. <https://biorender.com/p8xag3c>.



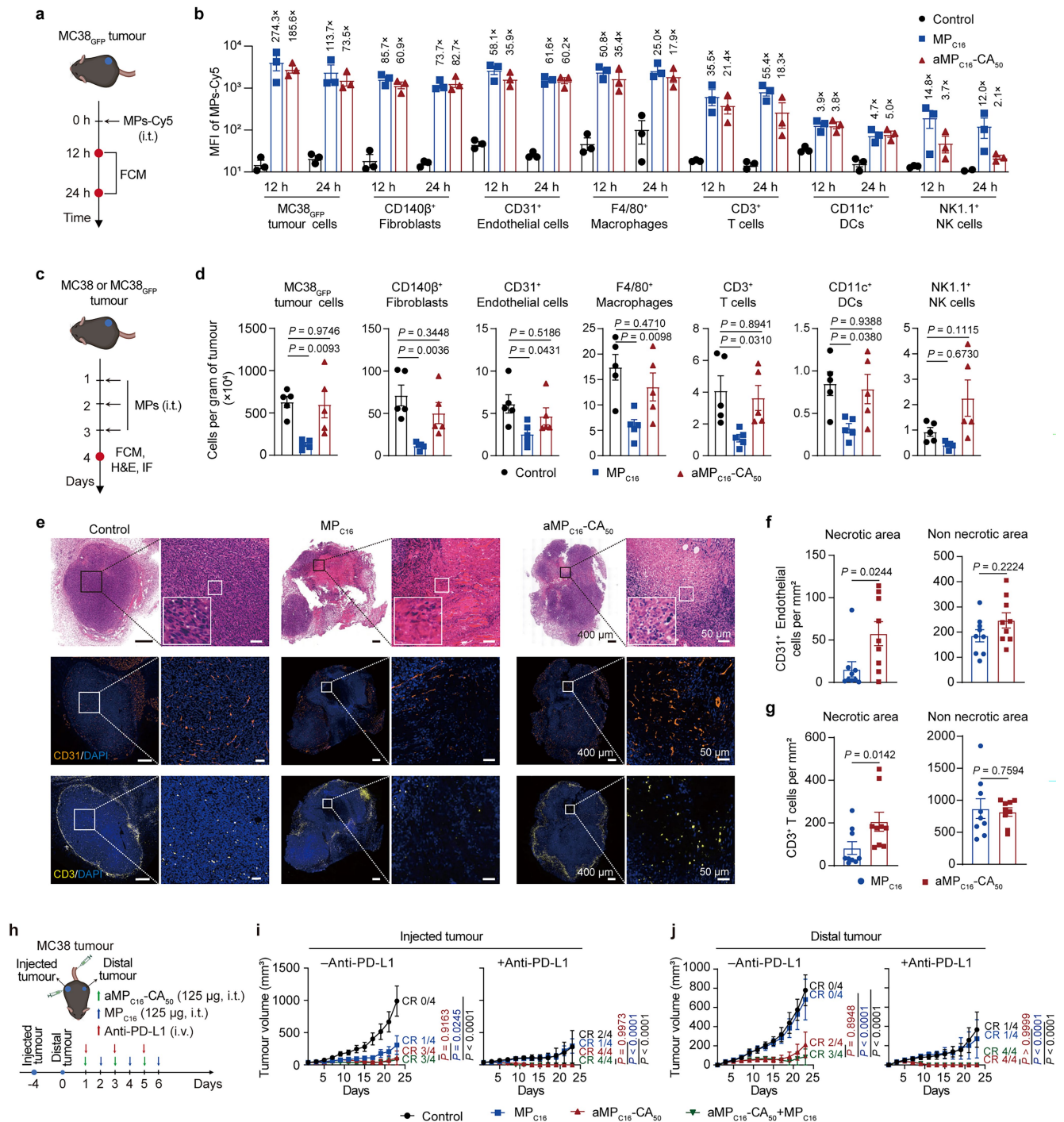
Extended Data Fig. 7 | pH-responsive kinetics of aMPs are crucial in modulating the process of membrane rupture and enhancing the immunogenicity of lytic tumour cells. **a**, Schematic illustration of aMP_{C16}-CA₃₀, aMP_{C16}-CA₇₀, and aMP_{C16}-AA₅₀, exhibiting varying pH-responsive kinetics. **b**, Time-dependent restoration of α -helical conformation of aMP_{C16}-A_x (100 μ g ml⁻¹) following incubation in buffers of different pH. **c**, Dye leakage from liposomes upon incubation with aMP_{C16}-A_x (160 μ g ml⁻¹) following preincubation under different pH conditions overnight. **d**, Concentration- and time-dependent cytotoxicity of aMP_{C16}-A_x against MC38 cells at pH 7.4 and 6.8 ($n = 3$ biological replicates). **e**, Confocal imaging of MC38_{LAMP1-mCherry} cells incubated with aMP_{C16}-A_x-FITC (160 μ g ml⁻¹, in green) at pH 6.8. Cells were treated with aMP_{C16}-CA₃₀-FITC for 10 min, with aMP_{C16}-CA₇₀-FITC for 1 h, or aMP_{C16}-AA₅₀-FITC for 1 h. Scale bars, 2 μ m. **f-j**, Time-lapse confocal imaging of

MC38_{Gal3-GFP} cells treated with aMP_{C16}-A_x (160 μ g ml⁻¹) and PI (red) at pH 6.8. **f**, Representative images. Yellow arrowheads indicate the same cell at different time points. Scale bar, 5 μ m. **g, h**, Percentage of MC38_{Gal3-GFP} cells exhibiting Gal3-GFP puncta (**g**) and PI-positive staining (**h**). **i**, Time for Gal3-GFP puncta appearance. **j**, Time interval between Gal3-GFP puncta appearance and PI-positive staining in each cell. **k, l**, Analysis of CD8⁺ T cells activation. OT-I T cells were co-cultured with BMDCs that had been pre-incubated with MC38_{OVA} cells treated with aMP_{C16}-A_x (160 μ g ml⁻¹) at pH 6.8 for 24 h. Flow cytometry analysis of CD8⁺ T-cell proliferation (**k**) and expression of IFN- γ , granzyme B, and perforin in CD8⁺ T cells (**l**). In **c, k, l**, data are mean $\pm$ s.d., and $n = 3$ biological replicates. Statistical significance was determined using one-way ANOVA with Tukey's multiple-comparison test (**k, l**). Representative images from at least two independent experiments are shown in **e, f**.



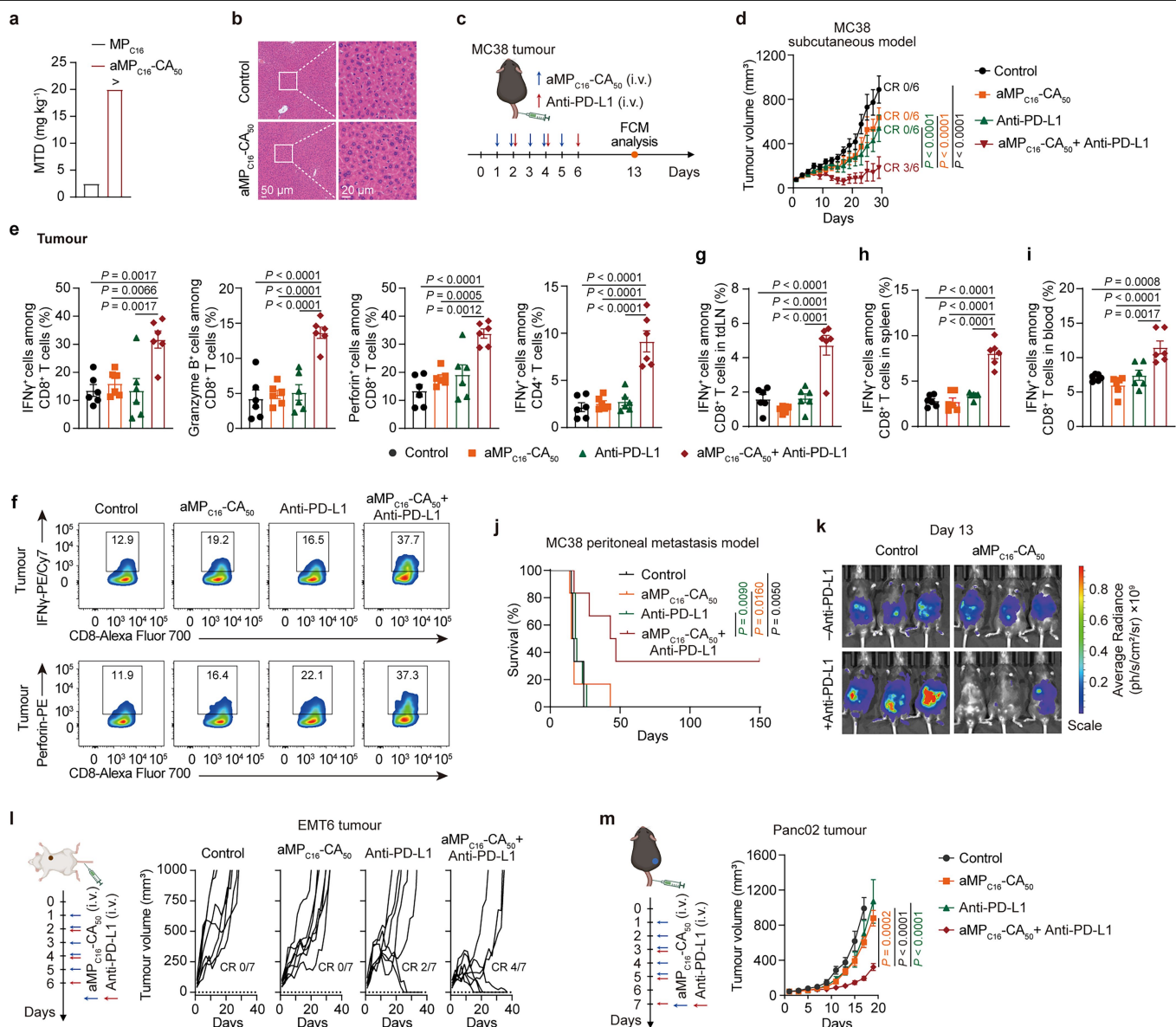
Extended Data Fig. 8 | Kinetically programmed aMPs potentiate anti-PD-L1 therapy in the MC38 bilateral. **a**, Experimental schedule for tumour inoculation and treatments of mice. Mice bearing bilateral MC38 tumours received i.t. injections of aMPs (125 µg per tumour for each injection) into the right flank tumours and the i.v. administration of anti-PD-L1 (0.75 mg kg⁻¹). **b-d**, Antitumour efficacy of combined treatment of aMP_{C16}-CA_x and anti-PD-L1 (x includes 30, 50, and 70). **e-g**, Antitumour efficacy of combined treatment of aMP_{C16}-AA₅₀ and anti-PD-L1 (A includes CA and AA). **h-j**, Antitumour efficacy of

combined treatment of aMP_{Cn}-CA₅₀ and anti-PD-L1 (Cn includes C6, C12, and C16). Chemical structures of aMPs (**b,e,h**) and tumour growth curves of the injected (**c,f,i**) and distal (**d,g,j**) tumours are shown. CR, complete response. In **c,d,f,g,i,j**, data are mean ± s.e.m., $n = 6$ mice, and statistical significance was determined using two-way ANOVA followed by Tukey's multiple-comparison test, comparing aMP_{C16}-CA₅₀ with the indicated groups. Diagram in **a** was created using BioRender; Xiong, M. <https://biorender.com/ap2k7ea>.



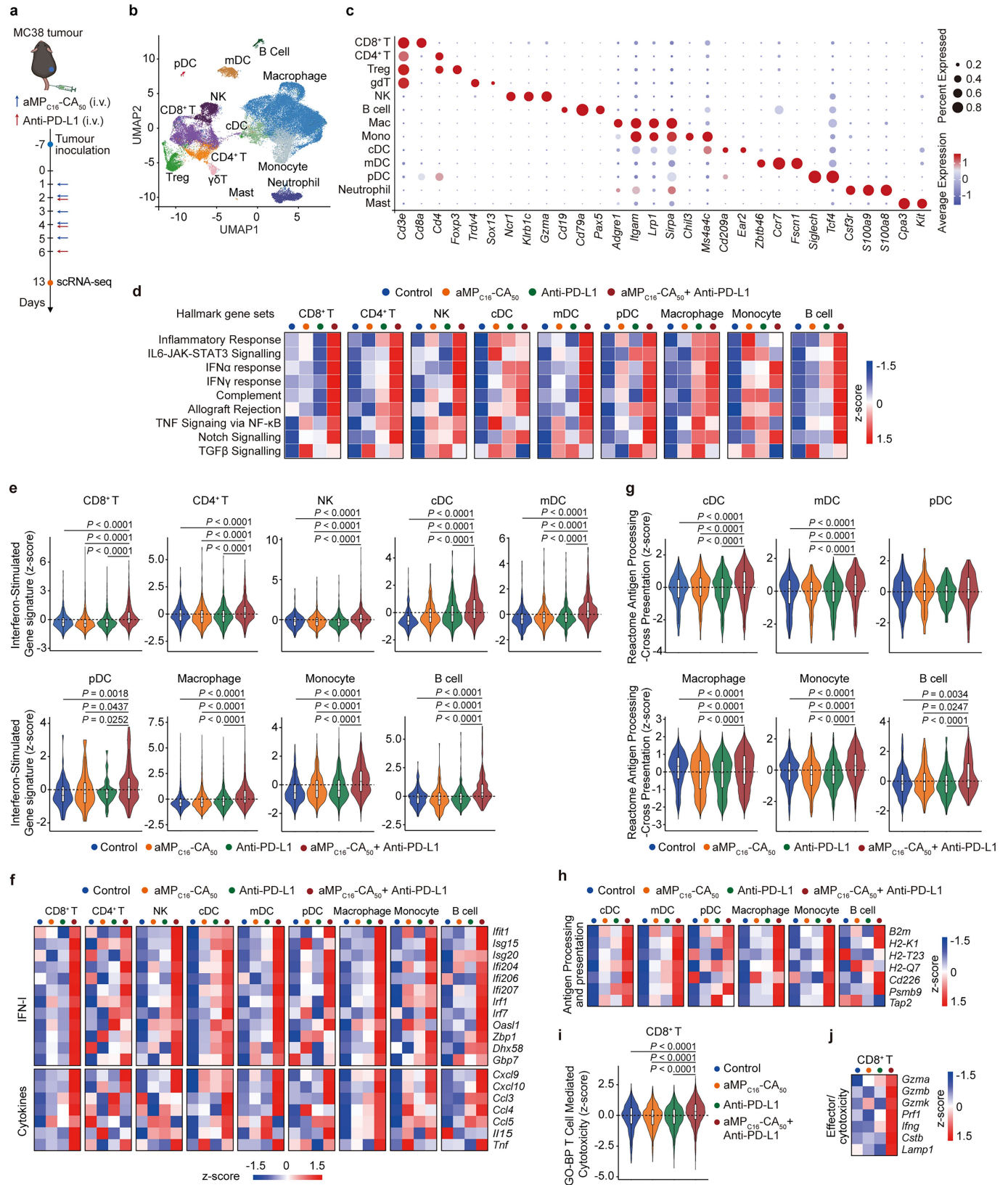
Extended Data Fig. 9 | Cellular uptake and cytotoxicity of aMP_{C16}-CA₅₀ in MC38. **a, b**, Cellular uptake in various viable cell populations in MC38_{GFP} tumours at 12 and 24 h following i.t. administration of aMP_{C16}-CA₅₀-Cy5 or MP_{C16}-Cy5 (125 μg per mouse). MC38_{GFP} cells express MyrPalm-GFP on the cell membranes. **c–g**, Cytotoxicity analysis of aMP_{C16}-CA₅₀ and MP_{C16} in MC38 tumours. **c**, Experimental design. Following three i.t. administrations of aMP_{C16}-CA₅₀ or MP_{C16} (125 μg per mouse for each injection), MC38_{GFP} or MC38 tumours were subjected to flow cytometry and histological analyses, respectively. **d**, Flow cytometry analysis of viable cell numbers across various cell types. **e–g**, H&E staining and immunofluorescent (IF) staining for CD31 and CD3 on consecutive tumour sections. **e**, Representative images. Scale bars, 400 and 50 μm for regular and magnified images, respectively. **f, g**, Quantification of CD31⁺ endothelial cells (**f**) and CD3⁺ T cells (**g**) in necrotic and non-necrotic

area. **h–j**, Alternating administration of aMP_{C16}-CA₅₀ and MP_{C16} did not compromise the antitumour efficacy of aMP_{C16}-CA₅₀. **h**, Experimental schedule for tumour inoculation and treatments. Mice bearing bilateral MC38 tumours received i.t. injections into the right flank tumours of aMP_{C16}-CA₅₀, MP_{C16}, or an alternating combination of both (125 μg per tumour for each injection). anti-PD-L1 was i.v. administrated at a dose of 0.75 mg kg^{−1}. **i, j**, Tumour growth curves of the injected (**i**) and distal (**j**) tumours. CR, complete response. In **b, d, f, g, i, j**, data are mean ± s.e.m., *n* = 3 mice (**b**), 5 mice (**d**), 9 mice (**f, g**), 4 mice (**i, j**). Statistical significance was determined using two-tailed Mann-Whitney *U* test (**f, g**), one-way ANOVA (**d**) and two-way ANOVA (**i, j**) with Tukey's multiple-comparison test. In **e**, representative images from 9 mice are shown. Diagrams in **a, c, h** were created using BioRender; Xiong, M. <https://biorender.com/ap2k7ea>.



Extended Data Fig. 10 | aMP_{c16}-CA₅₀ is systemically administrable to potentiate anti-PD-L1. **a**, MTD of intravenously administered aMP_{c16}-CA₅₀ and MP_{c16} in ICR mice. $n = 6$ mice. **b**, H&E staining of liver sections from ICR mice 24 h after aMP_{c16}-CA₅₀ administration. Scale bars, 50 μm (left) and 20 μm (right). Representative images from three mice are shown. **c–k**, Antitumour efficacy and T cell activation induced by combination therapy in MC38 tumour models. $n = 6$ mice. **c**, The schedule for treatments and sample collection for flow cytometry analysis. Tumour-bearing mice were i.v. administered with aMP_{c16}-CA₅₀ (15 mg kg⁻¹) and anti-PD-L1 (0.75 mg kg⁻¹). **d**, Growth curves of subcutaneous MC38 tumours. **e–i**, Flow cytometry analysis of T cells in the tumour (**e,f**), draining lymph node (**g**), spleen (**h**), and blood (**i**) of subcutaneous MC38 tumour-bearing mice. **e,g–i**, Percentages of IFN-γ, granzyme B-, and

perforin-positive cells among the CD8⁺ T cells and IFN-γ-positive cells among the CD4⁺ T cells. **f**, Representative plots showing IFN-γ and perforin expression in CD8⁺ T cells from tumour tissues. **j,k**, Kaplan–Meier survival curves (**j**) and bioluminescence imaging (**k**) of mice bearing peritoneal metastases of luciferase-expressing MC38 tumours. **l,m**, Growth curves of orthotopic EMT6 breast tumours (**l**, $n = 7$ mice) and subcutaneous Panc02 tumours (**m**, $n = 5$ mice). Tumour-bearing mice were i.v. administered with aMP_{c16}-CA₅₀ (15 mg kg⁻¹) and anti-PD-L1 (0.75 mg kg⁻¹ in **l** and 1 mg kg⁻¹ in **m**). In **d,e,g–i,m**, data are mean $\pm$ s.e.m.. Statistical significance was determined using two-way ANOVA with Tukey's multiple-comparison test (**d,m**), one-way ANOVA with Tukey's multiple-comparison test (**e,g–i**), and log-rank test (**j**). Diagrams in **c,l,m** were created using BioRender; Xiong, M. <https://biorender.com/ap2k7ea>.



Extended Data Fig. 11 | See next page for caption.

Extended Data Fig. 11 | scRNA-seq transcriptional profiling of CD45⁺ cells from MC38 tumours. **a**, The schedule for tumour inoculation, treatments, and sample collection for scRNA-seq analysis. Mice bearing subcutaneous MC38 tumours were i.v. administered with aMP_{CL6}-CA₅₀ (15 mg kg⁻¹) and anti-PD-L1 (0.75 mg kg⁻¹). *n* = 3 mice. **b**, Uniform manifold approximation and projection (UMAP) map of CD45⁺ cells. cDC, conventional DC; mDC, migratory DC; pDC, plasmacytoid DC. **c**, Bubble plots showing marker differentially expressed genes (DEGs) for major immune cell lineages. DEGs were identified using a two-sided Wilcoxon rank-sum test. **d**, Heatmaps of scaled GSVA scores for immune signatures. **e**, Violin plots of signature scores for Interferon-Stimulated Genes⁵⁰ (Supplementary Table 3). **f**, Heatmaps showing scaled expression of IFN-I-response and cytokines-associated genes. **g**, Violin plots showing signature

scores for Antigen Processing-Cross Presentation in APCs. **h**, Heatmaps showing scaled expression of antigen-processing and presentation genes in APCs. **i**, Violin plots showing signature scores for T cell-Mediated Cytotoxicity in CD8⁺ T cells. **j**, Heatmaps showing scaled expression of effector/cytotoxicity genes in CD8⁺ T cells. **In e, g, i**, box plots indicate median (middle line), interquartile range (box), and 1.5×IQR (whiskers); cell numbers (*n*, left to right): CD8⁺ T cell, 980/1888/2425/3706; CD4⁺ T cell, 305/548/425/685; NK cell, 208/637/668/1344; cDC, 478/334/403/729; mDC, 263/319/434/456; pDC, 66/59/71/83; macrophage, 8408/6602/5538/9485; monocyte, 720/749/934/2328; B cell, 218/165/183/118. Statistical significance was determined using Kruskal-Wallis H test with Dunn's post hoc test (**e, g, i**). Diagram in **a** was created using BioRender; Xiong, M. <https://biorender.com/ap2k7ea>.

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Software and code

Policy information about [availability of computer code](#)

Data collection	Tecan iControl (v2.0) was used for absorbance and fluorescence measurements. Malvern Zetasizer software (v7.12) was used to acquire size and zeta potential data. TopSpin (v3.5, v4.1.1) was used for the data collection of ¹ H-NMR spectra and TOCSY NMR spectra, respectively. MicroCal PEAQ-ITC Control Software (v1.1) was used to acquire ITC data. Chirascan Software (v4.5.1848.0) was used for secondary structure determination. Molecular simulations and analyses were performed using the Bax Group PDB Server, the Automated Topology Builder (ATB) server, PACKMOL, GAMESS-US, GROMACS (v2020), and Visual Molecular Dynamics (VMD). Talos L120C TEM (Thermo Fisher) and Tecnai G2 Spirit TEM (FEI) were used for morphology observation. BD FACSDiva Software (v9.0) was used to acquire flow cytometry data on BD LSRFortessa, BD FACSCelesta, and BD FACSCanto II flow cytometers (BD Biosciences). Cell images were acquired using CellSens Software (v2.2). Western blot images were acquired using a ChemiDoc imaging system (Bio-Rad). Organ section images were acquired with the TissueFAXS imaging system (TissueGnostics) and KF-FL-005 digital pathology scanner (KFBIO). Bioluminescence signals were acquired using an IVIS Lumina imaging system (PerkinElmer). qPCR data were obtained using a LightCycler 96 software (Roche Applied Science). Bulk RNA sequencing was performed on the MGISEQ-2000RS platform (BGI). Single-cell RNA-seq libraries were sequenced on NovaSeq X Plus (Illumina).
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Data analysis

Data analysis and figure preparation were performed using GraphPad Prism (v8.0, v10.6). NMR data were analyzed using MestReNova (v12.0.0-20080). ITC data were processed using MicroCal PEAQ-ITC analysis software (v1.1). CD spectra deconvolution software (v2.1.0.223) was used for secondary structure analysis. Simulation analysis was performed with Visual Molecular Dynamics (VMD). Flow cytometry data were analyzed using FlowJo v.10 software. Cell imaging data were analyzed using Olympus OlyVIA. Western blot images were analyzed with Image Lab software (Bio-Rad). H&E-stained section images were analyzed using CaseViewer (v2.1). Quantitative analysis of immunofluorescence images was performed using HALO image analysis software. Bioluminescence images were analyzed with Living Image software (v4.4). Bowtie2 (v2.4.5), RSEM (v1.3.1), DESeq2 (v1.34.0) in R, DAVID (<https://david.ncifcrf.gov/>), OmicStudio tools (<https://www.omicstudio.cn/tool>) and GSEA software (v4.3) were used for RNA-seq analysis. Cell Ranger (v8.0.0), Seurat R package (v5.1.0), GSVA R package (v1.50.5), MSigDB (v7.5.1), ggplot2 R package (v4.0.1) and AuCell R package v (1.28.0) were used for scRNA-seq analysis.

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio [guidelines for submitting code & software](#) for further information.

Data

Policy information about [availability of data](#)

All manuscripts must include a [data availability statement](#). This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our [policy](#)

RNA-seq data and scRNA-seq generated in this study have been deposited in the NCBI Sequence Read Archive (SRA) under the BioProject accession number PRJNA1091224, PRJNA1413508 and PRJNA1417626. Publicly available resources used for analysis include GRCm38 mouse genome assembly (https://www.ncbi.nlm.nih.gov/datasets/genome/GCF_000001635.26/), MSigDB for Hallmark, Gene Ontology (GO) and Reactome gene sets (<https://www.gsea-msigdb.org/gsea/msigdb/mouse/genesets.jsp>), and the DAVID web server for functional annotation (<https://david.ncifcrf.gov/>).

Research involving human participants, their data, or biological material

Policy information about studies with [human participants or human data](#). See also policy information about [sex, gender \(identity/presentation\)](#), [and sexual orientation](#) and [race, ethnicity and racism](#).

Reporting on sex and gender

Peripheral blood was collected from healthy donors who were confirmed to be HLA-A*02:01 positive, and no donor sex or gender information was provided.

Reporting on race, ethnicity, or other socially relevant groupings

This study does not include any variables on race, ethnicity, or other socially relevant groups.

Population characteristics

The donors were healthy individual screened for HLA-A*02:01 positivity. No other population characteristics were considered as variables in this study.

Recruitment

Healthy donors were recruited by Sun Yat-sen Memorial Hospital, Sun Yat-Sen University.

Ethics oversight

Human blood from healthy donors was obtained and processed using a protocol approved by the Institutional Review Board of Sun Yat-Sen Memorial Hospital, Sun Yat-Sen University (Approval No. SYSKY-2024-762-01), and an informed consent form was signed by the participants prior to blood collection.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size

For in vivo experiments, sample size was determined using preliminary data or previous publications (e.g., PMID: 37076617 and PMID: 40108474) to ensure they are sufficient for statistical comparison between different groups. For in vitro assays, sample sizes were determined according to common standards in the field. Sample sizes (n) for all in vitro and in vivo experiments are indicated in the figure legends.

Data exclusions

No data were excluded.

Replication

The number of replicates performed is indicated in the figure legend, where applicable

Randomization

Randomization was not applicable for in vitro studies. For in vivo tumour studies, tumour bearing mice were randomly assigned to match similar tumour sizes among groups. For the peritoneal metastasis model of MC38 tumour study and other in-vivo studies, mice were randomly allocated into experimental groups.

Blinding

Blinding was not performed. Because the data were evaluated through objective, quantitative measurements rather than subjective assessment.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☐	☒ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☐	☒ Animals and other organisms
☒	☐ Clinical data
☒	☐ Dual use research of concern
☒	☐ Plants

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☐	☒ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used

- Antibodies for flow cytometry: anti-CD11c-PE (BioLegend, clone N418, 1:200), anti-CD11c-APC (BioLegend, clone N418, 1:200), anti-I-A/I-E- Alexa Fluor 700 (BioLegend, clone M5/114.15.2, 1:200), anti-I-A/I-E-BV510 (BioLegend, clone M5/114.15.2, 1:200), anti-CD45.2-APC/Cy7 (BioLegend, clone 104, 1:200), anti-CD80-FITC (BioLegend, clone 16-10A1, 1:200), anti-CD86-APC (BioLegend, clone GL-1, 1:200), anti-CD40-PerCP/Cy5.5 (BioLegend, clone 3/23, 1:200), anti-SIINFELK-H2Kb-PE/Cy7 (Thermo Fisher, clone 25-D1.16, 25-5743-82, 1:200), anti-CD8-PE/Cy7 (BioLegend, clone 53-6.7, 1:200), anti-CD8-Alexa Fluor 700 (BioLegend, clone 53-6.7, 1:200), anti-CD8-FITC (BioLegend, clone 53-6.7, 1:200), anti-IFN- γ -PE/Cy7 (BioLegend, clone XMG1.2, 505826, 1:200), anti-perforin-PE (clone S16009B, BioLegend, 1:200), and anti-granzyme B-APC (BioLegend, clone QA16A02, 372204, 1:200), Rat IgG1k isotype control-PE/Cy7 (BioLegend, clone RTK2071, 400416, 1:200), Rat IgG2ak isotype control-PE (BioLegend, clone RTK2758, 400508, 1:200), and Mouse IgG1k isotype control-APC (BioLegend, clone MOPC-21, 400142, 1:200), Human TruStain FcX™ (BioLegend, 422301, 1:100), anti-CD69-APC/Cy7 (BioLegend, clone FN50, 310913, 1:200), anti-CD8-PE (BioLegend, clone RAT-T8, 300907, 1:200), and anti-TCR β -APC (BioLegend, clone H57-597, 1:200), anti-CD8-Alexa Fluor 700 (BioLegend, clone HIT8a, 300920, 1:200), anti-Granzyme B-BV421 (BioLegend, clone QA18A28, 396414, 1:200), anti-CD11b-BV785 (BioLegend, clone M1/70, 101243, 1:200), anti-CD3-FITC (BioLegend, clone 17A2, 100204, 1:100), anti-CD3-BV711 (BioLegend, clone 17A2, 100241, 1:100), anti-CD4-BV510 (BioLegend, clone RM4-5, 100559, 1:200), anti-CD4-PerCP/Cy5.5 (BioLegend, clone RM4-5, 116012, 1:200), anti-NK-1.1-PE/Dazzle 594 (BioLegend, clone S17016D, 156518, 1:200), anti-Nkp46-BV650 (BioLegend, clone 29A1.4, 137635, 1:200), anti-Gr-1-PerCP/Cy5.5 (BioLegend, clone RB6-8C5, 108428, 1:200), anti-F4/80-APC (BioLegend, clone BM8, 123116, 1:200), anti-F4/80-Alexa Fluor 700 (BioLegend, clone BM8, 123130, 1:200), anti-CD31-PE/Cy7 (Thermo Fisher, clone 390, 25-0311-82, 1:200), anti-CD140 β -PE (BioLegend, clone APB5, 136006, 1:200), anti-mouse CD16/32 antibody (BioLegend, clone 93, 101302, 1:200).
- Antibodies for immunoblot: 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).
- Antibodies for immunofluorescence staining: 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).
- Antibodies for T cell activation: anti-human CD3 (Novoprotein, GMP-A018), and anti-human CD28 (Novoprotein, GMP-A063).
- Antibodies for in vivo treatments: anti-PD-L1 (BioXcell, clone 10F.9G2, BE0101), anti-CD4 (BioXcell, clone GK1.5, BE0003), anti-CD8 α (BioXcell, clone 2.43, BE0061), rat IgG2b isotype control (BioXcell, clone LTF-2, BE0090).

Validation

The following antibodies have been validated for the specificity and application by the manufacturers (see detailed reference on the website).

- Antibodies for flowcytometry:
anti-CD11c-PE: <https://www.biolegend.com/en-gb/products/pe-anti-mouse-cd11c-antibody-1816>
anti-CD11c-APC: <https://www.biolegend.com/en-us/products/apc-anti-mouse-cd11c-antibody-1813>
anti-I-A/I-E- Alexa Fluor 700: <https://www.biolegend.com/en-gb/products/alexa-fluor-700-anti-mouse-i-a-i-e-antibody-3413>
anti-I-A/I-E-BV510: <https://www.biolegend.com/en-gb/products/brilliant-violet-510-anti-mouse-i-a-i-e-antibody-7997>

anti-CD45.2-APC/Cy7: <https://www.biolegend.com/en-gb/products/apc-cyanine7-anti-mouse-cd45-2-antibody-3906>
 anti-CD80-FITC: <https://www.biolegend.com/en-gb/products/fitc-anti-mouse-cd80-antibody-41>
 anti-CD86-APC: <https://www.biolegend.com/en-gb/products/apc-anti-mouse-cd86-antibody-2896>
 anti-CD40-PerCP/Cy5.5: <https://www.biolegend.com/en-gb/products/percp-cyanine5-5-anti-mouse-cd40-antibody-8103>
 anti-SIINFELK-H2Kb+PE/Cy7: <https://www.thermofisher.cn/cn/zh/antibody/product/OVA257-264-SIINFELK-peptide-bound-to-H-2Kb-Antibody-clone-eBio25-D1-16-25-D1-16-Monoclonal/25-5743-82>
 anti-CD8-PE/Cy7: <https://www.biolegend.com/en-gb/products/pe-cyanine7-anti-mouse-cd8a-antibody-1906>
 anti-CD8-Alexa Fluor 700: <https://www.biolegend.com/en-gb/products/alexa-fluor-700-anti-mouse-cd8a-antibody-3387>
 anti-CD8-FITC: <https://www.biolegend.com/en-gb/products/fitc-anti-mouse-cd8a-antibody-153>
 anti-IFN- γ -PE/Cy7: <https://www.biolegend.com/en-gb/products/pe-cyanine7-anti-mouse-ifn-gamma-antibody-5865>
 anti-perforin-PE: <https://www.biolegend.com/en-gb/products/pe-anti-mouse-perforin-antibody-15287>
 anti-Granzyme B-APC: <https://www.biolegend.com/en-gb/products/apc-anti-human-mouse-granzyme-b-recombinant-antibody-14429>
 Rat IgG1k isotype control-PE/Cy7: <https://www.biolegend.com/en-gb/products/pe-cyanine7-rat-igg1-kappa-isotype-ctrl-1934>
 Rat IgG2ak isotype control-PE: <https://www.biolegend.com/en-gb/products/pe-rat-igg2a-kappa-isotype-ctrl-1843>
 Mouse IgG1k isotype control-APC: <https://www.biolegend.com/en-gb/products/apc-mouse-igg1-kappa-isotype-ctrl-icfc-3033>
 Human TruStain FcX™: <https://www.biolegend.com/en-gb/products/human-trustain-fcx-fc-receptor-blocking-solution-6462>
 anti-CD69-APC/Cy7: <https://www.biolegend.com/en-gb/products/apc-cyanine7-anti-human-cd69-antibody-1917>
 anti-CD8-PE: <https://www.biolegend.com/en-gb/products/pe-anti-human-cd8a-antibody-762>
 anti-TCR β -APC: <https://www.biolegend.com/en-gb/products/apc-anti-mouse-tcr-beta-chain-antibody-268>
 anti-CD8-Alexa Fluor 700: <https://www.biolegend.com/en-gb/products/alexa-fluor-700-anti-human-cd8a-antibody-3434>
 anti-Granzyme B-BV421: <https://www.biolegend.com/en-gb/products/brilliant-violet-421-anti-humanmouse-granzyme-b-recombinant-antibody-17577>
 anti-CD11b-BV785: <https://www.biolegend.com/en-gb/products/brilliant-violet-785-anti-mouse-human-cd11b-antibody-7958>
 anti-CD3-FITC: <https://www.biolegend.com/en-gb/products/fitc-anti-mouse-cd3-antibody-45>
 anti-CD3-BV711: <https://www.biolegend.com/en-gb/products/brilliant-violet-711-anti-mouse-cd3-antibody-10022>
 anti-CD4-BV510: <https://www.biolegend.com/en-gb/products/brilliant-violet-510-anti-mouse-cd4-antibody-7991>
 anti-CD4-PerCP/Cy5.5: <https://www.biolegend.com/en-gb/products/percp-cyanine5-5-anti-mouse-cd4-antibody-9223>
 anti-NK-1.1-PE/Dazzle 594: <https://www.biolegend.com/en-gb/products/pe-dazzle-594-anti-mouse-nk-1-1-antibody-20720>
 anti-NKp46-BV650: <https://www.biolegend.com/en-gb/products/brilliant-violet-650-anti-mouse-cd335-nkp46-antibody-15729>
 anti-Gr-1-PerCP/Cy5.5: <https://www.biolegend.com/en-gb/products/percp-cyanine5-5-anti-mouse-ly-6g-ly-6c-gr-1-antibody-4286>
 anti-F4/80-APC: <https://www.biolegend.com/en-gb/products/apc-anti-mouse-f4-80-antibody-4071>
 anti-F4/80-Alexa Fluor 700: <https://www.biolegend.com/en-gb/products/alexa-fluor-700-anti-mouse-f4-80-antibody-6556>
 anti-CD31-PE/Cy7: <https://www.thermofisher.cn/cn/zh/antibody/product/CD31-PECAM-1-Antibody-clone-390-Monoclonal/25-0311-82>
 anti-CD140 β -PE: <https://www.biolegend.com/en-gb/products/pe-anti-mouse-cd140b-antibody-6256>

2. Antibodies for immunoblot:

Anti-RIPK1: https://www.cellsignal.cn/products/primary-antibodies/rip-d94c12-rabbit-monoclonal-antibody/3493?qs=keyword_redirect&qt=3493
 anti-p-RIPK1 (Ser166): <https://www.cellsignal.cn/products/primary-antibodies/phospho-rip-ser166-e7g6o-rabbit-monoclonal-antibody/53286>
 anti-RIPK3: <https://www.cellsignal.cn/products/primary-antibodies/rip3-d4g2a-rabbit-monoclonal-antibody/95702>
 anti-p-RIPK3 (Thr231/Ser232): <https://www.cellsignal.cn/products/primary-antibodies/phospho-rip3-thr231-ser232-e7s1r-rabbit-monoclonal-antibody/91702>
 anti-MLKL: <https://www.cellsignal.cn/products/primary-antibodies/mlkl-d6w1k-rabbit-monoclonal-antibody/37705>
 anti-p-MLKL(S345): <https://www.abcam.cn/products/primary-antibodies/mlkl-phospho-s345-antibody-epr95152-ab196436>
 anti-GSDME: <https://www.abcam.cn/products/primary-antibodies/dfna5-gsdme-antibody-epr19859-n-terminal-ab215191>
 anti-GSDMD: <https://www.abcam.cn/products/primary-antibodies/gsdmd-antibody-epr19828-ab209845>
 anti-PARP (Abcam, ab191217, 1:1000): <https://www.abcam.cn/products/primary-antibodies/parp1-antibody-epr18461-ab191217>
 anti-caspase-3: <https://www.cellsignal.cn/products/primary-antibodies/caspase-3-antibody/9662>
 anti-LC3: <https://www.ptgcn.com/results?category=&q=14600-1-AP&target=>
 anti-SQSTM1/p62: <https://www.abcam.cn/products/primary-antibodies/sqstm1-p62-antibody-epr4844-autophagosome-marker-ab109012>
 anti-GAPDH: <https://www.ptgcn.com/products/GAPDH-Antibody-60004-1-Ig.htm>
 anti- β -Actin: <https://www.ptgcn.com/products/Pan-Actin-Antibody-66009-1-Ig.htm>
 anti- α -Tublin: <https://www.ptgcn.com/products/TUBA1B-Antibody-11224-1-AP.htm>
 anti-HMGB1: <https://www.abcam.cn/products/primary-antibodies/hmgb1-antibody-epr3507-ab79823>
 anti-NINJ1: <https://www.thermofisher.cn/cn/zh/antibody/product/Ninjurin-1-Antibody-Polyclonal/PAS-95755>

3. Antibodies for immunofluorescence staining

anti-CD3: <https://www.cellsignal.cn/products/primary-antibodies/cd3-epsilon-e4t1b-rabbit-monoclonal-antibody/78588>
 anti-CD31: <https://www.abcam.cn/products/primary-antibodies/hmgb1-antibody-epr3507-ab79823>
 Alexa Fluor® 555 AffiniPure® Goat Anti-Rabbit IgG (H+L): <https://www.jacksonimmuno.com/catalog/products/111-565-144>.

4. Antibodies for T cell activation: anti-human CD3: <https://www.novoprotein.com/product-detail?productNumber=GMP-A018>
 anti-human CD28: <https://www.novoprotein.com/product-detail?productNumber=GMP-A063>

5. Antibodies for in vivo treatments: anti-PD-L1: <https://bioxcell.com/invivomab-anti-mouse-pd-l1-b7-h1-be0101>
 anti-CD4: https://bioxcell.com/invivomab-anti-mouse-cd4-be0003-1?queryID=593e82c86436aacab61d8fa25a5b5a7c&objectID=30483&indexName=bioxcell_live_default_products
 anti-CD8 α : <https://bioxcell.com/invivomab-anti-mouse-cd8-alpha-be0061>
 rat IgG2b isotype control: <https://bioxcell.com/invivomab-rat-igg2b-isotype-control-anti-keyhole-limpet-hemocyanin-be0090>.

Eukaryotic cell lines

Policy information about [cell lines and Sex and Gender in Research](#)

Cell line source(s)	The MC38, Panc02, EMT6, SKOV3 and LoVo cells were sourced from 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).
Authentication	MC38, Panc02, EMT6 and A549 cells were authenticated via short tandem repeat (STR) profiling. MC38-MyrPalm-GFP/mCherry cells, MC38-Gal3-GFP cells, Panc02-Gal3-GFP cells, EO771-Gal3-GFP cells, A549-Gal3-GFP cells, MC38-Gal3-GFP/LAMP1-mCherry cells, Panc02-Gal3-GFP/LAMP1-mCherry cells, MC38-LAMP1-mCherry cells, Panc02-LAMP1-mCherry cells and MC38-CTSB-mCherry cells were validated by confocal microscopy. Cell lines generated using CRISPR-Cas9 were validated by western blotting for the proteins of interest. The remaining cell lines were not authenticated.
Mycoplasma contamination	All cell lines were tested negative for mycoplasma contamination.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines were used.

Animals and other research organisms

Policy information about [studies involving animals](#); [ARRIVE guidelines](#) recommended for reporting animal research, and [Sex and Gender in Research](#)

Laboratory animals	Female BALB/c mice (6-8 weeks old), C57BL/6 mice (6-8 weeks old) and Institute of Cancer Research (ICR) mice (6-8 weeks old) were purchased from Hunan SJA Laboratory Animal Co., Ltd (China) or GemPharmatech Co., Ltd (China). OT-I (C57BL/6-Tg(TcraTcrb) 1100 Mjb/J) TCR transgenic mice sourced from the Jackson Laboratory were generously provided by Professor Tianmeng Sun of Jilin University (China). All mice were housed in pathogen-free conditions and kept in a room with controlled temperature (~22 °C) and humidity (45%-60%) under 12 h light/dark cycle. The procedures were approved by the South China University of Technology Animal Care and Use Committee.
Wild animals	The study did not involve wild animals.
Reporting on sex	Both sex of mice were used in this study.
Field-collected samples	The study did not involve samples collected from the field.
Ethics oversight	All experimental mouse procedures were approved by the Institutional Animal Care and Use Committee at South China University of Technology (Approval No. 2023032, 2025003 and AE-2025041).

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Plants

Seed stocks	Not involved
Novel plant genotypes	Not involved
Authentication	Not involved

Flow Cytometry

Plots

Confirm that:

- ☒ The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).
- ☒ The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).
- ☒ All plots are contour plots with outliers or pseudocolor plots.
- ☒ A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation

Spleen and tdlNs were mechanically dissociated by passing through a 70-µm stainless steel mesh to obtain single-cell suspensions. The tumour tissues were cut into small pieces and enzymatically digested with RPMI 1640 medium containing collagenase type IV, hyaluronidase and DNase I under gentle agitation at 37°C. The dissociated cell suspensions were subsequently passed through a 70-µm nylon mesh to obtain single-cell suspensions. For the isolation of tumour-infiltrating lymphocytes, cells were harvested via centrifugation and resuspended in 40% Percoll (GE Healthcare, USA) solution in PBS. The resuspended cells were then centrifuged at 800 × g for 20 min, and the tumour-infiltrating leukocytes were obtained from the pellet. Red blood cells were removed by ACK lysing buffer. For cell surface marker analysis, the cells were washed with PBA buffer (0.5% BSA in PBS), blocked with anti-mouse CD16/32 antibodies (Biolegend) on ice, and then incubated on ice with the designated antibodies. For intracellular cytokine analysis of tumour-infiltrating leukocytes, the isolated cells were pre-stimulated with a cell stimulation cocktail (Invitrogen) according to manufacturer's instructions prior to staining. Following surface marker staining, these cells were fixed and permeabilized using Fixation/Permeabilization Kit (BD Bioscience) according to manufacturer's instructions, and subsequently stained with antibodies against intracellular cytokines. For analysis of immune cells from in vitro co-culture experiments, cells were collected from culture plates after incubation and processed for phenotypic staining directly without any exogenous cytokine stimulation.

Instrument

Flow cytometry was performed on BD LSRFortessa, BD FACSCelesta or BD FACSCanto II instrument (BD Biosciences).

Software

Data were collected using FACSDiva (BD Bioscience).

Cell population abundance

Not applicable because cell sorting was not performed.

Gating strategy

FSC-A vs. SSC-A gate was used to identify the cell population. Singlets were separated from doublets using FSC-A vs. FSC-H gating. DAPI or Fixable Viability Dye was used to eliminate dead cells. Target populations were further determined by specific antibodies, which were able to distinguish from negative populations.

- ☒ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.