Engineered bioorthogonal POLY PROTAC nanoparticles for tumour-specific protein degradation and precise cancer therapy

Heterobifunctional PROteolysis TArgeting Chimeras (PROTACs) hold promising potential for cancer therapy, as they can degrade oncoproteins, particularly undruggable targets\(^1\). PROTACs are generally composed of a warhead that binds to the protein of interest (POI), a ligand-hijacking endogenous E3 ubiquitin ligase, and a linker connecting the warhead and the ligand\(^2\). PROTACs can label the POI with ubiquitin by recognising the E3 ligase and subsequently degrade the POI through the ubiquitin-proteasome system (UPS)\(^3\). Compared to small molecule inhibitors, PROTACs can potentially degrade any intracellular protein, including undruggable targets (e.g., transcription factors and scaffold proteins)\(^4\). Furthermore, PROTACs are potent agents that can circumvent acquired drug resistance by degrading whole proteins after a short drug exposure time and a low dosage\(^5\). Despite being promising, conventional small molecular PROTACs generally display unfavourable pharmacokinetics and lack tumour specificity, which might cause systemic toxicity due to their non-specific distribution in normal tissues\(^6\). Thus, it remains a formidable challenge to achieve tumour-specific delivery and potentiate the antitumor potency of conventional PROTACs.

To achieve tumour-targeted delivery of PROTACs, several ligand modification strategies (e.g., antibody-PROTACs, folate-PROTACs and aptamer-PROTAC conjugates) have been

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investigated in recent years \(^1\)–\(^2\). These decorated PROTACs have shown increased cellular uptake in vitro. In particular, aptamer-

PROTAC conjugates have displayed increased tumour accumulation and antitumor potency in vivo compared to conventional PROTACs \(^2\). Nevertheless, these ligand-PROTAC conjugates suffer from low serum stability, limited tumour penetration and heterogeneous expression of the receptors in different tumour cells and cancer types. Furthermore, opto-PROTACs were developed for ultraviolet light-inducible protein degradation \(^2\)–\(^4\). These photoactivatable PROTACs have been demonstrated to spatio-temporally control protein degradation in vitro \(^5\)–\(^7\). However, clinical translation of opto- PROTACs is restricted by the poor tissue penetration profile of ultraviolet light with short wavelength (e.g., 360 nm). Therefore, precise PROTAC delivery to the tumour and efficient POI degradation inside tumour cells remain formidable challenges.

In this work we rationally engineer a polymeric PROTAC (POLY-PROTAC) nanoplatform for tumour-targeted degradation of the bromodomain and extraterminal (BET) protein BRD4. We first synthesise four von Hippel-Lindau (VHL)-based small molecular PROTACs and then design a series of reduction-activatable POLY-

PROTACs and self-assemble into micellar nanoparticles (NPs) for systemic PROTAC delivery (Fig. 1a). We subsequently engineer a dibenzocyclooctyne (DBCO)-loaded pretargeted NP to enhance intratumoral accumulation and retention of azide-modified POLY-

PROTAC NPs via in situ click reaction. Upon internalisation into the tumour cells, the POLY-PROTAC NPs release the PROTAC payload via glutathione (GSH)-mediated reduction of the disulfide bond (Fig. 1b). We demonstrate that the clickable POLY-PROTAC NPs synergistically induce apoptosis of tumour cells when combined with photodynamic therapy (PDT) in a mouse model of MDA-MB-231 breast cancer (Fig. 1c). This study might provide a
generalisable nanoplatform for tumour-specific PROTAC delivery and potentiated cancer therapy.

Results

Synthesis and characterisation of the POLY-PROTAC NPs

BET family proteins, in particular BRD4, have been investigated as promising antitumor targets due to their crucial role in gene transcription. To design a BRD4-targeted POLY-PROTAC, we selected the VHL ligand for PROTAC synthesis since the hydroxyl group of VHL can be reversibly caged via a disulphide spacer. The ability of the modified PROTACs to bind VHL can be restored by GSH-triggered cleavage of the disulphide bond in the cytosol of tumour cells.

We first synthesised four VHL-based PROTACs by adjusting the chemical structures of the VHL ligand and linkers (Fig. 2a,

![Synthesis and characterisation of the BRD4 PROTAC and POLY-PROTAC NPs.](image)

**Fig. 2 | Synthesis and characterisation of the BRD4 PROTAC and POLY-PROTAC NPs.** a Synthesis of the BRD4-targeted VHL PROTACs. b Western blot assay of PROTAC-mediated BRD4 degradation and c-Myc downregulation in MDA-MB-231 breast tumour cells after 24 h of incubation. c Western blot assay of determined half BRD4 degradation concentration (DC50) values of the PROTACs (n = 3 biologically independent cells). d PROTACs efficiently inhibited the proliferation of MDA-MB-231 cells in a dose-dependent manner in vitro (inset shows the IC50 values of the indicated PROTACs and JQ1). Cells were incubated with the PROTACs or JQ1 for 72 h before being subjected to the CCK-8 assay (n = 4 biologically independent cells). e Schematic illustration of the GSH-triggered activation of ARV771 from Me-ARV771. f HPLC chromatograms of the reduction-mediated ARV771 restoration and g quantitative ARV771 release percentages from reduction-activatable Me-ARV771 via incubation with 5.0 mM DTT. h, i Representative DLS data and TEM images of the PGD7 NPs at pH 7.4 and i pH 6.0 (scale bar = 50 nm). j Acid-activatable fluorescence profile of PPy-labelled PGD7 NPs (the fluorescence intensity was normalised to that determined at pH 7.4). Inset shows the fluorescence image of the PGD7 NP suspensions at different pH values. k GSH-triggered ARV771 release from the PGD7 NPs at pH 7.4 and 6.0 (with or without 10 mM GSH addition) (n = 3 independent experiments). All data are presented as mean ± SD.
Supplementary Figs. 1–11). Western blot assays validated that all four PROTACs remarkably degraded BRD4 in MDA-MB-231 breast cancer cells in vitro and consequently suppressed downstream c-Myc expression. Noticeably, the compounds ARV771 and MZI reduced BRD4 expression more efficiently than their analogues. The half-degradation concentration (DC_{50}) of ARV771 was 1.9-fold lower than that of mARV771 (Fig. 2b, c and Supplementary Fig. 12). The CCK-8 assay showed that all four PROTACs comparably inhibited the proliferation of MDA-MB-231 cells. Of note, the half inhibition concentrations (IC_{50}) of the PROTACs were tens of times lower than that of the BRD4 inhibitor JQ1 (Fig. 2d), suggesting that the BRD4 degraders more significantly impaired the proliferation of the tumour cells than the BRD4 inhibitor.

The hydroxyl group on the VHL ligand plays a crucial role in VHL protein binding by forming hydrogen bonds with HIS-115 and SER-111 in the binding pocket of the VHL protein. The hydroxyl groups of ARV771 and MZI were thus methacrylated with a disulﬁde spacer (termed Me-ARV771 and Me-MZI) (Fig. 2e) to reversibly disrupt the interaction with the VHL protein and abolish the protein degradation performance of the PROTAC. An ethylene glycol spacer that is inert to reduction was employed to synthesise the GSH-nonresponsive control (namely, Me-ARV771). Successful synthesis of the methacrylate-modiﬁed PROTACs was validated by proton nuclear magnetic resonance (1H-NMR) and mass spectrometry (MS) measurements (Supplementary Fig. 13–17). High-performance liquid chromatography (HPLC) examination showed that ARV771 was completely regenerated from Me-ARV771 upon 6.0 h of incubation with dithiothreitol (DTT), verifying the superior reduction sensitivity of the disulﬁde bond- incorporated Me-ARV771 (Fig. 2f, g).

With the reduction-activatable Me-ARV771 and Me-MZI in hand, we next sought to synthesise POLY-PROTACs via PEG-induced RAFT copolymerisation of Me-ARV771 (or Me-MZI) and the aci-dionisable 2- (diisopropylamino)ethyl methacrylate (DPA) monomer for intracellular delivery of the BRD4 PROTACs. The resultant POLY-PROTACs were termed according to the PROTAC molecule used: PD7 (based on Me-ARV771) or PDM (based on Me-MZI). An MMP-2-cleavable GPLGLAG (GG) peptide spacer34,35, was introduced into the PD7 POLY-PROTAC for increased cellular uptake and deep tumour penetration ability. The tumour penetration proﬁle of the PPa-labelled POLY-PROTAC NPs was then investigated in a three-dimensional (3D) multicellular spheroid (MCS) MDA-MB-231 tumour model in vitro. CLSM images showed that after 12 h of incubation, the MMP-2-insensitive PD7 NPs were primarily entrapped in the peripheral areas and marginally penetrated into the deep areas of the MCSs (Fig. 3d, e). In contrast, the MMP-2-cleavable PD7 NPs showed great diffusion into the central areas of the MCSs with ~ 3.0-fold higher ﬂuorescence intensity at a scanning depth of 50 μm (Fig. 3f), implying that the sheddable POLY-PROTAC NPs had a remarkable tumour penetration proﬁle.

BRD4 degradation by the POLY-PROTAC NPs in vitro

To demonstrate the advantage of the MMP-2-sheddable POLY-PROTAC for increased cellular uptake and deep tumour penetration (Figs. 3a, 3b and Supplementary Figs. 18–23). Two MZI-based POLY-PROTACs without the GG peptide spacer (PDGM) and with the GG peptide spacer (PDGDM) were also synthesised for comparison (Supplementary Figs. 24 and 25). The POLY-PROTAC NPs, which might facilitate PROTAC release inside the tumour cells. Pretreating the MMP-2-sheddable POLY-PROTAC NPs with MMP-2 for 1 h to cleave the PEG corona as reported previously38. MDA-MB-231 breast tumour cells were then incubated with PD7 or dePEGylated PDG7 NPs for the desired time durations. Flow cytometry measurements identiﬁed an ~ 2.5-fold higher intracellular ﬂuorescence signal in the PDG7 group than in the PDA7 group (Fig. 3b). Confocal laser scanning microscopy (CLSM) examination further revealed remarkably higher intracellular uptake of the PDG7 NPs than the PDA7 control when examined after 12 h of incubation (Fig. 3c and Supplementary Fig. 32), validating that the MMP-2-triggered dePEGylation promoted internalisation of the POLY-PROTAC NPs.

The tumour penetration proﬁle of the PPa-labelled POLY-PROTAC NPs was then investigated in a three-dimensional (3D) multicellular spheroid (MCS) MDA-MB-231 tumour model in vitro. CLSM images showed that after 12 h of incubation, the MMP-2-insensitive PD7 NPs were primarily entrapped in the peripheral areas and marginally penetrated into the deep areas of the MCSs (Fig. 3d, e). In contrast, the sheddable PDG7 NPs showed great diffusion into the central areas of the MCSs with ~ 3.0-fold higher ﬂuorescence intensity at a scanning depth of 50 μm (Fig. 3f), implying that the sheddable POLY-PROTAC NPs had a remarkable tumour penetration profile.

With the MMP-2-responsive POLY-PROTAC NPs in hand, we next evaluated their BRD4 degradation performance in vitro. After 24 h of incubation, the GSH-activatable POLY-PROTACs of ARV771 and MZI efﬁciently degraded the BRD4 protein in MDA-MB-231 tumour cells in vitro (Fig. 3g–i). In contrast, the PGDO7 POLY-PROTAC bearing an ethylene glycol linker negligibly affected BRD4 protein expression (Fig. 3k), validating the crucial role of GSH-triggered reduction of the disulﬁde bond and restoration of the VHL ligand for protein degradation inside the tumour cells. Pretreating the MMP-2-sheddable POLY-PROTAC NPs with MMP-2 remarkably reduced the DC_{50} of the PGD7 and PGDM NPs, which were 1.9- and 3.1-fold lower than their MMP-2-insensitive PD7 and PDM counterparts, respectively, and comparable to those of free ARV771 and MZI (Fig. 3l and Supplementary Fig. 33). This could be attributed to the increased cellular uptake of the PGD7 and PGDM NPs due to MMP-2-mediated cleavage of the PEG corona.

Cotreatment with the proteasome inhibitor MG132 abolished the protein degradation ability of the small molecular PROTACs (e.g., MZI and ARV771) and the POLY-PROTAC NPs (e.g., PDG and PDG7) (Fig. 3m), verifying the ubiquitin-proteasome-dependent BRD4 degradation proﬁle of the POLY-PROTAC NPs. The CCK-8 assay further
Fig. 3 | Stimuli-activatable POLY-PROTAC NPs displayed increased cellular uptake, tumour penetration and BRD4 degradation performance in vitro. 

a Cartoon illustration of the MMP-2-triggered cleavage of the PEG corona and promoted cellular uptake of the PGD7 NPs in vitro. 
b Flow cytometric analysis of PGDA7 and PDA7 NPs cellular uptake in vitro (the NPs were pretreated with 0.2 mg/mL MMP-2 for 1 h) (n = 3 biologically independent cells). 
c CLSM examination of the intracellular distribution of the POLY-PROTAC NPs after 12 h of incubation (scale bar = 20 μm). 
d–f MMP-2-responsive PGDA7 NPs displayed increased penetration into MDA-MB-231 MCSs in vitro compared with their MMP-2-nonresponsive PDA7 counterpart. 
e CLSM examination of PGDA7 and PDA7 NPs distribution after 12 h of incubation in vitro. 
f 2.5-D reconstruction of the CLSM images at a scanning depth of 50 μm. 
g–j Reduction-activatable POLY-PROTAC NPs efficiently degraded the BRD4 protein in MDA-MB-231 cells in vitro. Western blot assay of BRD4 degradation in MDA-MB-231 cells with various GSH-sensitive POLY-PROTAC NPs in vitro after 24 h of incubation. 
k Western blot assay of BRD4 expression in PGDO7 NP-treated MDA-MB-231 cells in vitro. 
l DC_{50} of the POLY-PROTAC NP-mediated degradation of BRD4 in MDA-MB-231 cells in vitro (n = 4 biologically independent cells). 
m POLY-PROTAC NPs degraded the POI via the ubiquitin-proteasome system. Western blot assay of BRD4 expression in MDA-MB-231 cells with or without MG132 incubation (MZ1/ARV771 concentrations of 1.0 μM and MG132 concentration of 5.0 mM). 

PGDM and PGD7 NPs efficiently inhibited the proliferation of MDA-MB-231 cells in vitro (n = 4 biologically independent cells) (the POLY-PROTAC NPs were named according to the PROTAC and components integrated. P: PEG chain; G: GPLGLAG peptide; D: acid-activatable DPA group; 7: disulfide bond-bearing ARV771 methacrylate; M: disulfide bond-bearing MZ1 methacrylate; O7: ethylene group-bearing ARV771 methacrylate; A: PPa. See Supplementary Fig. 31 for NPs compositions). All data are presented as mean ± SD.

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revealed increased cytotoxicity of the PGDM and PGD7 NPs compared with that of the MMP-2 non-responsive PGD07 control after 72 h of incubation (Fig. 3n). Notably, the MMP-2 liable POLY-PROTAC NPs displayed half inhibitory concentrations comparable to that of the parental molecule (Fig. 2d), suggesting that the PROTAC payload can be readily released inside the tumour cells via GSH-mediated cleavage of the disulfide bond. Collectively, the above data demonstrated that the POLY-PROTAC NPs with sheddable PEG coronas and reduction-liable linkers efficiently degraded the POI and suppressed tumour cell proliferation in vitro.

**Biodistribution and antitumor performance of the POLY-PROTAC NPs in vivo**

The tumour-specific accumulation and penetration of the POLY-PROTAC NPs were next investigated in an MDA-MB-231 breast tumour-bearing BALB/c nude mouse model in vivo. MMP-2 sheddable PGDA7 and the MMP-2 insensitive PDA7 analogue NPs were administered via intravenous (i.v.) injection at an identical ARV771 dose of 10 mg/kg and PPa dose of 5.0 mg/kg when the tumour volume reached 200 mm³. In vivo fluorescence imaging displayed clear tumour distribution of the PGDA7 NPs over time (Fig. 4a), which can be explained by the

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**Fig. 4 | Stimuli-activatable POLY-PROTAC NPs specifically accumulated at the tumour site and suppressed tumour growth in vivo.** a) Fluorescence images of POLY-PROTAC NPs distribution in MDA-MB-231 tumour-bearing nude mice in vivo. MMP-2-activatable PGDA7 NPs specifically accumulated at the tumour site. b) Normalised fluorescence intensities of the tumour tissue (n = 3 biologically independent mice) Statistical analysis of was performed by two-sided unpaired t-test. Statistical significance of was calculated by survival curve comparison with Log-rank (Mantel-Cox) test. i) Western blot assay of PGD7 NP-induced BRD4 degradation and caspase-3 activation in MDA-MB-231 tumours in vivo (the tumours were harvested on the second day after five cycles of treatment), and i) tumour cells in vitro as a function of ARV771 concentration. j) Immunohistochemical staining of the tumour sections at the end of the antitumor study (scale bar = 100 μm). k) I PGD7 NPs degraded the BRD4 protein and activated caspase-3 in vivo. l) Semiquantitation of IHC-determined BRD4 expression in the tumour sections (n = 5 biologically independent mice). Statistical analysis was performed by two-sided unpaired t-test. All data are presented as mean ± SD.

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enhanced permeability and retention effect of the NPs40. Remarkably, the PGDA7 NPs showed much higher intratumoral accumulation and slower blood clearance than the MMP-2-insensitive PDA7 control at all examined time points. For instance, the tumour fluorescence in the PGDA7 group was 2.0-fold higher than that in the PDA7 control group at 36 h post-injection (Fig. 4b).

The tumour-specific distribution of the PGDA7 NPs was confirmed by ex vivo fluorescence imaging of the major organs (e.g., heart, liver, spleen, lung and kidney) and the tumour tissue at 48 h post-injection (Fig. 4c). CLSM examination of the tumour section further illustrated that the PDA7 NPs were distributed in the peripheral area of the blood vessels. In contrast, the PGDA7 NPs diffused throughout the tumour tissue (Fig. 4d). Taken together, the fluorescence imaging and CLSM examination data verified increased tumour accumulation and penetration of the PGDA7 NPs via MMP-2-mediated cleavage of the PEG corona.

We subsequently explored the antitumor efficacy of the POLY-PROTAC NPs in an MDA-MB-231 tumour model in vivo. The tumour-bearing BALB/c nude mice were randomly grouped when the tumour volumes reached 100 mm³ and i.v. injected with PBS, ARV771 or the PGD7 NPs at an identical ARV771 dosage of 10 mg/kg. The treatments were repeated every three days for a total of five injections (Fig. 4e).

Free ARV771 negligibly affected MDA-MB-231 tumour growth compared to the PBS control. In contrast, the PGD7 NPs significantly delayed tumour growth by ~50% and consequently prolonged the survival of the tumour-bearing mice (Fig. 4f, g).

To elucidate the mechanism underlying the antitumor performance of the POLY-PROTAC NPs, BRD4 degradation in lysates from the tumours of the PGD7 group were examined by western blot assay. Free ARV771 displayed a negligible influence on BRD4 expression in tumour xenografts. In contrast, PGD7 NPs remarkably suppressed BRD4 expression in vivo by ~80% in the tumour tissue (Fig. 4h and Supplementary Fig. 34a) due to the improved delivery efficacy of ARV771 from the POLY-PROTAC NPs. Notably, PGD7 NPs cleaved caspase-3 1.5-fold more efficiently than ARV771 in the tumour tissue in vitro (Fig. 4i and Supplementary Fig. 34b), implying apoptosis of the tumour cells since caspase-3 is a crucial executor of cellular apoptosis41. Notably, free ARV771 and the PGD7 NPs comparably activated caspase-3 in MDA-MB-231 tumour cells (Fig. 4i and Supplementary Fig. 34c–e). Therefore, the PGD7 NP-mediated caspase-3 activation profile could be attributed to the BRD4 degradation property of ARV771.

BRD4 degradation-induced apoptosis of tumour cells in vivo was verified by haematoxylin-eosin (H&E) staining of the tumour sections (Fig. 4j). Immunohistochemistry (IHC) examination of the tumour sections showed that free ARV771 negligibly affected BRD4 expression (Fig. 4k). In contrast, PGD7 NP remarkably suppressed BRD4 expression with an ~4-fold lower BRD4-positive area than free ARV771 (Fig. 4l). The experimental groups displayed similar body weight changes during the whole experimental period compared to the PBS group (Supplementary Fig. 35a). Furthermore, the H&E images revealed negligible histopathological damage to the major organs (e.g., heart, liver, spleen, lung and kidney) (Supplementary Fig. 35b), verifying that the PGD7 POLY-PROTAC NPs had good biosafety.

The bioorthogonal click reaction amplified the tumour distribution of the POLY-PROTAC NPs in vivo

Insufficient tumour distribution of PROTACs is one of the bottlenecks for PROTAC-based cancer therapy. To further prompt tumour-specific accumulation of the POLY-PROTAC NPs via a copper-free click reaction in the biological milieu41, we next designed an extracellular tumour acidity-activatable pretargeted NPs for tumour-targeted delivery of the dibenzocyclooctyne (DBCO) groups (Fig. 3a). The pretargeted NPs were prepared by the self-assembly of DBCO-modified mPEG-b-poly(ethylene propyl amine) (namely, PED) diblock copolymer (Supplementary Figs. 36 and 37 for PED diblock copolymer synthesis).

The PED pretargeted NPs displayed a homogeneous and spherical morphology with an average hydrodynamic diameter of ~60 nm at neutral pH (i.e., 7.4) (Fig. 3b) and with dramatic dissociation at the acidic pH of 6.5 (Fig. 3c), which mimics the acidic tumour microenvironment (e.g., pH = 6.5–6.8)33. The fluorescence of the PPA-conjugated PED NPs (Supplementary Fig. 38 for polymer synthesis) was quenched under neutral conditions. In contrast, the fluorescence of PPA was recovered at pH values below 6.6 via the extracellular acid-induced dissociation of the pretargeted NPs (Fig. 3d), validating the superior extracellular acid-responsive property of the PED NPs.

Subsequently, an azide group was modified on the hydrophilic PEG head of the PGDH diblock copolymer (N3-PGDH) (Supplementary Fig. 39) to prepare azide-functionalized PGD7 POLY-PROTAC (namely, N6@PGD7) NPs. The azide group on the surface of the N6@PGD7 NPs was expected to perform in-situ click reaction with DBCO groups in the acidic tumour microenvironment. Both the PED and N6@PGD7 NPs displayed good serum stability in 10% (V/V) foetal bovine serum containing neutral solution (pH 7.4) (Supplementary Fig. 40). DLS examination showed a uniform hydrodynamic diameter and narrow particle size distribution when the PED NPs and the N6@PGD7 POLY-PROTAC NPs were mixed under neutral pH conditions (Fig. 3e), implying that the PED and N6@PGD7 NPs could remain inert during blood circulation to avoid unfavourable interactions by encapsulating the DBCO groups inside the hydrophobic core of the PED NPs. In contrast, DLS and TEM examinations revealed amorphous aggregates with a broad size distribution when the PED and N6@PGD7 NPs were coincubated at an acidic pH of 6.5 (Fig. 3f), validating the occurrence of a multivalent click reaction between the DBCO-grafted PED copolymer and azide-modified POLY-PROTAC NPs to form cross-linked nanostructures.

In vivo fluorescence imaging displayed a clear intratumoural fluorescence signal when the PED pretargeted NPs were i.v. injected into MDA-MB-231 tumour-bearing nude mice (Fig. 3g), which was caused by tumour acidity-triggered protonation of the tertiary amine of EPA and subsequent dissociation of the PED NPs. CLSM examination of the tumour sections showed that the PED pretargeted NPs colocalized well with the cell membrane (labelled with wheat germ agglutinin (WGA)) 2–4 h post-injection, verifying that the PED NPs dissociated and exposed the DBCO groups in the extracellular matrix (ECM) of the tumour tissue (Fig. 3h); this facilitates the click reaction between the DBCO and the azide groups on the surface of the POLY-PROTAC NPs in the ECM.

To evaluate whether the in situ click reaction enhances tumour-targeted PROTAC delivery in vivo, N6@PGD7 NPs integrating ARV771 POLY-PROTAC and PPA were prepared by coassembling N6@PGDH, PGD7 and PGD diblock copolymers (Supplementary Fig. 3i), which was i.v. administered 2 h after pretargeted NP injection. The PED + N6@PGD7 group displayed a much brighter intratumoural fluorescence signal than that observe in the N6@PGDA7–injected mice (Fig. 3i). For instance, the PED + N6@PGD7 group showed an ~2.0-fold higher fluorescence signal than the N6@PGDA7 NP group at 24–36 h post-injection (Fig. 3k).

The increased tumour distribution of the POLY-PROTAC NPs was validated by the ex vivo fluorescence imaging of the major organs and tumour tissues 48 h post-injection (Fig. 3l). CLSM examination of the tumour sections demonstrated that the pretargeted PED NPs dramatically increased tumour accumulation and penetration of the N6@PGDA7 POLY-PROTAC NPs (Fig. 3j). HPLC examination of ARV771 distribution in vivo further demonstrated increased intratumoural distribution and retention of the POLY-PROTAC NPs via an in situ click
reaction. In comparison with free ARV771 and N@g3677@PGDA7 NPs, the combination of PED + N@g3677@PGDA7 NP remarkably enhanced the tumour distribution of ARV771 by 3.9- and 1.9-fold, respectively, when examined at 36 h post-injection (Fig. 5m). The data from both the fluorescence imaging and HPLC analyses provided consistent evidence that the in situ click reaction significantly enhanced the accumulation and retention of ARV771 PROTAC at the tumour site.

**Bioorthogonal POLY-PROTAC NPs regressed breast tumour growth in vivo**

Given the significantly improved tumour distribution of the clickable POLY-PROTAC NPs, we next investigated their antitumor performance in the in vivo MDA-MB-231 tumour model (Fig. 6a). Compared to ARV771 or N@g3677@PGDA7 NPs alone, PED + N@g3677@PGDA7 much more efficiently delayed ~70% of the MDA-MB-231 tumour growth due to
increased ARV771 distribution in the tumour tissue (Fig. 6b). However, PED + N6@PGD7 marginally prolonged the survival time of the tumour-bearing mice compared to treatment with the N6@PGD7 NPs due to tumour relapse during the late stage of the antitumor study (Fig. 6c).

In previous studies, we explored intracellular acid-activatable PDT to circumvent multidrug resistance of breast tumours.31 Herein, we next sought to explore the potential of the bioorthogonal POLY-PROTAC NPs for PDT-promoted BRD4 degradation therapy (Fig. 6d). Upon 671 nm laser irradiation, the ARV771-free PGDA NPs remarkably induced the generation of reactive oxygen species and activated the caspase-3 protein in MBA-MB-231 tumour cells in vitro, validating the photoactivity of the PPA-labelled NPs (Supplementary Figs. 42 and 43).

The combination of Laser + PGDA7 NPs activated the caspase-3 protein in MBA-MB-231 tumour cells more efficiently than PDT (Laser + PGDA7) or BRD4 degradation with ARV771 treatment (Fig. 6e), implying the improved apoptosis induction performance of PDT combined with BRD4 degradation in vitro.

The antitumor performance of combining PDT with BRD4 degradation was next investigated in vivo. MBA-MB-231 tumour-bearing BALB/c nude mice were randomly grouped when the tumour volumes reached ~ 100 mm3, and they were then treated with PBS, ARV771, PED + N6@PGDA7 + Laser, PED + N6@PGD7, PED + PGDA7 + Laser, or PED + N6@PGDA7 + Laser (Fig. 6f). The N6@PGDA7 NPs were i.v. administered 2 h post-injection of the PED NPs, and 671 nm laser irradiation was applied 36 h post-injection of the N6@PGDA7 NPs. Figure 6g demonstrates that PDT or ARV771 administration alone marginally suppressed the proliferation of MBA-MB-231 tumours. In contrast, the combination of the bioorthogonal NPs (PED + N6@PGDA7) and PDT dramatically repressed 95% of tumour growth, which was 1.5-fold more efficient than BRD4 degradation alone caused by PED + N6@PGDA7 treatment.

Furthermore, the combination of PED pretargeted NPs with N6@PGDA7 and PDT prolonged the survival of the tumour-bearing mice by 40% compared to those in the PED + N6@PGDA7 group, with 40% of the animals surviving 100 days post-treatment (Fig. 6h). TUNEL staining of the tumour sections revealed notable apoptosis of the tumour cells from the mice in the PED + N6@PGDA7 + PDT group, suggesting that the combination of PDT and BRD4 degradation with the bioorthogonal NPs cumulatively induced apoptosis of the tumour cells in vivo (Fig. 6i). Semiquantitative analysis of the TUNEL staining data further revealed that PED + N6@PGDA7 + Laser treatment more efficiently induced apoptosis of the tumour cells than ARV771 and PED + N6@PGDA7 by 15.2- and 4.2-fold, respectively (Supplementary Fig. 44).

Treatment-induced apoptosis of the tumour cells was further confirmed by ex vivo H&E staining of the tumour sections (Fig. 6k). Moreover, negligible body weight loss and histopathological damage to the major organs were observed during the experimental period, verifying the satisfactory biosafety of the POLY-PROTAC NPs (Fig. 6l and Supplementary Fig. 44).

Immunohistochemical examination of the tumour tissue demonstrated significantly decreased BRD4 expression in vivo (Fig. 6l). Semiquantitative analysis of the IHC images further determined that PED + N6@PGDA7 treatment more efficiently downregulated BRD4 expression (by 2.6-fold) than N6@PGDA7 NP alone, validating that the increased intratumoural distribution of ARV771 via the in situ bioorthogonal click reaction contributed to BRD4 degradation in vivo (Fig. 6m).

Notably, the combination of PDT and PED + N6@PGDA7 further promoted BRD4 degradation compared to PED + N6@PGDA7 treatment due to PDT-enhanced intracellular release of the ARV771 PROTAC in vivo (Fig. 6m, n). Additionally, combinatorial therapy with PED + N6@PGDA7 + Laser more efficiently activated caspase-3 (1.5-fold) and degraded BRD4 (3.0-fold) than PED + N6@PGDA7, verifying the synergistic apoptosis-induction profile caused by combined PDT and BRD4 degradation (Fig. 6o).

**Discussion**

Heterobifunctional PROTACs with the ability to degrade proteins have been recently exploited for cancer therapy. However, the clinical translation of small molecular PROTACs suffers from low bioavailability and insufficient tumour specificity. It is therefore highly desirable to develop novel PROTACs for tumour-specific protein degradation while minimising off-target, off-tumour adverse effects. A tumour microenvironment-activatable POLY-PROTAC nanoplatform was thus engineered herein for tumour-specific delivery and potentiation of the antitumor performance of PROTACs.

In comparison with their small molecular PROTAC counterparts, the POLY-PROTAC NPs reported in this study possess several distinct advantages. First, the POLY-PROTAC NPs with MMP-2-labile PEG coronas elongated the blood circulation of the small molecular PROTACs, while the PEG coronas were cleaved via intratumoural MMP-2 to facilitate PROTAC tumour-specific accumulation and retention. Second, the POLY-PROTAC NPs can disintegrate in the intratumoural acidic microenvironment. The PROTAC prodrug can thus be restored in the cytosol via GSH-mediated cleavage of the disulfide bond. Furthermore, we demonstrated that the POLY-PROTAC NPs can be adapted for bioorthogonal click reaction-enforced tumour specificity. Remarkably, the azide-modified POLY-PROTAC NPs showed 3.9-fold higher tumour accumulation than their small molecule counterparts via an in situ click reaction with the pretargeted NPs, which therefore further boosted PROTAC-based cancer therapy. Moreover, other kinds of stimuli-activatable chemical bonds (e.g., thiolacetic acid bonds)44,45 can be utilised to achieve spatial-temporal PROTAC activation. Apart from PDT, the POLY-PROTAC nanoplatform can be combined with other kinds of therapeutic modalities (e.g., radiotherapy or chemotherapy), and these are both under investigation in our laboratory for potentiated antitumor performance.

In summary, a POLY-PROTAC prodrug strategy was developed for the tumour-specific delivery of PROTACs in this study. We
demonstrated that this POLY-PROTAC nanoplatform can be activated by multiple stimuli (extracellular MMP-2, intracellular acidity and reductive conditions), which can achieve increased tumour accumulation, deep tumour penetration and enhanced protein degradation performance compared with its small molecule counterpart. The POLY-PROTAC nanoplatform with an extracellular tumour acidity-triggered bioorthogonal click reaction remarkably enhanced tumour-specific PROTAC delivery. This clickable POLY-PROTAC nanoplatform can be further applied with PDT for protein degradation and combination therapy, as this combinatorial treatment suppressed over 95%...
of MDA-MB-231 TNBC tumour growth. The extracellular acidic microenvironment containing MMP-2 has been well documented in various solid tumours. Therefore, this acid-activatable bioorthogonal POLY- PROTAC nanoplatform can further be employed for combination therapy to treat a broad spectrum of cancers by integrating multiple therapeutic regimens (e.g., chemotherapeutics and immunotherapeutics). Taken together, the proposed POLY- PROTAC nanoplatform might provide a novel avenue to potentiate PROTAC-based cancer therapy.

Methods

Materials

1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI), 1-Hydroxybenzotriazole (HOBT), 4-Dimethylaminopyridine (DMAP), Trifluoroacetic acid (TFA), N,N-Diisopropylethylamine (DIEA), N,N-Dimethylacetamide (anhydrous, DMAC), Dichloromethane (anhydrous, DCM), Dimethyl sulfoxide (anhydrous, DMSO), N,N-Dimethylformamide (anhydrous, DMF), 2,2-‘-Azobis (2-methylpropanitrile) (AIBN), and methanol (HPLC, MeOH) were purchased from J&K Chemicals (Shanghai, China). 4-Cyano-4-(dodecylsulanythiocarbonyl) sufferoglycan (ACP), 2,3,3-tetramethyl-3-azabicyclo[3.2.1]oct-2-ene-2-carboxylic acid (CDP), 2-(diisopropylamino) ethyl methacrylate (DPA), IV Gelatinase, Trisethylene glycol and tert-butyl carbamate (Tulsa, OK). BD Matrigel, Tris-Base, glycine, SDS, Peroxidase-Conjugated Goat Anti-Mouse IgG (H + L) (CAT:33201ES60) (Dalian, China). Foetal bovine serum (FBS) was purchased from Gibco (ThermoFisher, USA), and the MDA-MB-231 human breast cancer cell line was obtained from the cell bank of Chinese Academy of Sciences (Shanghai, China). All other reagents and solvents were purchased from YEASEN (Shanghai, China). The anti-BRD4 antibody (ab8226), anti-β-tubulin antibody (ab8226), anti-β-actin antibody (ab8226), anti-GAPDH antibody (ab8245), anti-α-tubulin antibody (ab87087) were all purchased from Abcam (Shanghai). All other reagents and solvents were purchased from SinoPharm Chemical Reagent Co., Ltd. (Shanghai, China).

Preparation of the POLY- PROTAC and pretargeted nanoparticles

Briefly, mPEG113-GALGLPG-b-P(DPA46-r-ARV7712) (2.0 mg) and mPEG113-GALGLPG-b-P(DPA48-r-HEMA2) (1.0 mg) were dissolved in 100 μL of DMF, and the mixture was added into 1 mL of DI water under ultrasonication to obtain the POLY- PROTAC nanoparticles (termed as PGD7 nanoparticles). The organic solvent was removed by dialyzing against DI water. One set of the POLY- PROTAC nanoparticles were prepared by following the same procedure described above: POLY-ARV77 nanoparticles (PD7): mPEG113-b-P(DPA46-r-ARV7712) + mPEG113-b-P(DPA48-r-HEMA2); MMP-2 liable POLY-ARV77 nanoparticles (PGDA7): mPEG113-GALGLPG-b-P(DPA46-r-ARV7712) + mPEG113-GALGLPG-b-P(DPA48-r-HEMA2); MMP-2 liable POLY-MZI nanoparticles (PDM): mPEG113-b-P(DPA42-r-MZI2) + mPEG113-b-P(DPA48-r-HEMA2); MMP-2 liable POLY-MZI nanoparticles (PGDM): mPEG113-GALGLPG-b-P(DPA46-r-MZI2) + mPEG113-GALGLPG-b-P(DPA48-r-HEMA2); DBCO-loaded pretargeted nanoparticles (PEA): mPEG113-b-P(EPA60-r-DBCO3) (namely PED) and mPEG113-b-P(EPA60-r-PPa3); To prepare the azide-functionalized POLY-ARV77 (N6@PGD7): mPEG113-GALGLPG-b-P(DPA46-r-ARV7712) (1.7 mg) + mPEG113-GALGLPG-b-P(DPA48-r-PPa3) (0.85 mg) + N6@PGD7 (0.45 mg) were dissolved into 100 μL of DMF, and added into 1 mL of DI water under ultrasonication. Then, DMF was removed via dialyzing against DI water to obtain the N6@PGD7 nanoparticles.

Physio-chemical characterisation of the POLY- PROTAC, N6@POLY- PROTAC and pretargeted nanoparticles

Hydrodynamic diameter and morphology of all the nanoparticles were investigated by DLS (Zetasizer Nano ZS90, Malvern Instrument, UK) and TEM examination (Talos L120C, USA, 120 kV).

The fluorescence emission profile of the PPa-labelled micelle suspension was examined by microplate reader (Perkin Elmer Enspire, USA), and the fluorescence images were recorded by IVIS imaging system (Xenogen, Alameda, CA).

To demonstrate reduction sensitivity of the POLY- PROTAC nanoparticles, ARV771 release from ARV771 methyl methacrylate (Me-ARV771) was investigated in vitro. Briefly, Me-ARV771 (50 μM) was incubated with 10 mM of DTT (MeOH: H2O = 1:1, v/v) for pre-determined time duration at 37 °C, the concentration of ARV771 released was examined by HPLC measurement (mobile phase: A: MeOH, C: H2O with 0.1% TFA; flow rate: 1 mL/min; column temperature: 30 °C; UV: 254 nm; eluate gradient: 0-10 min, from 5%A to 95% A; 15-20 min, from 95%A to 5% A).

Cell lines and animals

MDA-MB-231 human breast cancer cell line was obtained from the cell bank of Chinese Academy of Sciences (Shanghai, China). The cells were incubated in complete DMEM cell culture medium containing FBS 10% (V/V), penicillin G sodium and streptomycin sulfate (100 U/mL), and incubated at 37 °C under a humidified atmosphere containing 5% of CO2. All cell culture studies were performed in the logarithmic phase of cell growth in vitro.

Balb/c nude mice (female, 4-week old, 18 - 20 g) were obtained from Shanghai Experimental Animal Center (Shanghai, China). Animals were housed under SPF conditions in groups of 4-5 mice per cage, and maintained at a temperature of ~25 °C in a humidity-controlled environment with a 12 h light/dark cycle, with free access to standard food and water. All animal procedures were carried out under the guidelines approved by the Institutional Animal Care and Use Committee (IACUC) of Shanghai Institute of Material Medica, Chinese Academy of Sciences.

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The PROTAC release profile of the POLY-PROTAC nanoparticle in vitro
To evaluate ARV771 release profile of the POLY-ARV771 nanoparticle, the suspension of PGD7 nanoparticles was dialysed against 1% (wt) Tween 80-containing buffer solution at 37 °C (MWCO 3500 Da; e.g., pH 6.0 without GSH, pH 6.0 + 10 mM GSH; pH 7.4 without GSH, pH 7.4 + 10 mM. was added in each buffer). ARV771 concentration in the buffer solution was examined by HPLC measurement at the predetermined time points (e.g., 0.5, 1.0, 2.0, 4.0, 8.0, 12 and 24 h).

Western blot assay
To investigate PROTAC-induced protein degradation in vitro, MBA-MB-231 tumour cells were incubated with free PROTACs or POLY-PROTAC nanoparticles at the predetermined condition. The cells were then lysed in RIPA lysis buffer (50 mM Tris-HCl pH 7.4, 150 mM NaCl, 1% NP-40, 0.1% SDS) including 1 mM of phenylmethanesulfonyl fluoride (PMSF). The protein lysate was obtained by centrifugation at 12000 g for 15 min at 4 °C. Then, the lysates were denatured at 100 °C and resolved by SDS-polyacrylamide gel electrophoresis (SDS-PAGE). The gels were blotted onto PVDF membrane (Merck Millipore) and blocked by 5% BSA buffer for 2 h at room temperature. After incubated with the indicated primary antibodies (anti-β-tubulin antibody, ab78078, 1:1000; anti-GAPDH antibody, ab8245, 1:1000; anti-actin antibody, ab8226, 1:1000; anti-β-tubulin antibody, ab78078, 1:1000) at 4 °C overnight, the membrane was washed several times with TBST buffer, incubated with corresponding secondary antibody (Peroxidase-Conjugated Goat Anti-Rabbit IgG (H + L), CAT:33101ES60, 1:5000 and Peroxidase-Conjugated Goat Anti-Mouse IgG (H + L), CAT:33201ES60, 1:5000) in 5% BSA solution for 1 h at room temperature. The membranes were washed several times with TBST buffer, incubated with corresponding secondary antibody (Peroxidase-Conjugated Goat Anti-Rabbit IgG (H + L), CAT:33101ES60, 1:5000; Anti-Mouse IgG (H + L), CAT:33201ES60, 1:5000) in 5% BSA solution for 1 h at room temperature. The membrane was washed several times with TBST buffer, incubated with corresponding secondary antibody (Peroxidase-Conjugated Goat Anti-Rabbit IgG (H + L), CAT:33101ES60, 1:5000; Anti-Mouse IgG (H + L), CAT:33201ES60, 1:5000) in 5% BSA solution for 1 h at room temperature. The samples were then imaged with Bio-Rad Chemi Doc XR5 imaging system.

Cell proliferation assay
To investigate the cytotoxicity of the POLY-PROTAC nanoparticles in vitro, 1 × 10⁴ MBA-MB-231 cells were cultured in 96-well cell culture plate for 24 h, the cells were then treated with free PROTAC or POLY-PROTAC nanoparticles at predetermined condition for 72 h. The cell viability was then examined by CCK-8 assay.

Cellular uptake and tumour penetration analysis in vitro
To investigate cellular uptake profile of the POLY-PROTAC nanoparticles, MBA-MB-231 cells cultured in 24-well tissue culture plate were incubated with various POLY-PROTAC nanoparticles at the identical PPa concentration of 5.0 μM for the desired time durations (e.g., 2, 4, 8, 12, 24 h, respectively), the intracellular fluorescence intensity were then examined by flow cytometry (BD FACS Fortessa, BD, USA). The intracellular distribution of the POLY-PROTAC nanoparticles was investigated by CLSM examination.

3D multicellular spheroid (MCS) of the MDA-MB-231 breast tumour was employed to illustrate the tumour penetration profiles of the POLY-PROTAC nanoparticles in vitro. Briefly, the 3D tumour spheroids were prepared by culturing MBA-MB-231 cells in 48-well tissue culture plate (2000 cells/well) on % agarose gel. The 3D tumour spheroids were then incubated with the PGDA7 and PDA7 (MMP-2 pretreated) nanoparticles for 12 h. Nanoparticle distribution inside the tumour spheroids was then investigated by CLSM examination at the predetermined time points.

Photoactivity of the POLY-PROTAC nanoparticles in vitro
To investigate photoactivity of the PPa-modified POLY-PROTAC nanoparticles, 671 nm laser irradiation-induced ROS generation was examined. Briefly, the PGDA suspension with different PPa concentrations (e.g., 2.5, 5.0, or 10 μM) was incubated in pH 6.0 or pH 7.4 buffer solution for 30 min, and then added with singlet oxygen sensor green (SOSG, 5.0 μM). The micellar suspension was then irradiated with 671 nm laser at different photodensity (e.g., 100, 200 or 400 mW/cm²) for 1 min, and the fluorescence intensity of SOSG was detected by microplate reader (Perkin Elmer Enspire, USA).

To examine the photoactivity in vitro, MDA-MB-231 cells were cultured in 24-well tissue culture plate for 24 h, incubated with the PGDA nanoparticles for 12 h, and then incubated with DCFH-DA (10 μM) for 30 min. The cells were thereafter irradiated with 671 nm laser for 1 min at varied photodensity (e.g., 100, 200, 400 mW/cm²), and the intracellular fluorescence intensity of DCF was analysed by flow cytometry. Laser irradiation induced intracellular ROS generation was validated by CLSM examination.

Biodistribution of the nanoparticles in vivo
MDA-MB-231 breast tumour model was established by subcutaneously injecting 100 μL of cell suspension (1 × 10⁶ cells, DMEM: Matrigel = 1:1, v/v) into the fat pad of the Balb/c nude mice (female, 4 weeks, 18 ~ 20 g). The tumour-bearing mice were randomly grouped when the tumour volume reached ~200 mm³ and intravenously (i.v.) injected with PPa-labelled POLY-PROTAC nanoparticles at an identical PPa dose of 5.0 mg/kg. Then, fluorescence imaging was collected with the IVIS imaging system at predetermined time points (Xenogen, Alameda, CA). The mice were sacrificed at 48 h post-injection, the major organs and tumour tissue were harvested for fluorescence imaging ex-vivo. Afterwards, the tumour tissues were fixed, sectioned and stained with identified antibodies and DAPI, the fluorescence signal of the tumour sections were measured by CLSM examination.

Bioorthogonal click reaction-enhanced tumour distribution of the POLY-PROTAC nanoparticles in vivo
To investigate whether in-situ click reaction increased tumour accumulation of the POLY-PROTAC nanoparticles, the tumour-bearing nude mice were divided into three groups when the tumour volume reached ~200 mm³ and intravenously (i.v.) injected with PPa-labelled POLY-PROTAC nanoparticles at an identical PPa dose of 5.0 mg/kg, and the N₆₀@PGDA7 nanoparticle was i.v. injected at a DBCO dose of 1.0 mg/kg, and the N₆₀@PGDA7 nanoparticle was i.v. injected 2 h post PED administration at an ARV771 dose of 10 mg/kg. The biodistribution of PED and N₆₀@PGDA7 was then examined by IVIS fluorescence imaging systems in vivo.

To evaluate in-situ bioorthogonal click reaction enhanced tumour distribution of PROTAC, the tumour-bearing mice were i.v. injected with the POLY-PROTAC nanoparticles and sacrificed at the predetermined time points (12, 24, 36, 48 h). The major organs (e.g., heart, liver, spleen, lung and kidney) and tumour tissue were harvested and homogenised. ARV771 was extracted with methanol, examined by HPLC measurement and normalised with tissue mass.

Anti-tumour study in vivo
The anti-tumour performance of the POLY-PROTAC nanoparticle was evaluated in MDA-MB-231 orthotopic tumour model in vivo. The female Balb/c nude mice were s.b. injected with 100 μL of cell suspension (1 × 10⁶ cells, DMEM: Matrigel = 1:1, v/v). The tumour volume reached 100 mm³ at 15-days post inoculation. The tumour-bearing mice were randomly grouped (n = 5), and i.v. injected with PBS, ARV771, or PGD7 nanoparticle at an identical ARV771 dose of 10 mg/kg for five cycles at a time interval of 3 days. The tumour volume was monitored for 30-days post treatment.

To investigate the antitumor performance of the bioorthogonal POLY-PROTAC nanoparticles, the tumour-bearing mice were randomly grouped when the tumour volume reached 100 mm³ (n = 5), the tumour-bearing mice were then i.v. injected with PBS, ARV771, PGD7, or PED + N₆₀@PGD7 every three days at an identical ARV771 dose of 10 mg/kg, and DBCO dose of 1.0 mg/kg for five cycles (PED was injected 2 h pre POLY-PROTAC nanoparticle administration).
To investigate the antitumor performance of POLY-PROTAC nanoparticle-performed combinatory BRD4 degradation and PDT, the POLY-PROTAC nanoparticles were labelled with PPa for PDT. The tumour-bearing mice were grouped when the tumour volume reached 100 mm³ (n = 5), and then i.v. injected with PBS, ARV771, PED + N₅@PGDA, PED + N₅@PGD7 or PED + PGDA7 at the identical ARV771 dose of 10.0 mg/kg, PPa dose of 5.0 mg/kg and DBCO dose of 1.0 mg/kg, respectively (PED was i.v. injected 2 h pre-administration of the N₅@PGD7 nanoparticles). Thirty-six hours post nanoparticle injection, the mice of the PED + N₅@PGDA and PED + PGDA7 groups were treated with 671 nm laser irradiation at photodensity of 400 mW/cm² for 5 min. The treatment was repeated for five cycles at a time interval of three days. The tumour volume and body weights of the animals were monitored during the whole experimental period. The mice were considered death when the tumour volume reached 1500 mm³ according to the animal ethics of our institute and animal welfare. The tumours and the major organs (e.g., hearts, livers, lungs, spleens and kidneys) of mice were harvested at the end of the antitumor studies, and examined by H&E and TUNEL staining. BRD4 degradation in the tumour tissue was validated by western blot assay of the tumour lysate. The tumour volume was calculated with the formula below:

\[ V = \frac{L \times W \times W}{2} \] (L, the longest dimension; W, the shortest dimension).

**Statistical analysis**

All data were given as Mean ± SD. The GraphPad Prism software 6.01 was used for the statistical analyses. Two-tailed Student’s t-test was used for the statistical comparison between the two groups. One-way analysis of variance (ANOVA) with a Tukey post hoc test was used for statistical comparison among multiple (more than two) groups. Two-sided log-rank (Mantel-Cox) test was used for the statistical comparison of the survival study.

**Reporting summary**

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

**Data availability**

The authors declare that the data supporting the findings of this study are available within the article, source data, and its Supplementary Information. The source data underlying Figs. 2, 3, 4, 5, 6, supplementary Figs. 12, 32, 33, 34, 35, 40, 41, 42, 43, 44, and western blot are provided with this paper. A reporting summary for this article is available as a Supplementary Information file. Source data are provided with this paper.

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Competing interests
The authors declare no competing interests.

Additional information
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