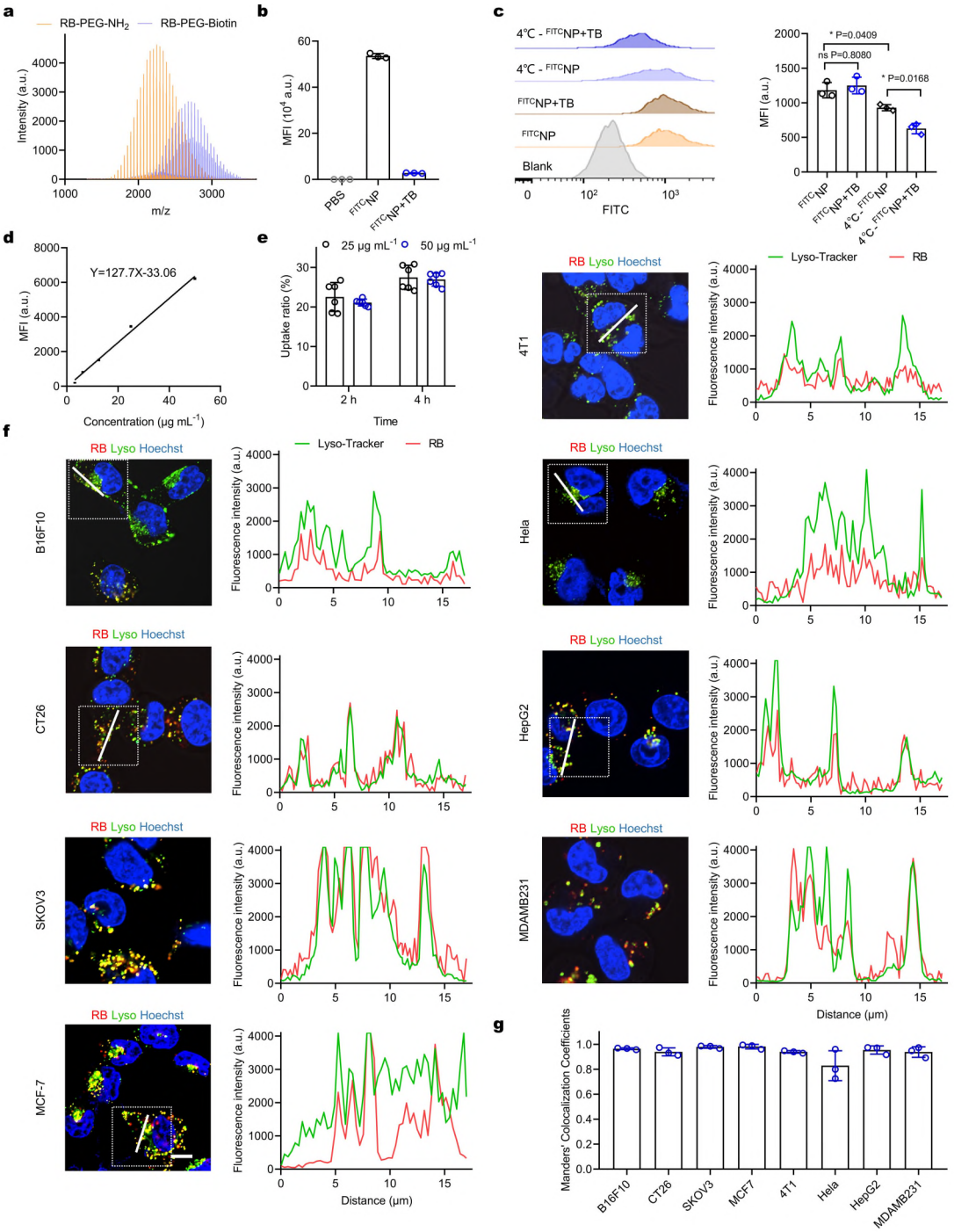
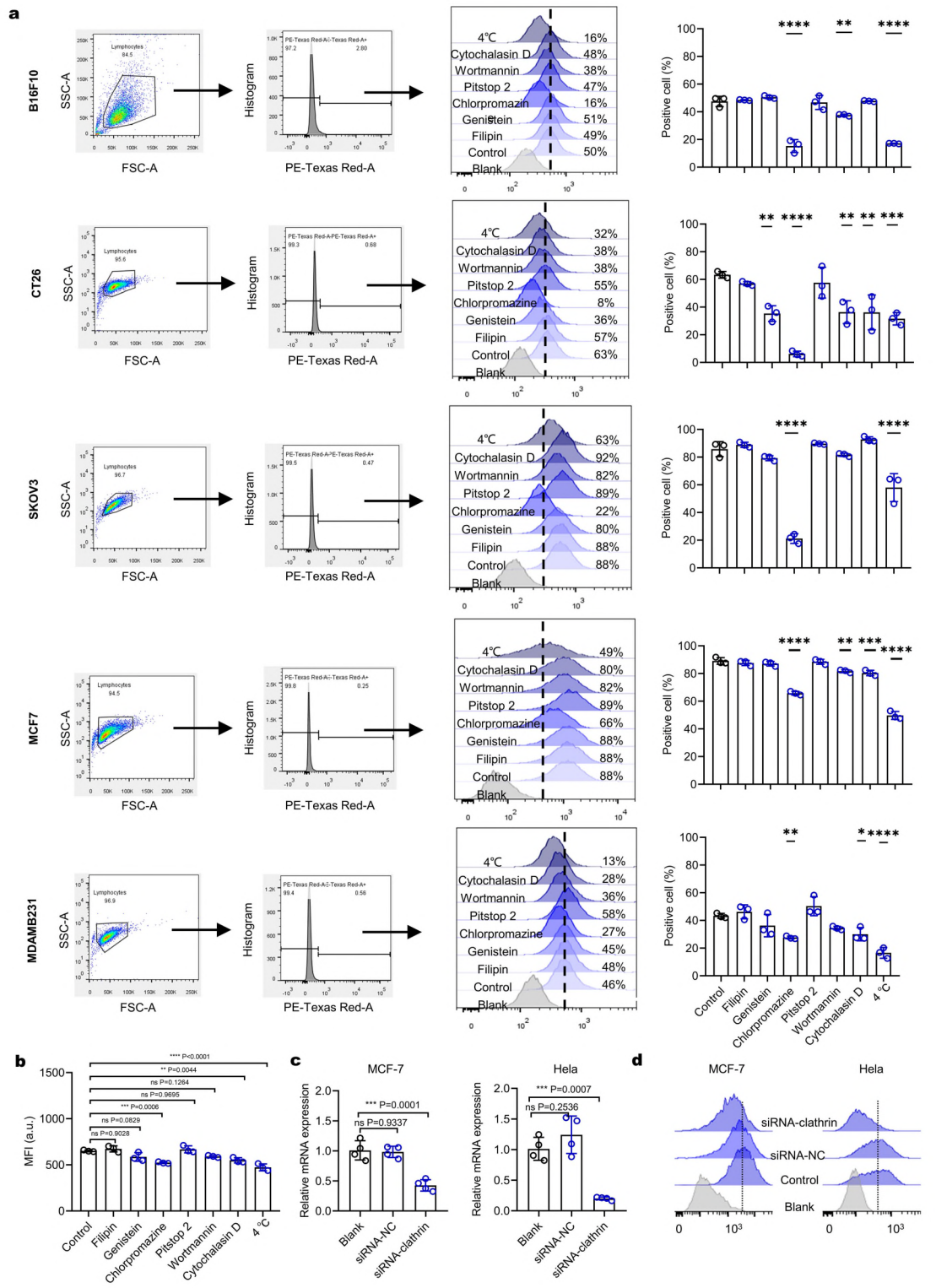


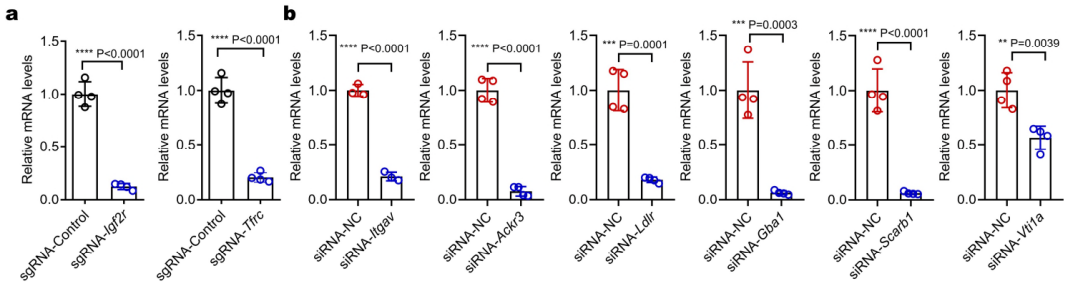
Supplementary Fig. 1 Lysosome-targeting endocytosis of ^{RB}NPs. a, MALDI-TOF mass spectrum of RB-PEG-Biotin and RB-PEG-NH_2. **b,** Fluorescence intensity of ^{FITC}NP (50 μg mL^{-1}) in the presence or absence of 0.01% Trypan Blue (TB) (n = 3 biologically independent experiments). Ex: 494 nm; Em: 525 nm. **c,** Flow cytometry analysis of B16F10 cells co-incubated with ^{FITC}NP (50 μg mL^{-1}) at 37 ºC or 4 ºC for 1.5 hours, followed by 0.01% TB treatment (n = 3 biologically independent experiments,

each counting 10,000 cells). **d,** Standard curve for fluorescence intensity versus ^{RB}NP concentration. **e,** Ratios of internalized ^{RB}NPs after co-incubation with B16F10 cells for 2 or 4 hours (n = 6 biologically independent experiments). **f,** Live-cell images of different cell lines treated with ^{RB}NPs (100 μg mL^{-1}) for 4 hours and the resulting fluorescence intensity profiles along the line plotted in the boxes. Scale bar, 5 μm. The images are representative of n = 3 biological replicates. **g,** Manders' colocalization coefficients (MCC) measuring the colocalization of ^{RB}NPs and lysosomes in different cell lines (n = 3 biologically independent experiments). Data are presented as mean values ± SD where relevant. *P* values were determined by one-way ANOVA with Tukey's multiple comparisons test. ns, no significant; *$p < 0.05$. Source data are provided as a Source Data file.

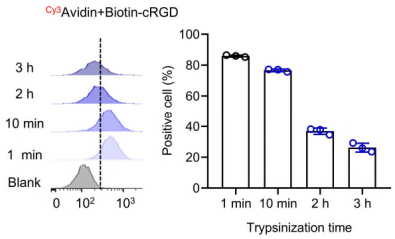


Supplementary Fig. 2 Determination of the endocytic pathway of ^{RB}NP. a, Flow cytometry gating strategy for analyzing RB-positive cells (n = 3 biologically independent experiments, each counting 10,000 cells). **b,** Effects of endocytic inhibitors on cellular uptake of ^{RB}NPs in MDAMB231 cells in terms of the RB intensity measured by flow cytometry (n = 3 biologically independent experiments, each counting 10,000

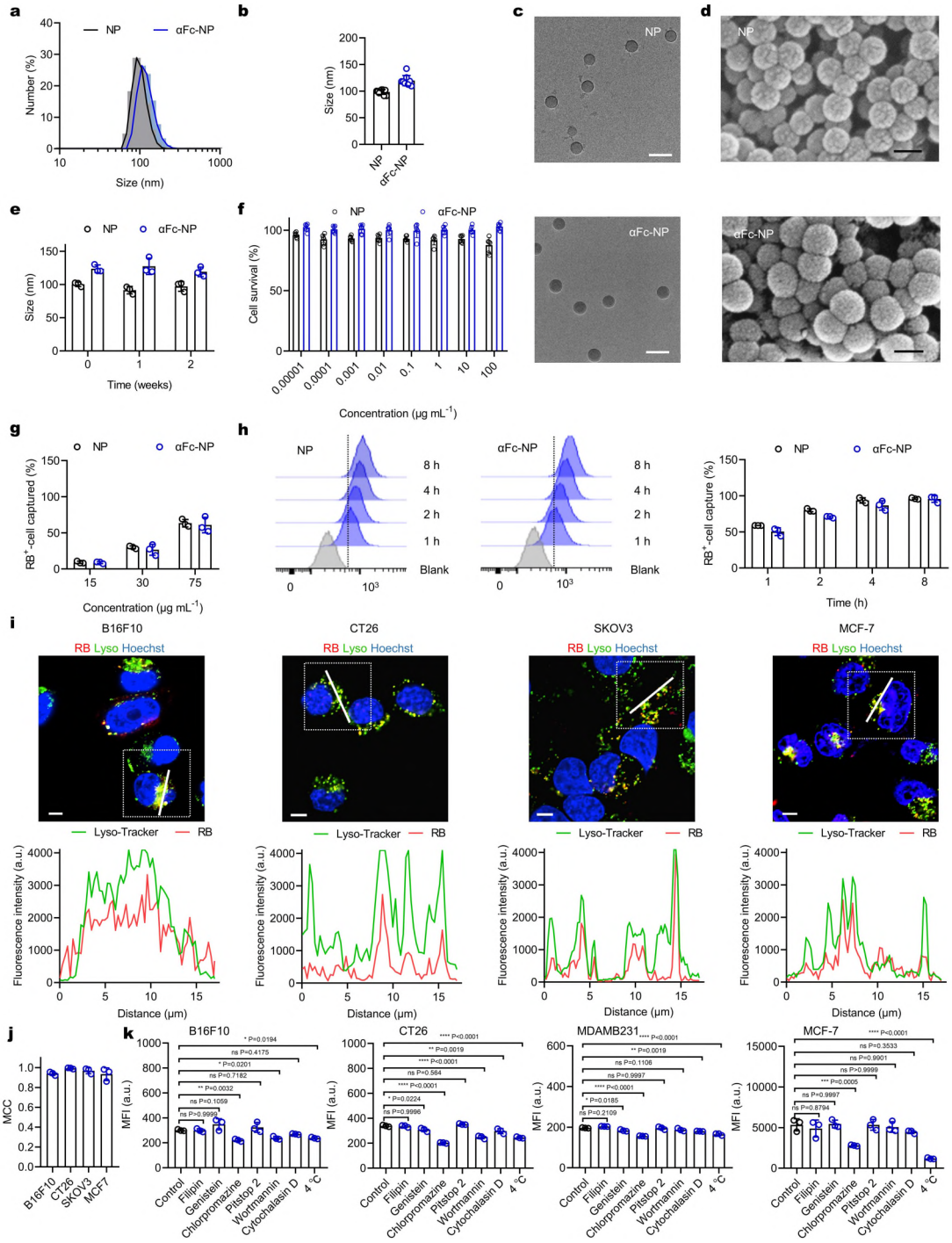
cells). **c,** Validation of clathrin knockdown using RT-qPCR. MCF-7 cells were treated with 120 nM of siRNA-NC or siRNA-clathrin for 48 hours, while Hela cells were treated with 160 nM of siRNA-NC or siRNA-clathrin for 60 hours (n = 4 biologically independent experiments, each counting 10,000 cells). **d,** Cellular uptake of ^{RB}NPs in siRNA-treated MCF-7 cells or Hela cells after 2-hour incubation The uptake profiles are representative of n = 3 biological replicates. Data are presented as mean values ± SD where relevant. *P* values were determined by one-way ANOVA with Dunnett's *post hoc* test. *$p < 0.05$; **$p < 0.01$; ****$p < 0.0001$. Source data are provided as a Source Data file.



Supplementary Fig. 3 Targeted gene silencing *via* CRISPR interference or RNA interference. a, Validation of *Igf2r* (n = 4 biological replicates) or *Tfrc* (n = 4 biological replicates) knockout in B16F10 cells using RT-qPCR. **b,** Validation of *Itgav* (n = 3 biological replicates), *Ackr3* (n = 4 biological replicates), *Ldlr* (n = 4 biological replicates), *Gba1* (n = 4 biological replicates), *Scarb1* (n = 4 biological replicates), or *Vti1a* (n = 4 biological replicates) knockdown in B16F10 cells using RT-qPCR. *P* values were determined by unpaired two-tailed *t*-tests. Data are presented as mean values ± SD where relevant. $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$. Source data are provided as a Source Data file.

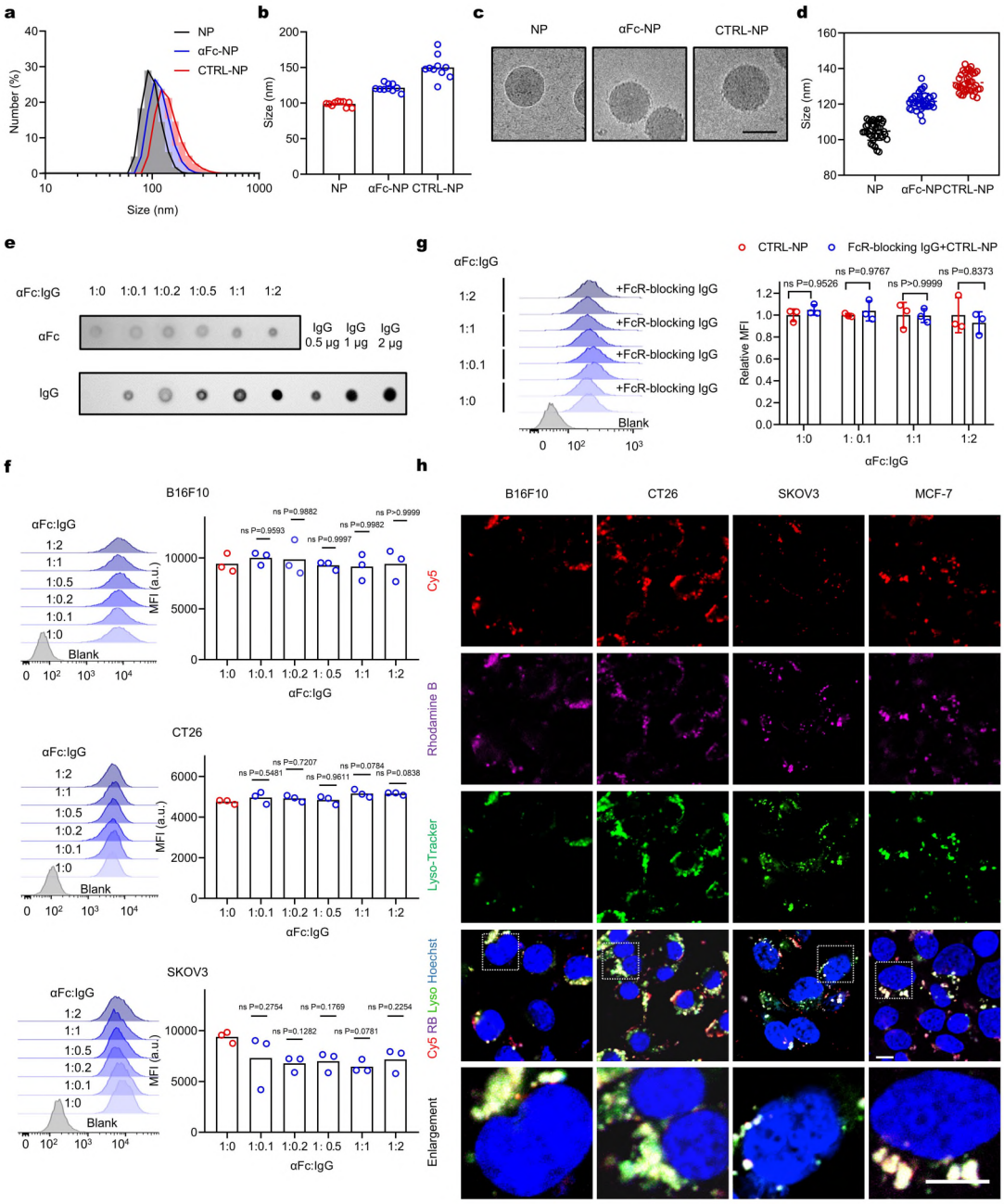


Supplementary Fig. 4 Cellular uptake of ^{Cy3}Avidin (50 nM) mediated by biotin-cRGD (200 nM) after co-incubation with B16F10 cells for 4 hours. Cells were pre-treated with trypsin within 3 hours (n = 3 biologically independent experiments, each counting 5,000 cells). Data are presented as mean values ± SD where relevant. Source data are provided as a Source Data file.



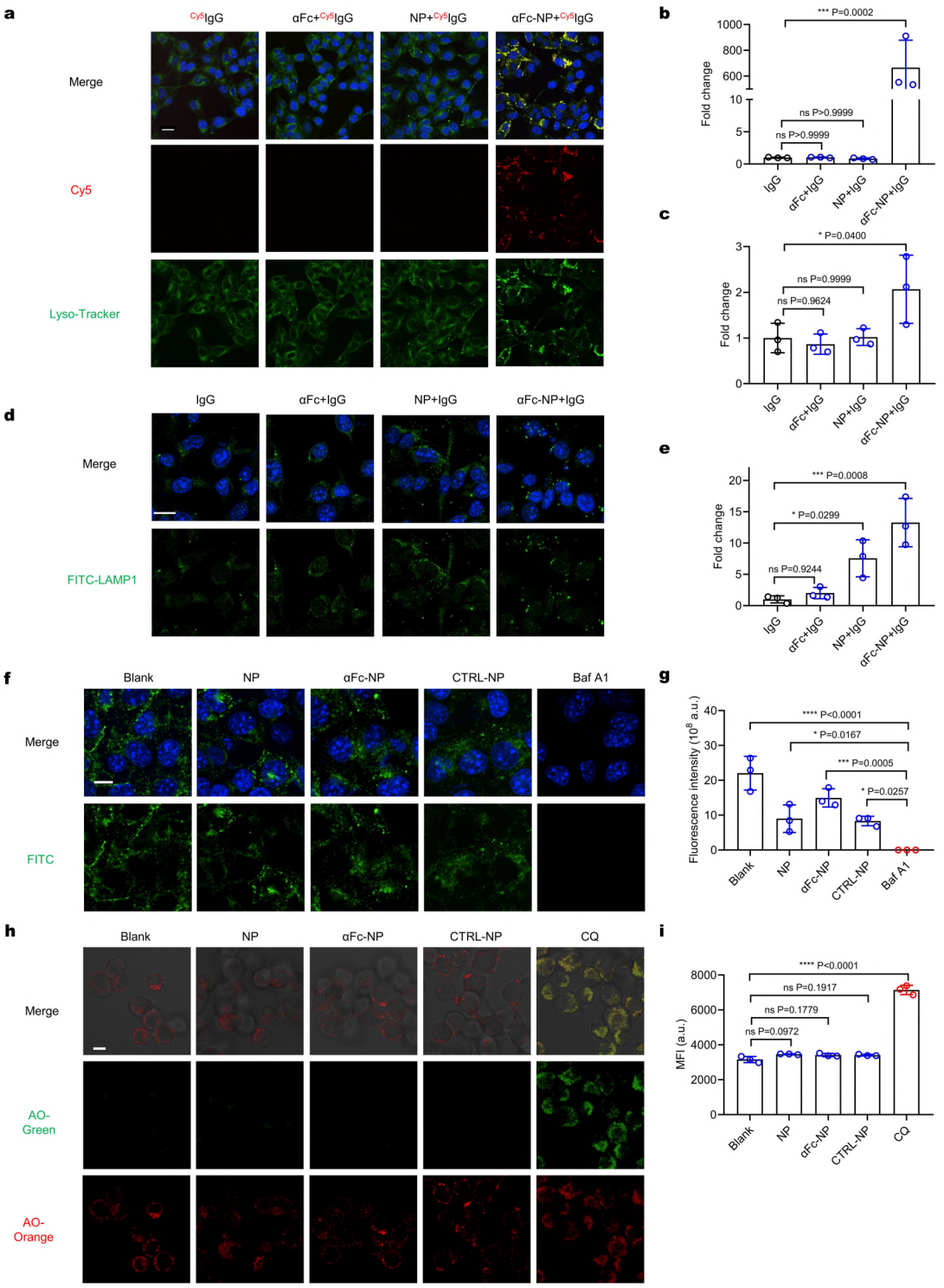
Supplementary Fig. 5 Characterization and cellular uptake of αFc-NPs. a, Size distribution of NP and αFc-NP determined by dynamic light scatting (DLS). **b** Average size of NP and αFc-NP measured by DLS (n = 8 independent replicates). **c, d,** Representative TEM (**c**) and SEM (**d**) images of NP and αFc-NP. Scale bar, 200 nm. The TEM and SEM images are representative of n = 3 independent replicates. **e,** Stability test of NP and αFc-NP by measuring the change in particle size after storage

for the indicated durations at 4 °C (n = 3 independent replicates). **f,** Cytotoxicity of NP and αFc-NP on B16F10 cells determined by the CCK-8 assay (n = 6). **g, h,** Flow cytometry analysis of cellular uptake of ^{RB}NP or αFc-^{RB}NP in B16F10 cells at the indicated concentrations within 2 hours (**g**) or at the concentration of 100 μg mL^{-1} within the indicated time durations (**h**) (n = 3 biologically independent experiments, each counting 10,000 cells). **i,** Live-cell images of different cell lines treated with αFc-^{RB}NP (100 μg mL^{-1}) for 4 hours and the resulting fluorescence intensity profiles along the line plotted in the boxes. Scale bar, 5 μm. The images are representative of n = 3 biological replicates. **j,** Manders' colocalization coefficients (MCC) measuring the colocalization of αFc-^{RB}NPs within lysosomes in different cell lines (n = 3 biologically independent experiments). **k,** Effects of endocytic inhibitors on cellular uptake of αFc-^{RB}NP in B16F10, CT26, MCF-7, and MDAMB231 cells in terms of the RB intensity measured by flow cytometry (n = 3 biologically independent experiments, each counting 10,000 cells). *P* values were determined by one-way ANOVA with Dunnett's *post hoc* test. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ****$p < 0.0001$. Data are presented as mean values ± SD where relevant. Source data are provided as a Source Data file.



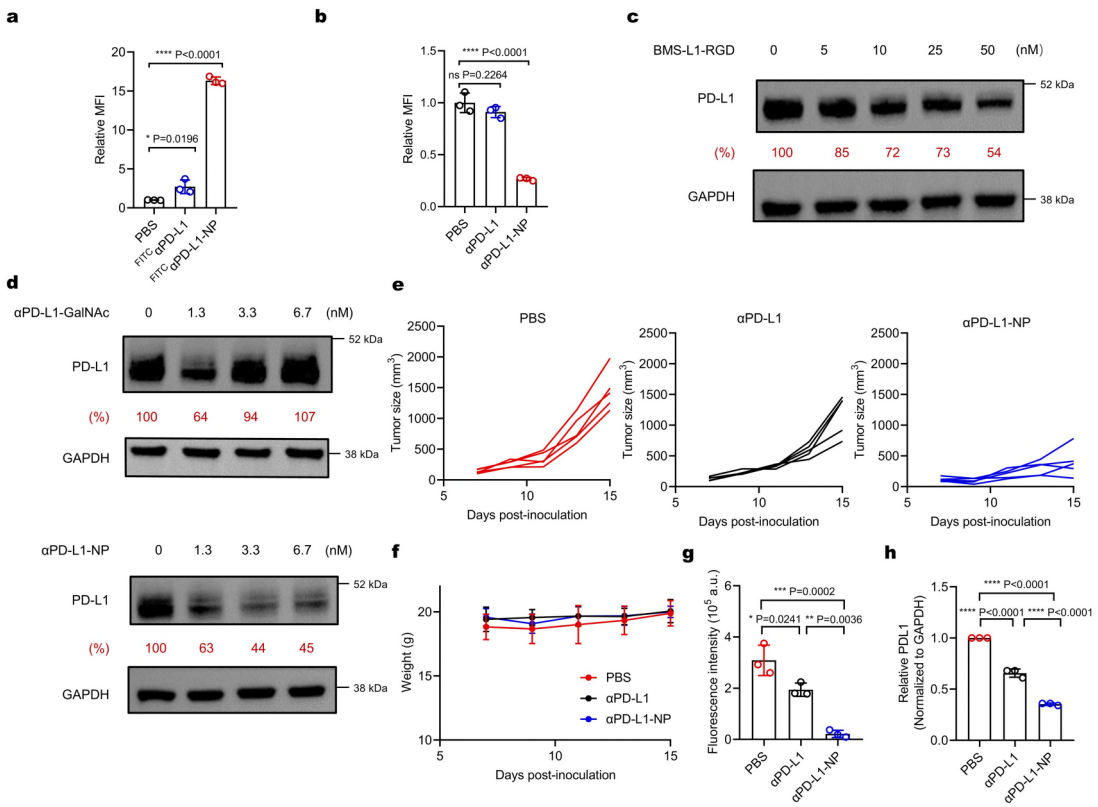
Supplementary Fig. 6 Characterization and lysosome-targeting ability of CTRL-NP. a, Size distribution of NP, αFc-NP, and CTRL-NP determined by DLS. **b,** Average size of NP, αFc-NP, and CTRL-NP measured by DLS (n = 10 biologically independent experiments). **c,** Representative Cryo-TEM images of NP, αFc-NP, and CTRL-NP. Scale bar, 100 nm. The Cryo-TEM images are representative of n = 3 independent replicates. **d,** Particle size distribution analysis of Cryo-TEM images with ImageJ (n = 37 nanoparticles). **e,** Dot blot assay for quantifying the αFc and IgG concentrations in CTRL-NPs with varying αFc:IgG ratios. **f,** Cellular uptake of CTRL-^{RB}NPs with

varying αFc:IgG molar ratios in B16F10, CT26, and SKOV3 cells within 4 hours (n = 3 biologically independent experiments, each counting 10,000 cells). *P* values were determined by one-way ANOVA with Dunnett's *post hoc* test. ns, no significant. **g,** Cellular uptake of CTRL-^{RB}NPs (50 μg mL^{-1}) with varying αFc:IgG molar ratios in FcR-preblocked B16F10 cells within 4 hours. B16F10 cells were pre-treated with mouse FcR-blocking antibody for 10 hours (n = 3 biologically independent experiments, each counting 10,000 cells). *P* values were determined by *t*-test. ns, no significant. **h,** Live-cell images of different cell lines treated with ^{Cy5}CTRL-^{RB}NPs (50 μg mL^{-1}) for 10 hours. Scale bar, 10 μm. The images are representative of n = 3 biological replicates. Data are presented as mean values ± SD where relevant. Source data are provided as a Source Data file.



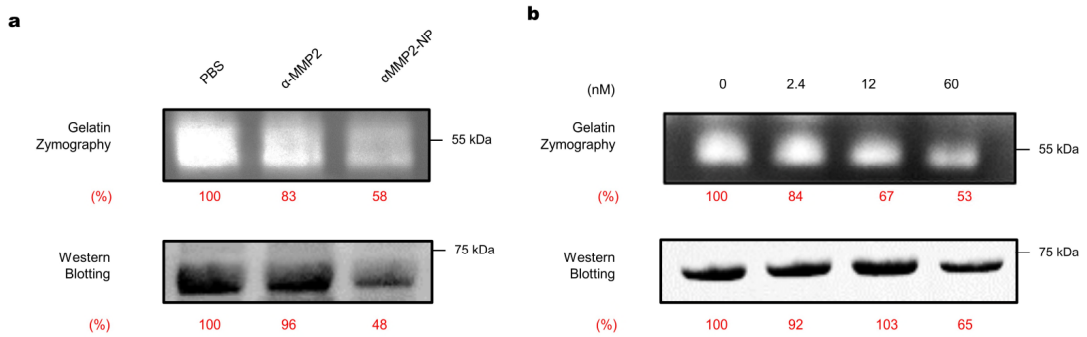
Supplementary Fig. 7 Effects of MONOTAB on lysosomal function. a, Live-cell images of B16F10 cells treated with ^{Cy5}IgG, ^{Cy5}IgG + αFc, ^{Cy5}IgG + NP, or ^{Cy5}IgG + αFc-NP for 10 hours. The corresponding concentrations of ^{Cy5}IgG, αFc, and NP were 2, 2, and 50 μg mL^{-1}, respectively. Scale bar, 20 μm. These images are representative of n = 3 biological replicates. **b, c,** Fold changes in MFI of the Cy5 (**b**) or LysoTracker (**c**) signal in **a** (n = 3 biologically independent experiments). **d,** Immunofluorescence

analysis of LAMP1 in B16F10 cells treated with IgG, IgG + αFc, IgG + NP, or IgG + αFc-NP for 10 hours. The corresponding concentrations of ^{Cy5}IgG, αFc, and NP were 2, 2, and 50 μg mL^{-1}, respectively. Scale bar, 20 μm. These images are representative of n = 3 biological replicates. **e,** Fold changes in MFI of the FITC signal in **d** within individual cells calculated from triplicate samples. **f,** Live-cell images of B16F10 cells treated with 50 μg mL^{-1} of NP, αFc-NP, or CTRL-NP for 10 hours or 100 nM of Bafilomycin A1(Baf A1) for 1 hour, then loaded with DQ Green BSA for 10 hours. Scale bar, 10 μm. These images are representative of n = 3 biological replicates. **g,** Quantification of green fluorescence in **f** using ImageJ (n = 3 biologically independent experiments). **h i,** Live-cell images (**h**) and flow cytometry analysis (**i**) of B16F10 cells treated with 50 μg mL^{-1} of NP, αFc-NP, or CTRL-NP for 10 hours or 10 μM of chloroquine (CQ) plus 50 μM of $ZnCl_2$ for 30 minutes, then stained with acridine orange for 15 minutes (n = 3 biologically independent experiments, each counting 10,000 cells). Scale bar, 10 μm. These images are representative of n = 3 biological replicates. Data are presented as mean values ± SD where relevant. *P* values were determined by one-way ANOVA with Dunnett's *post hoc* test. ns, no significant; *$p < 0.05$; ***$p < 0.001$; ****$p < 0.0001$. Source data are provided as a Source Data file.

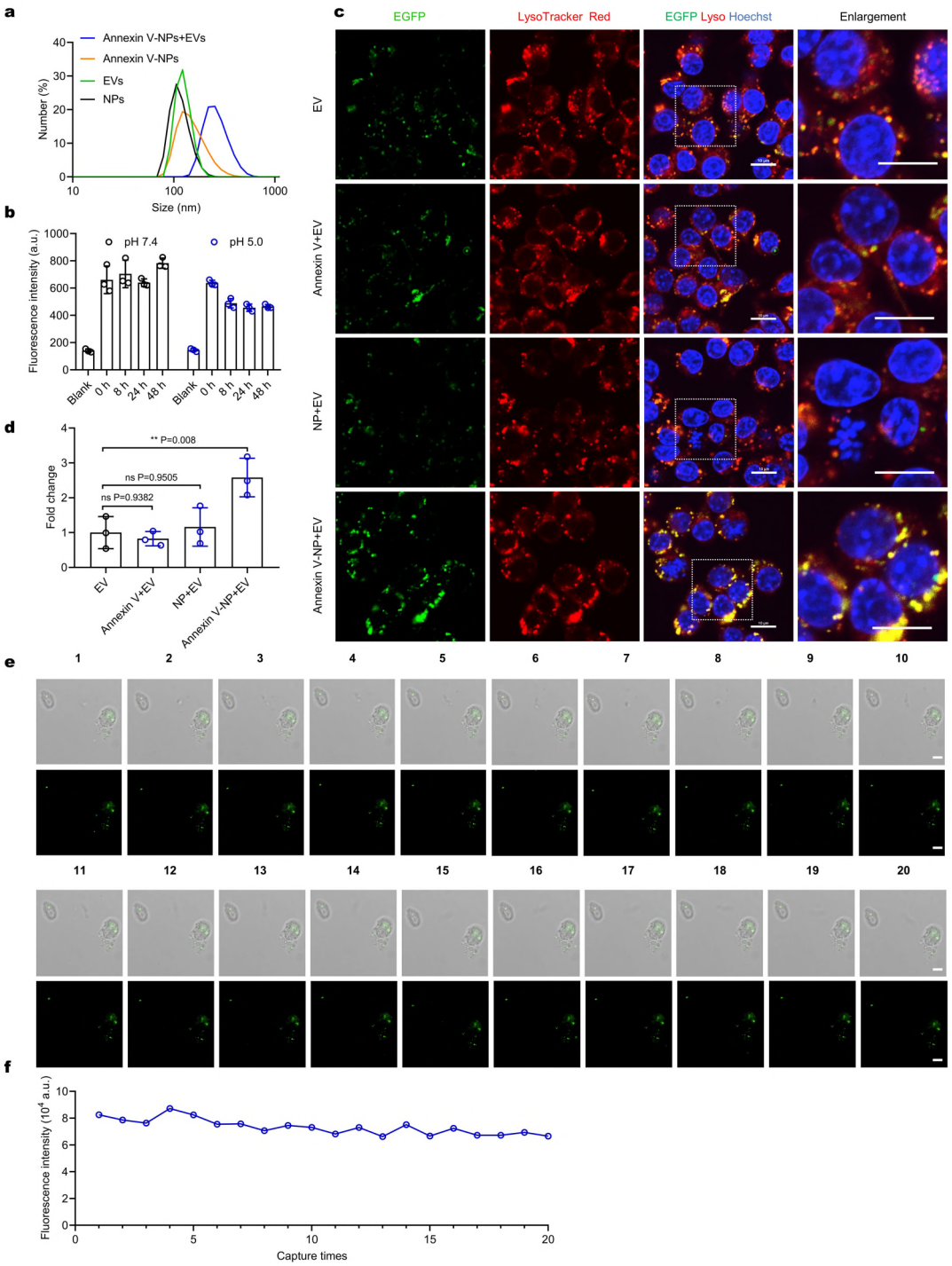


Supplementary Fig. 8 Degradation of PD-L1 *in vitro* and *in vivo*. a, Fold changes in MFI of the FITC signal in B16F10 cells with the treatment of ^{FITC}αPD-L1 (3.3 nM) or ^{FITC}αPD-L1-NP (^{FITC}PD-L1-equiv., 3.3 nM) for 4 hours (n = 3 biologically independent experiments). **b,** Quantitative image analysis of PD-L1 degradation as shown in **Fig. 3e** (n = 3 biologically independent experiments). **c,** Western blot analysis of PD-L1 degradation in B16F10 cells after co-incubation with BMS-L1-RGD at the indicated concentrations for 24 hours. The blots are representative of n = 3 biological replicates. The uncropped blots are included in the Source Data file. **d,** Western blot analysis of PD-L1 degradation in Hepa1-6 cells after co-incubation with αPD-L1-GalNAc or αPD-L1-NP at the indicated concentrations for 24 hours. The blots are representative of n = 3 biological replicates. The samples derived from the same experiment and the blots were processed in parallel. The uncropped blots are included in the Source Data file. **e,** Tumor growth curves for individual mice in each treatment group (n = 5 mice per group). **f,** Average body weight change of mice in each group. **g,** Quantitative image analysis of the PD-L1 level in tumor sections as shown in **Fig. 3n** (n = 3 biologically independent experiments). **h,** Densitometric analysis of the PD-L1 band as shown in **Fig. 3o** (n = 3

biologically independent experiments). Data are presented as mean values ± SD where relevant. *P* values were determined by one-way ANOVA with Tukey's multiple comparisons test. ns, no significant; *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ****$p < 0.0001$. Source data are provided as a Source Data file.



Supplementary Fig. 9 Degradation of MMP2 mediated by αMMP2-NP. a, Gelatin zymography and Western blot analysis of the culture medium of MCF-7 cells after the treatment with PBS, αMMP2 (12 nM), or αMMP2-NP (α-MMP2-equiv. 12 nM) for 12 hours. Equal amounts of the medium were applied to gel electrophoresis. The blots are representative of n = 3 biologial replicates. The uncropped blots and gels are included in the Source Data file. **b,** Gelatin zymography and Western blot analysis of the culture medium of MDAMB231 cells treated with varying concentrations of αMMP2-NP for 12 hours. Equal amounts of the medium were applied to gel electrophoresis. The blots are representative of n = 3 biologial replicates. The uncropped blots and gels are included in the Source Data file. Source data are provided as a Source Data file.



Supplementary Fig. 10 Degradation of EVs mediated by Annexin V-NPs. a, Particle size of NP, EV, Annexin V-NP, and Annexin V-NP + EV measured by DLS. **b,** Fluorescence intensity change of ^{EGFP}EVs (6×10^9 particles mL^{-1}) incubated at pH 7.4 or pH 5.0 for different durations (0, 8, 24 and 48 h) (n = 3 biologically independent experiments). Blank refers to the background fluorescence in the buffer. **c,** Live-cell images of RAW264.7 cells incubated with EVs, Annexin-V + EVs, NPs + EVs, or

Annexin-V-NPs + EVs for 8 hours. The corresponding concentrations of EV, Annexin-V, and NP were 1.3×10^8 particles mL^{-1}, 0.4 μg mL^{-1}, and 50 μg mL^{-1}, respectively. Scale bar, 10 μm. The images are representative of n = 3 biological replicates. **d,** Fold changes in MFI of the EGFP signal as shown in **c** (n = 3). **e,** Confocal tracking of the EGFP signal in ECDHCC1-PalmGRET cell debris with 20 rounds of image capture. Scale bar, 5 μm. **f,** Quantitative image analysis of the EGFP signal as shown in **e**. *P* values were determined by one-way ANOVA with Dunnett's *post hoc* test. ns, no significance; ***p* < 0.01. Data are presented as mean values ± SD where relevant. Source data are provided as a Source Data file.

Supplementary Table 1. Comparison of existing approaches in terms PD-L1 degradation

Existing approach	Concentration	Duration	Degradation
LYTAC[1]	25 nM (Atz)	48 h	73%
GalNAc-LYTAC[2]	25 nM (Atz)	48 h	7%
Atz-EndoTag[3]	200 nM (Atz)	48 h	77%
Covalent LYTAC platform[4]	1 μM (DBCO-PDL1 aptamer)	12 h	72%
KineTAC[5]	100 nM (Atz)	24 h	67%
	50 nM (Atz)	24 h	55%
	10 nM (Atz)	24 h	37%
AbTAC[6]	10 nM (Atz)	24 h	63%
IFLD[7]	25 nM (BMS-8)	24 h	73%
PROTAB[8]	13.3 nM (Atz)	24 h	near-complete
GlueTAC[9]	100 nM	12 h	89%
SignalTAC[10]	100 nM (Az)	48 h	55%
CDTAC[11]	6 μM (BMS-1166)	24 h	76%
IGF2-peptide based LYTAC[12]	100 nM (C5 antibody)	22 h	55%
	50 nM (C5 antibody)	22 h	75%
ROTAC[13]	R2PD1 protein	24 h	90%
iLYTACs[14]	200 nM	48 h	55%
TransTAC[15]	25 nM (Atz Fab)	/	98%
GLP1-LYTAC[16]	100 nM (HS636)	48 h	82%
LYTACA[17]	100 μM (fm-TPP)	12h	42%
	100 μM (nf-TPP)	12h	70%
MONOTAB	**6.6 nM**	**24 h**	**92%**
	3.3 nM	**48 h**	**95%**

Supplementary Table 2. Reagents and Sources

Regent	Source	Regent	Source
Streptavidin-conjugated and carboxyl-functionalized polystyrene nanoparticles (25mg mL^{-1})	XINQIAO Bio	DAPI	Aladdin
Rhodamine PEG Amine (RB-PEG_{2K}-NH_2)	Ponsure	Paraformaldehyde	Aladdin
Fluorescein PEG Amine (FITC-PEG_{2K}-NH_2)	Ponsure	Genistein	Aladdin
Biotin-PEG4-NHS Ester	LEO Biochem	Cytochalasin D	Aladdin
BCA protein assay kit	Beyotime	Filipin	Aladdin
Membrane and cytosol protein extraction kit	Beyotime	Chlorpromazine	Aladdin
sodium dodecyl sulfate (SDS)	Beyotime	Pitstop 2	Aladdin
Tween 20	Beyotime	Wortmannin	Aladdin
Tris-HCl (pH=7.5)	Beyotime	Leupeptin	Macklin
Gelatin from porcine skin	Beyotime	mitomycin C	Macklin
Coomassie Blue Fast Staining Solution	Beyotime	Bafilomycin A1	TargetMol
RIPA Lysis Buffer	Beyotime	Triton-X-100	YUANYE Bio
Phenylmethanesulfonyl fluoride (PMSF, 100 mM)	Beyotime	2×S6 Universal SYBR qPCR Mix	EnzyArtisan
BeyoECL Star	Beyotime	HyperScript III 1st Strand cDNA Synthesis Kit with gDNA Remover	EnzyArtisan
Crystal Violet Nonahydrate	TCI	RNAsimple Total RNA Kit	TianGen
RNAiMAX Transfection Reagent	Thermo Fisher	CCK-8 assay kit	Meilunbio
LysoTracker™ Green DND-26	Thermo Fisher	Prestained Protein Ladder	Meilunbio
LysoTracker™ Red DND-99	Thermo Fisher	Fetal bovine serum (FBS)	Gibco
Hoechst 33342	Thermo Fisher	100×penicillin/streptomycin	GENOM Bio
RPMI-1640	Thermo Fisher	BMS8	MedChemExpress
DMEM	Thermo Fisher	Tri-$GalNAc(OAc)_3$-NH_2	CincoBio
Hexafluorophosphate Azabenzotriazole Tetramethyl Uronium (TATU)	BidePharm	1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDCI)	BidePharm
N-Boc-1,3-propanediamine	BidePharm	5-Hexynoic acid	BidePharm
Imidazole-1-sulfonyl azide hydrochloride	BidePharm	Tris(benzyltriazolylmethyl)amine (TBTA)	BidePharm
cRGD	BidePharm	4-Dimethylaminopyridine (DMAP)	BidePharm
NHS-C6-DBCO	BidePharm	Sodium ascorbate (NaVc)	BidePharm
N-Hydroxysuccinimide (NHS)	BidePharm	N-boc-1,3-propanediamine	Aikonchem
Copper(I) bromide (CuBr)	BidePharm	NHS-$(PEG)_4$-azide	Aikonchem

Supplementary Table 3. Antibody information and concentrations

Antibody	Source (#)	Usage, Dilution
Goat Anti-Rabbit IgG H&L (Biotin)	Abcam (ab97198)	Functional (6 nM)
Goat Anti-Mouse H&L (Biotin)	Abcam (ab207996)	Functional (6 nM)
Rabbit IgG	Elabscience (E-AB-1128)	Functional (0.6-12 nM)
Mouse IgG	Abmart (B30010M)	Functional (0.6-12 nM)
Rabbit IgG-Cy5	Yaji Biological	Functional (3.3 nM)
Anti-PDL1 antibody	Abcam (ab213480)	Functional (0.7-6.7 nM); WB, 1:1000
Anti-PDL1 antibody	Proteintech (66248-1-lg)	IF, 1:200; WB, 1:1000
Anti-MMP2 antibody	Cell Signaling Technology (13132)	WB, 1:1000
Anti-MMP2 antibody	Abcam (ab92536)	Functional (2.4-60 nM); WB, 1:1000
Biotin-Annexin V	Biolegend (640904)	Functional (0.4 μg mL^{-1})
Annexin V-Biotin Reagent	Elabscience (E-CK-A110)	Functional (8 μL mL^{-1})
FITC Anti-mouse CD274/PD-L1 Antibody	Elabscience (E-AB-F1132UC)	Functional (3.3 nM)
Goat Anti-Mouse IgG H&L (Alexa Fluor® 647)	Abcam (ab150115)	IF, 1:1000
Goat Anti-Rabbit IgG H&L (FITC conjugated)	Elabscience (E-AB-1014)	IF, 1:100
EEA1 (C45B10) Rabbit mAb	Cell Signaling Technology (3288)	WB, 1:1000
Rab7 (D95F2)XP® Rabbit mAb	Cell Signaling Technology (9367)	WB, 1:1000
LAMP1 (D2D11)XP® Rabbit mAb	Cell Signaling Technology (9091)	IF, 1:400
CD107a / LAMP1 Monoclonal antibody	Proteintech (67300-1-lg)	WB, 1:5000
Anti-GAPDH Recombinant Rabbit Monoclonal Antibody	HUABIO (ET16014)	WB, 1:1000
HRP-labeled Goat Anti-Rabbit IgG (H+L)	Beyotime	WB, 1:2000
HRP-labeled Goat Anti-Mouse IgG (H+L)	Beyotime	WB, 1:2000

Supplementary Table 4. RNA sequences and PCR primers

gRNA sequence (5’ to 3’)

	Oligo1	Oligo2
Mouse *Igf2r* gRNA	CACCGATCAACGTGTGTAGAGACAT	AAACATGTCTCTACACACGTTGATC
Mouse *Tfrc* gRNA	CACCGCTACACGCTTACAATAGCCC	AAACGGGCTATTGTAAGCGTGTAGC

siRNA sequence (5’ to 3’)

	Oligo1	Oligo2
Mouse *Itgav* siRNA	AAGGACUUUGGGAAUGGUUTT	AACCAUUCCCAAAGUCCUUTT
Mouse *Ackr3* siRNA	UACACUACAUCCCGUUUACTT	GUAAACGGGAUGUAGUGUATT
Mouse *Ldlr* siRNA	GCAACAUCUCUGGAAGAUUTT	AAUCUGUCCAGUAGAUGUUGCTT
Mouse *Gba1* siRNA	ACGGAAUUGAAUAUAACAUTT	AUGUUAUAUUCAAUUCCGUTT
Mouse *Scarb1* siRNA	GGGAUGAACAACUCGAAUUTT	AAUUCGAGUUGUUCAUCCCTT
Mouse *Vti1a* siRNA	CAGAUGGAUUUGGAAGUUCTT	GAACUUCCAAAUCCAUCUGTT

PCR Primers

	Forward sequence	Reverse sequence
Mouse *Igf2r*	GGGAAGCTGTTGACTCCAAAA	GCAGCCCATAGTGGTGTTGAA
Mouse *Tfrc*	GTTTCTGCCAGCCCCTTATTAT	GCAAGGAAAGGATATGCAGCA
Mouse *Itgav*	CCGTGGACTTCTTCGAGCC	CTGTTGAATCAAACTCAATGGGC
Mouse *Ackr3*	GACCGCTATCTCTCCATCACCT	GTTGGAAGCAGATGTGACCGTC
Mouse *Ldlr*	TGACTCAGACGAACAAGGCTG	ATCTAGGCAATCTCGGTCTCC
Mouse *Gba1*	GCCAGTTGTGACTTCTCCATCC	CGTGAGGACATCTTCAGGGCTT
Mouse *Scarb1*	TTTGGAGTGGTAGTAAAAAGGGC	TGACATCAGGGACTCAGAGTAG
Mouse *Vti1a*	TGACATCAGGGACTCAGAGTAG	CCCTTCCTTGAACTCATAAGCC

Supplementary Table 5. Sequence of the used EGFP variant

Sense strand (5' to 3')	ATGGTGAGCAAGGGCGAGGAGCTGTTCACCGGGGTGGTGCCCATCCTGG TCGAGCTGGACGGCGACGTAAACGGCCACAAGTTCAGCGTGTCCGGCGA GGGCGAGGGCGATGCCACCTACGGCAAGCTGACCCTGAAGTTCATCTGC ACCACCGGCAAGCTGCCCGTGCCCTGGCCCACCCTCGTGACCACCCTGA CCTACGGCGTGCAGTGCTTCAGCCGCTACCCCGACCACATGAAGCAGCAC GACTTCTTCAAGTCCGCCATGCCCGAAGGCTACGTCCAGGAGCGCACCAT CTTCTTCAAGGACGACGGCAACTACAAGACCCGCGCCGAGGTGAAGTTCG AGGGCGACACCCTGGTGAACCGCATCGAGCTGAAGGGCATCGACTTCAA GGAGGACGGCAACATCCTGGGGCACAAGCTGGAGTACAACTACAACAGCC ACAACGTCTATATCATGGCCGACAAGCAGAAGAACGGCATCAAGGTGAACT TCAAGATCCGCCACAACATCGAGGACGGCAGCGTGCAGCTCGCCGACCAC TACCAGCAGAACACCCCCATCGGCGACGGCCCCGTGCTGCTGCCCGACAA CCACTACCTGAGCACCCAGTCCGCCCTGAGCAAAGACCCCAACGAGAAGC GCGATCACATGGTCCTGCTGGAGTTCGTGACCGCCGCCGGGATCACTCTC GGCATGGACGAGCTGTACAAG
Anti-sense strand (5' to 3')	CTTGTACAGCTCGTCCATGCCGAGAGTGATCCCGGCGGCGGTCACGAACT CCAGCAGGACCATGTGATCGCGCTTCTCGTTGGGGTCTTTGCTCAGGGCG GACTGGGTGCTCAGGTAGTGGTTGTCGGGCAGCAGCACGGGGCCGTCGC CGATGGGGGTGTTCTGCTGGTAGTGGTCGGCGAGCTGCACGCTGCCGTCC TCGATGTTGTGGCGGATCTTGAAGTTCACCTTGATGCCGTTCTTCTGCTTGT CGGCCATGATATAGACGTTGTGGCTGTTGTAGTTGTACTCCAGCTTGTGCCC CAGGATGTTGCCGTCCTCCTTGAAGTCGATGCCCTTCAGCTCGATGCGGTT CACCAGGGTGTCGCCCTCGAACTTCACCTCGGCGCGGGTCTTGTAGTTGC CGTCGTCCTTGAAGAAGATGGTGCGCTCCTGGACGTAGCCTTCGGGCATG GCGGACTTGAAGAAGTCGTGCTGCTTCATGTGGTCGGGGTAGCGGCTGAA GCACTGCACGCCGTAGGTCAGGGTGGTCACGAGGGTGGGCCAGGGCACG GGCAGCTTGCCGGTGGTGCAGATGAACTTCAGGGTCAGCTTGCCGTAGGT GGCATCGCCCTCGCCCTCGCCGGACACGCTGAACTTGTGGCCGTTTACGT CGCCGTCCAGCTCGACCAGGATGGGCACCACCCCGGTGAACAGCTCCTC GCCCTTGCTCACCAT

Supplementary methods

Preparation of ^{RB}NP/ ^{FITC}NP

Procedure for RB-biotin and FITC-biotin conjugation. RB-PEG$_{2K}$-amine (25.0 mg) or FITC-PEG$_{2K}$-amine (100.0 mg) was mixed with biotin-PEG$_4$-NHS (7.0 mg, 0.012 mmol) in an Eppendorf tube, and 1 mL of $NaHCO_3$ solution (pH ~8.5) was added. The reaction was incubated at room temperature in the dark for 8 hours and then filtered through a Sephadex G15 column to yield RB-PEG-Biotin and FITC-PEG-Biotin.

Procedure for RB or FITC labelling of NP. RB-PEG-Biotin (5 mM) or FITC-PEG-Biotin (20 mM) was mixed with a 25 mg mL^{-1} solution of streptavidin-modified NPs (XINQIAO Bio, Hangzhou, China) at the ratio of 1:1 (v/v) and incubated on an incubator overnight, at 4 ℃ in the dark. The reaction mixture was then ultracentrifuged at 15,000 g for 5 minutes to pellet ^{RB}NPs or ^{FITC}NPs.

Preparation of αFc-NP and αFc-^{RB}NP

Biotinylated goat anti-rabbit or anti-mouse IgG H&L (1 mg mL^{-1}) was mixed with NPs or ^{RB}NPs (25 mg mL^{-1}) at the molar ratio of 1:1 and incubated on a shaker for 8 hours at 4 ℃. The reaction mixture was then ultracentrifuged at 15,000 g for 5 minutes to pellet αFc-NPs or αFc-^{RB}NPs.

General procedure for preparation of MONOTABs

αFc-NPs or αFc-^{RB}NPs were mixed with control IgG or targeted antibodies at the αFc:IgG molar ratio of 1:2, unless otherwise described, and incubated on a shaker for 2 hours at 4℃. The reaction mixture was ultracentrifuged at 15,000 g for 5 minutes to pellet MONOTABs.

Cytotoxicity of NP and αFc-NP against B16F10 cells

B16F10 cells were plated into a 96-well plate (5,000 cells per well) one day before the experiment and then incubated with serial concentrations of NP or αFc-NP for 24 hours. Subsequently, culture medium in each well was replaced with 80 μL of the CCK-8 solution (Meilunbio, # MA0218-3) and incubated for 3 hours. The absorbance at 450

nm of each well was measured using a Synergy H1 microplate reader (BioTek). The cell viability was calculated as previously described.

Clathrin knockdown with siRNA

Cells were plated in a 6-well plate (400,000 cells per well) one day before the experiment and then transfected with 120 nM of clathrin siRNA (s476: 5'-CGGUUGCUCUUGUUACGGATT-3', GenePharma) and Lipofectamine RNAiMAX (Invitrogen) according to the manufacturers' instructions. A non-specific Silencer Select siRNA (NC-siRNA) was used as the negative control.

Cell sample processing for TEM analysis

Double fixation. Cell samples were first fixed with 2.5% glutaraldehyde in phosphate buffer (0.1 M, pH 7.0) for more than 4 hours. The samples were then washed with phosphate buffer (0.1 M, pH 7.0) three times for 15 minutes each. Postfixation was performed with 1% OsO_4 in phosphate buffer (0.1 M, pH 7.0) for 1-2 hours, followed by three 15-minute washes with phosphate buffer (0.1 M, pH 7.0).

Dehydration. The fixed samples were first dehydrated using a graded series of ethanol (30%, 50%, 70%, and 80%) for 15 minutes each, followed by a graded series of acetone (90% and 95%) for 15 minutes each. Afterward, the samples were dehydrated twice using absolute acetone for 20 min respectively.

Infiltration. The specimen was placed in a 1:1 mixture of absolute acetone and the final Spurr resin mixture for 1 hour at room temperature. Afterward, the specimen was transferred to a 1:3 mixture of absolute acetone and the final resin mixture for 3 hours and then placed in the final Spurr resin mixture overnight.

Embedding, ultrathin sectioning, staining and examination. The specimen was placed in an Eppendorf tube containing the Spurr resin and heated at 70℃ for more than 9 hours. Subsequently, the specimen was sectioned using the Leica EM UC7 ultratome. The sections were stained with uranyl acetate for 5 minutes and alkaline lead citrate for 10 minutes, respectively. The sections were examined using a Hitachi H-7650 TEM.

Confocal microscopy for lysosome biogenesis

B16F10 cells were seeded into a Nunc glass bottom dish (100,000 cells per dish) one day before the experiment and then incubated with IgG (2 μg mL^{-1}), IgG (2 μg mL^{-1}) + αFc (2 μg mL^{-1}), IgG (2 μg mL^{-1}) + NP (50 μg mL^{-1}), or IgG (2 μg mL^{-1}) + αFc-NP (50 μg mL^{-1}) for 10 hours. Subsequently, the cells were washed with PBS, fixed with methanol for 5 minutes, and washed with PBS three times. After blocking with 10% goat serum for 1 hour at room temperature, the samples were incubated with a 1:400 dilution of rabbit LAMP1 monoclonal antibody (CST, #9091T) overnight at 4 ℃ and then a 1:1000 dilution of FITC-conjugated goat anti-rabbit IgG (Elabscience, #E-AB-1014) for 1 hour at room temperature. After washing with PBS, the cells were stained with DAPI (2 μg mL^{-1}) for 20 minutes, washed, and imaged using a Nikon AX R confocal microscope. DAPI was excited using a 405 nm laser and visualized at 427~475 nm; FITC was excited using a 488 nm laser and visualized at 505~544 nm.

NP-mediated ^{FITC}αPD-L1 internalization

B16F10 cells were seeded into a Nunc glass bottom dish (100,000 cells per dish) one day before the experiment and then incubated with 3.3 nM of ^{FITC}αPD-L1 or ^{FITC}αPD-L1-NP for 4 hours. Subsequently, the cells were washed with PBS three times and incubated with 2 μM of Hoechst 33342 for 30 minutes and 100 nM of LysoTracker DND99 for 15 minutes. After washing with PBS three times, the cells were imaged using a Nikon AX R confocal microscope. FITC was excited using a 488 nm laser and visualized at 505~544 nm.

Confocal microscopy for PD-L1 degradation

B16F10 cells were seeded into a Nunc glass bottom dish (100,000 cells per dish) one day before the experiment and then incubated with 3.3 nM of αPD-L1-NP or control treatments for 24 hours. Subsequently, the cells were washed with PBS, fixed with 4% paraformaldehyde (PFA) for 15 minutes, and washed with PBS three times. After permeabilization with 0.1% Triton X-100 for 15 minutes at room temperature, the cells were blocked in 10% goat serum in PBS for 1 hour at room temperature and incubated

with a 1:200 dilution of mouse PD-L1/CD274 monoclonal antibody (Proteintech, #66248-1) for 2 hours at room temperature. After washing with PBS, the cells were incubated with a 1:1000 dilution of Alexa Fluor 647-conjugated goat anti-mouse IgG (Abcam, #ab150115) for 1 hour at room temperature. Following another round of washing with PBS, the cells were stained with DAPI (2 μg mL^{-1}) for 20 minutes. The cells were then washed with PBS and imaged using a Nikon AX R confocal microscope. DAPI was excited using a 405 nm laser and visualized at 427~475 nm; Alexa Fluor 647 was excited using a 639 nm laser and visualized at 662~737 nm.

Immunofluorescence of PD-L1 in tumor sections

The collected tumor tissues were embedded in optimal cutting temperature (OCT) compound (Sakura, #4583), then snap-frozen in liquid nitrogen and Cryo-sectioned into 4-μm-thick slices. The sections were immersed in ethanol for 10 minutes and washed three times with PBS for 5 minutes each. Antigen recovery was performed by heating the sections in the Tris-EDTA retrieval solution (HaoKebio, HKI0004) at 95-100 ºC for 10 minutes. After cooling to room temperature, the sections were washed three times with PBS for 5 minutes each and then treated with 3% H_2O_2 for 25 minutes at room temperature to inactivate endogenous peroxidase, followed by three washes with PBS. Subsequently, the sections were blocked with 3% BSA for 30 minutes, then incubated overnight at 4 ºC with αPD-L1 antibody (Abcam, ab213480). After three washes with PBS, the sections were incubated with polyHRP-conjugated secondary antibody (HaoKebio, HKI0029) for 50 minutes, washed three times with PBS and then stained with Fare signal amplifier (Flare 570, HaoKebio, HKI0015) for 3-5 minutes. Following three washes with PBS, the sections were stained with DAPI for 8 minutes and washed three times with PBS. The slides were sealed with Antifade Mounting Medium (HaoKebio, HKI0007-1) and imaged using a Nikon AX R confocal microscope. DAPI was excited using a 350 nm laser and visualized at 420 nm; Flare 570 was excited using a 550 nm laser and visualized at 570 nm.

MMP2 content analysis by Western blot

Cells were plated in a 6-well plate (200,000 cells per) and incubated in full medium for 24 hours. Subsequently, the medium was replaced with a serum-free medium and the cells were cultured for an additional 24 hours. Afterward, the cells were incubated with αMMP2-NP or control treatment for 12 hours. The medium was collected and concentrated with a Millipore centrifugal filter unit (3 kDa MWCO). The protein concentration in the concentrated samples was determined using the BCA assay. Subsequently, the samples were applied to Western blot analysis or gelatin zymography as described below.

Production of ^{EGFP}EVs

Procedure for generating ECDHCC1-PalmGRET cells. ECDHCC1 cells were transfected with pLenti-PalmGRET-EGFP plasmids (Addgene plasmid #158221) using a lentiviral vector. The engineered cell line was designated as ECDHCC1-PalmGRET and maintained in high-glucose DMEM supplemented with 10% FBS and 1% penicillin/streptomycin.

Procedure for isolating ^{EGFP}EVs. Prior to EV isolation, ECDHCC1-PalmGRET cells were plated at a density of 1000,000 cells per cm^2 and cultured in high-glucose DMEM supplemented with 10% EV-depleted FBS. To obtain the conditioned medium, the cells were collected at 80–90% confluency and subjected to sequential centrifugation at 300 g and 2000 g at 4 °C for 10 minutes to remove cells and cell debris, respectively. The conditioned medium was then ultracentrifuged at 100,000 g at 4 °C for 70 minutes to pellet the ^{EGFP}EVs. The ^{EGFP}EV pellet was resuspended in double-0.10 μm-filtered PBS. Fresh ^{EGFP}EV aliquots were stored at −80 °C or subjected to Nanoparticle Tracking Analysis for measuring the particle concentration.

Photobleaching test of ^{EGFP}EVs

ECDHCC1-PalmGRET cell debris were transferred into a Nunc glass bottom dish and imaged locally with continuous laser scanning using a confocal microscope. EGFP was excited using a 488 nm laser and visualized at 505~544 nm.

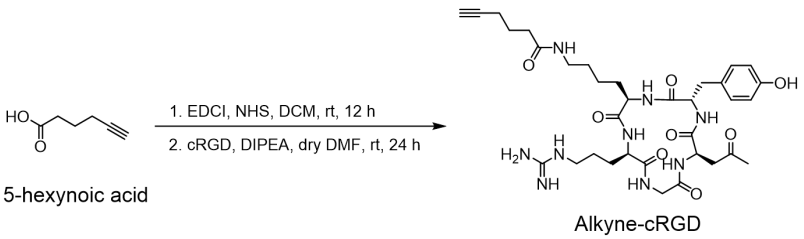
Fluorescence analysis of EV endocytosis and exocytosis

Cells were plated in a 6-well plate (200,000 cells per well) one day before the experiment and then incubated with EVs (1.3×10^8 particles mL^{-1}), Annexin-V (0.4 μg mL^{-1}) + EVs, NPs (50 μg mL^{-1}) + EVs, or Annexin-V-NPs (Annexin-V-equiv. 0.4 μg mL^{-1}, NP-equiv. 50 μg mL^{-1}) + EVs for 8 hours. Subsequently, the culture medium was collected and the cells were further cultured in fresh serum-free medium for 10 hours. The fluorescence intensity of the collected medium was measured using a microplate spectrophotometer for analysis of ^{EGFP}EV endocytosis. After 10-hour incubation, the serum-free medium was also collected for analysis of ^{EGFP}EV exocytosis. EGFP was excited by 488 nm laser and visualized at 540 nm.

Synthetic procedures

Synthesis of Biotin-cRGD. Biotin-cRGD was synthesized following the previously described methods with modifications[7].

1) Synthesis of alkyne-cRGD



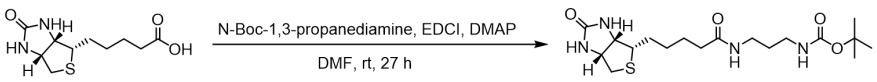
5-hexynoic acid

Alkyne-cRGD

The synthesis of alkyne-cRGD involves two reactions. First, EDCI (920 mg, 4.8 mmol) and NHS (552 mg, 4.8 mmol) were added to the solution of 5-hexynoic acid (449 mg, 4.0 mmol) in DCM (20 mL). The reaction was allowed to stir at room temperature for 12 hours, followed by removal of solvent under vacuum. The mixture was resuspended in water (10 mL) and extracted with DCM three times. The combined organic phase was dried over anhydrous Na_2SO_4, filtered, and concentrated under vacuum to give the intermediate product. The intermediate product (62 mg, 0.23 mmol) was then mixed with cRGD (11.4 mg, 0.014 mmol) and DIPEA (15.5 μL, 0.09 mmol) in anhydrous DMF (0.5 mL). After stirring at room temperature under N_2 atmosphere for 24 hours, the solvent was removed under vacuum and the crude product was purified by RP-HPLC (5-45 min: 0-100% ACN, t_R = 17.5 min) to afford alkyne-cRGD. MS (ESI)

m/z: calcd. for $C_{33}H_{47}N_9O_9$ $[M+H]^+$ 714.34, found 714.10.

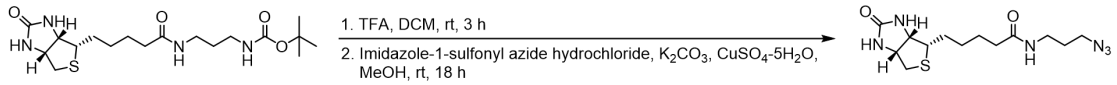
2) Synthesis of Biotin-L1- NHBoc



Biotin Biotin-L1-NHBoc

EDCI (2.15 g, 2.24 mmol) was added to the mixture of biotin (2.17 g, 1.78 mmol), N-Boc-1,3-propanediamine (1.54 g, 1.77 mmol), and DMAP (0.13 g, 0.21 mmol) in DMF (80 mL). After stirring at room temperature for 27 hours, the solvent was evaporated under vacuum and the residue was purified by flash column chromatography on silica to afford BMS8-L1-NHBoc as a white solid (3.28 g, 93%). ^{1}H NMR (400 MHz, Methanol-d_4) δ 4.49 (dd, J = 7.9, 4.9 Hz, 1H), 4.31 (dd, J = 8.0, 4.5 Hz, 1H), 3.24-3.18 (m, 3H), 3.06 (t, J = 6.8 Hz, 2H), 2.93 (dd, J = 12.8, 5.0 Hz, 1H), 2.70 (d, J = 12.7 Hz, 1H), 2.20 (t, J = 7.4 Hz, 2H), 1.78-1.55 (m, 6H), 1.47-1.42 (m, 11H).

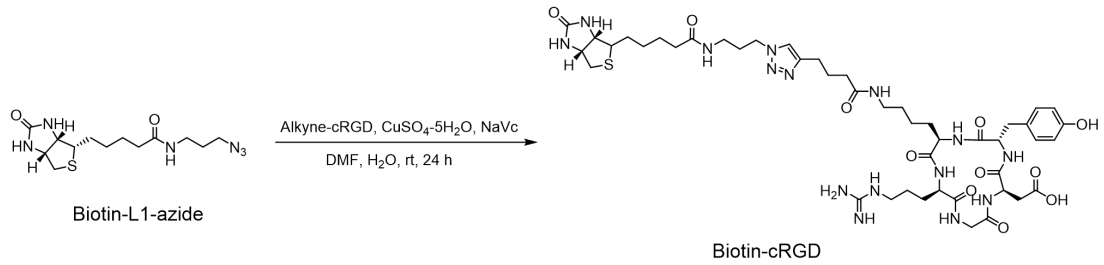
3) Synthesis of Biotin-L1- azide



Biotin-L1-NHBoc Biotin-L1-azide

Biotin-L1-NHBoc (1.60 g, 4 mmol) was dissolved in a solution of DCM (34 mL) and TFA (17 mL). After stirring at room temperature for 2 hours, the solvent was evaporated under vacuum and the intermediate product was redissolved in 20 mL of MeOH, followed by the addition of imidazole-1-sulfonyl azide hydrochloride (1.01 g, 4.8 mmol), K_2CO_3 (4.42 g, 32 mmol), and $CuSO_4 \cdot 5H_2O$ (0.04 g, 0.16 mmol). The reaction was allowed to stir at room temperature for 18 hours. Subsequently, the mixture was filtered through celite and the filtrate was concentrated under vacuum. The residue was purified by flash column chromatography on silica to afford Biotin-L1-azide as a white solid (1.29 g, 98%). ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.23 (s, 1H), 4.49 (dd, J = 7.9, 4.8 Hz, 1H), 4.30 (dd, J = 7.9, 4.5 Hz, 1H), 3.81 (s, 1H), 3.36 (t, J = 6.7 Hz, 2H), 3.27-3.16 (m, 3H), 2.93 (dd, J = 12.8, 5.0 Hz, 1H), 2.71 (d, J = 12.7 Hz, 1H), 2.21 (t, J = 7.4 Hz, 2H), 1.82-1.54 (m, 6H), 1.49-1.40 (m, 2H).

4) Synthesis of biotin-cRGD



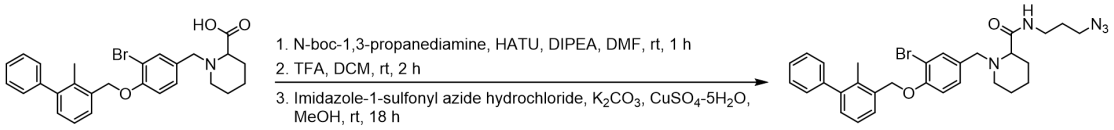
Biotin-L1-azide

Biotin-cRGD

An aqueous solution (1.35 mL) containing $CuSO_4 \cdot 5H_2O$ (1.9 mg, 0.008 mmol) and NaVc (10 mg, 0.05 mmol) were added to the mixture of Biotin-L1-azide (5.3 mg, 0.016 mmol) and alkyne-cRGD (9 mg, 0.013 mmol) in DMF (0.45 mL). After stirring at room temperature for 24 hours, the mixture was directly purified by RP-HPLC (5-35 min: 0-50% ACN, t_R = 20.6 min) to afford biotin-cRGD as a white powder after free-drying. MS (ESI) m/z: calcd. for $C_{46}H_{69}N_{15}O_{11}S$ $[M+CF_3COO]^-$ 1152.50, found 1152.20.

Synthesis of BMS-L1-RGD

1) Synthesis of BMS8-L1-azide

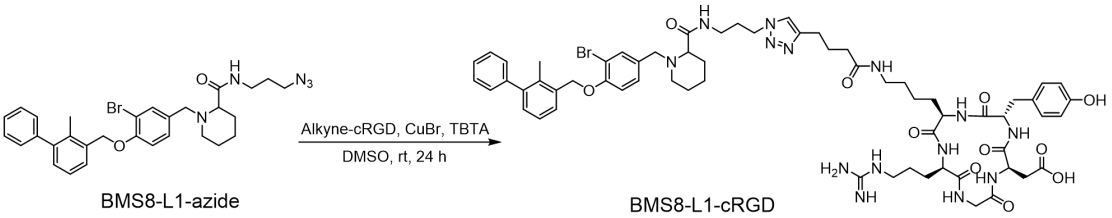


BMS-8

BMS8-L1-azide

The synthesis of BMS8-L1-azide involves three reactions. First, DIPEA (87 μL, 0.50 mmol) was added to a mixture of BMS-8 (50 mg, 0.10 mmol), N-boc-1,3-propanediamine (19 mg, 0.11 mmol), and HATU (38 mg, 0.10 mmol) in anhydrous DMF (4.0 mL). The solution was stirred at room temperature under N_2 atmosphere for 1 hour and then concentrated under vacuum. The intermediate product was obtained by flash column chromatography on silica and then reacted with TFA (320 μL) in DCM (1.3 mL) at room temperature for 2 hours. After removal of TFA under vacuum, the residue was redissolved in MeOH, followed by the addition of imidazole-1-sulfonyl azide hydrochloride (63 mg, 0.30 mmol), K_2CO_3 (4 mg, 0.60 mmol), and $CuSO_4 \cdot 5H_2O$ (0.8 mg, 0.003 mmol). The reaction was allowed to stir at room temperature for 18 hours and then concentrated under vacuum. The mixture was purified by flash column chromatography on silica to afford BMS8-L1-azide as a white solid (41 mg, 70%). MS (ESI) m/z: calcd. for $C_{30}H_{34}BrN_5O_2$ $[M+H]^+$ 576.20, found 576.00.

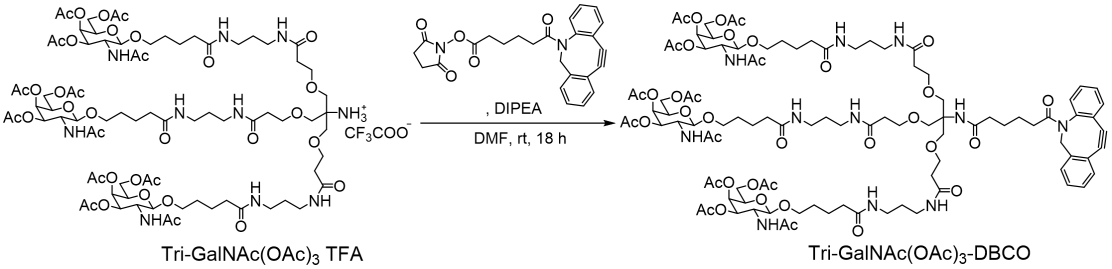
2) Synthesis of BMS-L1-RGD.



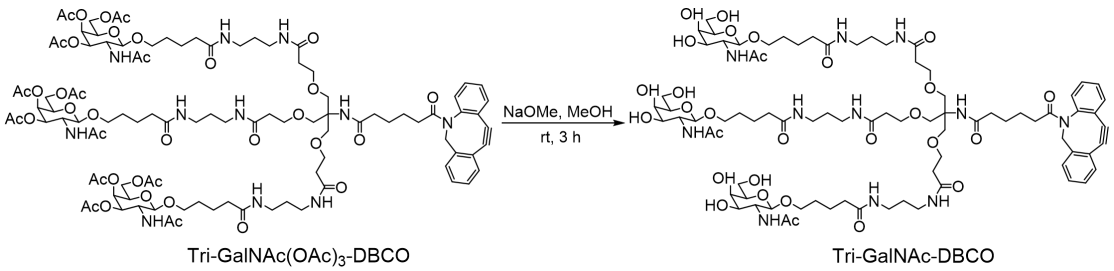
BMS8-L1-cRGD was synthesized following the previously described methods with modification[7]. BMS8-L1-azide (6.44 mg, 11.2 μmol), alkyne-cRGD (8.04 mg, 11.2 μmol), CuBr (3.2 mg, 22.4 μmol), and TBTA (12 mg, 22.4 μmol) were dissolved in 0.5 mL DMSO under N_2 atmosphere. The solution was stirred at room temperature for 24 hours, and the mixture was directly purified by RP-HPLC (5-23 min: 0-45% ACN, 23-33 min: 45% ACN, t_R = 26.5 min) to afford BMS-L1-cRGD as a white powder after free-drying. MS (ESI) m/z: calcd. for $C_{63}H_{81}BrN_{14}O_{11}$ $[M+H]^+$ 1291.54, found 1291.80; $[M+2H]^{2+}$ 646.28, found 646.40; $[M+2K+Na]^{3+}$ 463.82, found 463.40.

Synthesis of αPD-L1-GalNAc

1) Synthesis of Tri-GalNAc(OAc)$_3$-DBCO

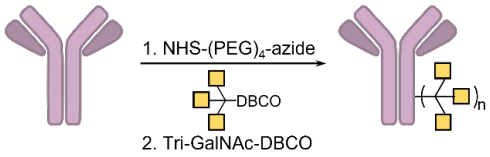


Tri-GalNAc(OAc)$_3$-DBCO was synthesized following the previously described methods with modification[2]. DIPEA (35 μL, 0.2 mmol) was added to the mixture of Tri-GalNAc(OAc)$_3$ TFA (100 mg, 0.053 mmol) and NHS-C_6-DBCO (22 mg, 0.05 mmol) in DMF (600 μL). After stirring at room temperature for 18 hours, the solvent was evaporated under vacuum and the residue was purified by flash column chromatography on silica to afford Tri-GalNAc(OAc)$_3$-DBCO as a white solid (43 mg, 37%). HRMS (ESI) m/z: calcd. for $C_{100}H_{145}N_{11}O_{38}$ $[M+2H]^{2+}$ 1055.4966, found 1055.4991; $[M+3H]^{3+}$ 704.0002, found 704.0006.



Tri-GalNAc(OAc)$_3$-DBCO (60.6 mg, 0.03 mmol) was dissolved in methanol (0.58 mL) and 0.5 M of sodium methoxide in MeOH was added until pH reached 9~10. The reaction was allowed to stir at room temperature for 3 hours. Upon completion, the reaction was quenched with formic acid and concentrated under vacuum to give Tri-GalNAc-DBCO. The product was used directly without further purification.

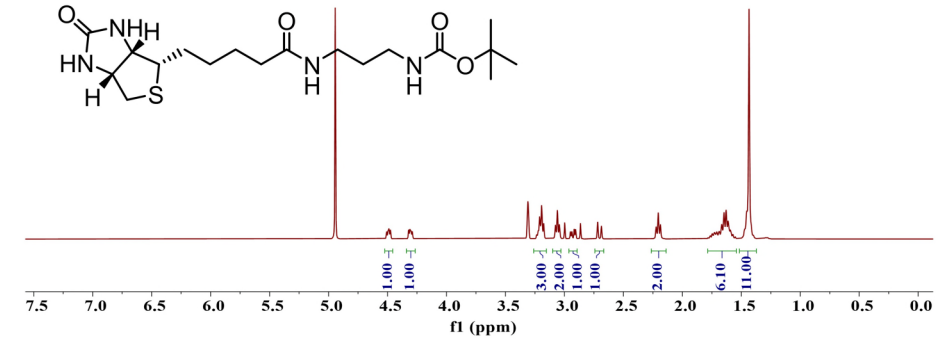
2) Synthesis of αPD-L1-GalNAc

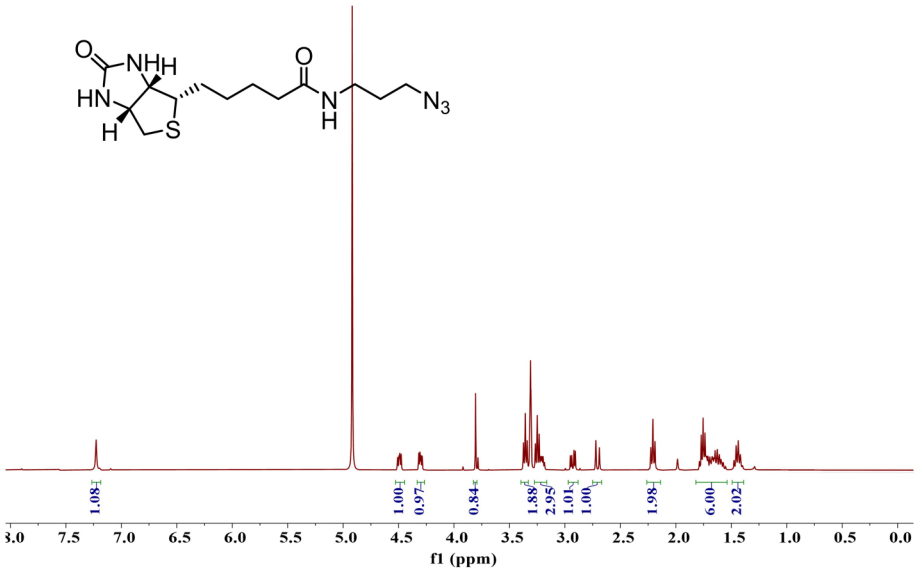


Antibody azide labeling. αPD-L1 (BioXCell, BE0101) was mixed with 25 equiv. of NHS-(PEG)$_4$-azide (20 mg mL^{-1} in DMSO). After incubation overnight at room temperature, the mixture was filtered through a 30K ultrafiltration spin column to yield αPD-L1-(PEG)$_4$-azide.

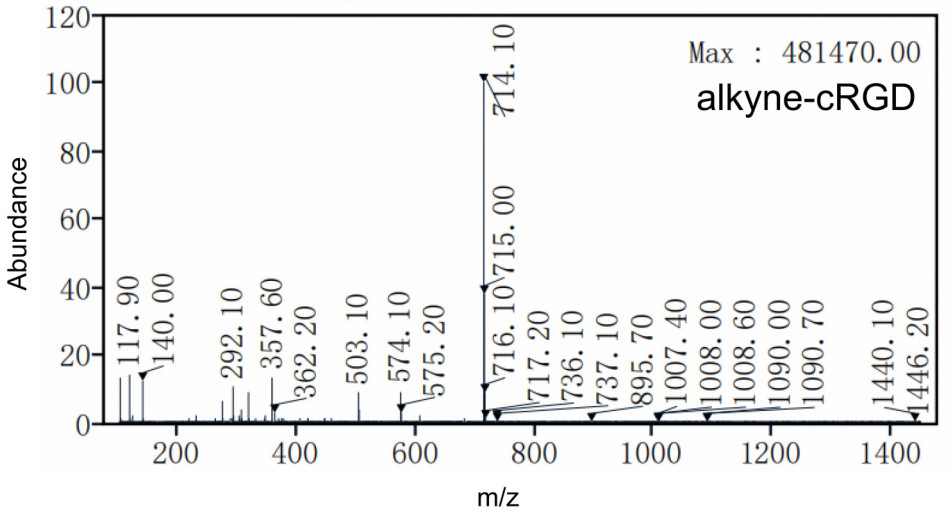
Antibody GalNAc labeling. Tri-GalNAc-DBCO (100 equiv.) was weighed into an Eppendorf tube, and a solution of αPD-L1-(PEG)$_4$-N$_3$ (2 mg mL^{-1}) was added. The solution was allowed to incubate at room temperature in the dark for 3 days and was filtered through a 30K ultrafiltration spin column to give αPD-L1-GalNAc.

^{1}H NMR spectra





MS spectra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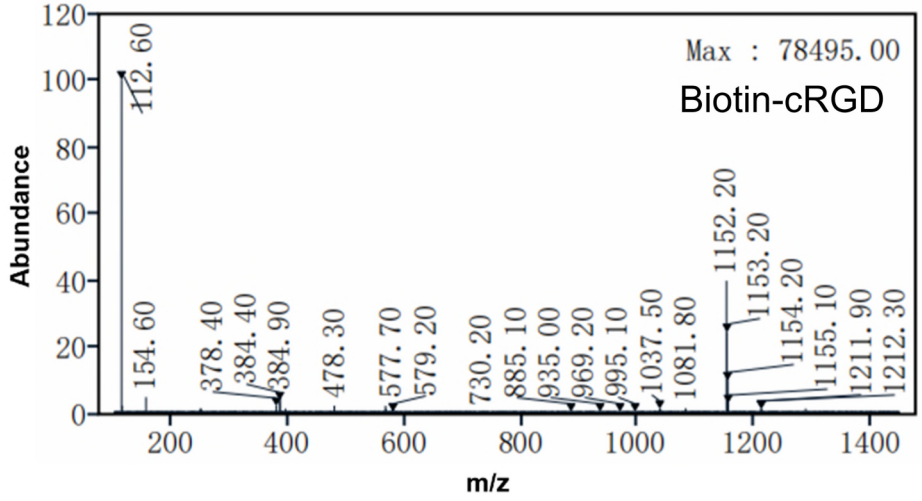
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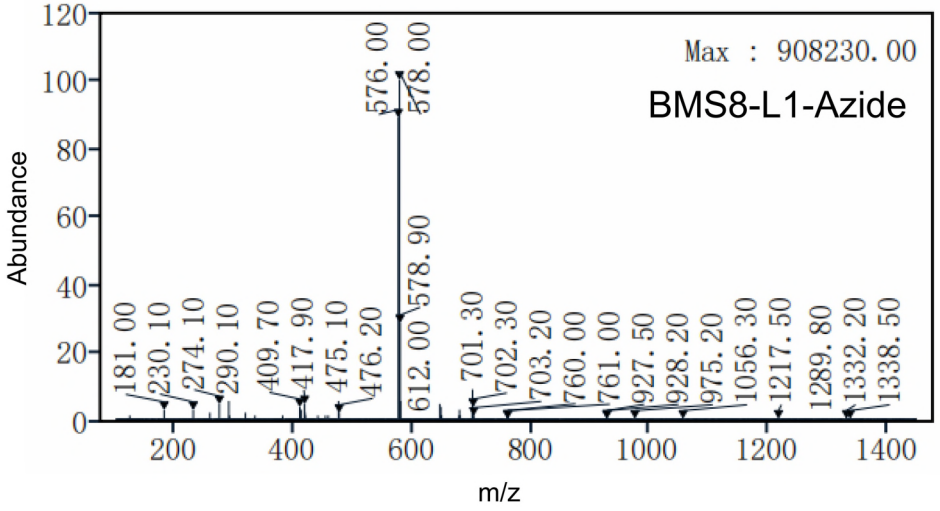
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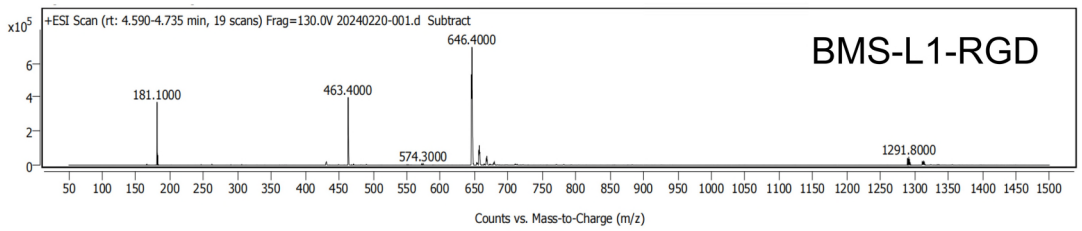
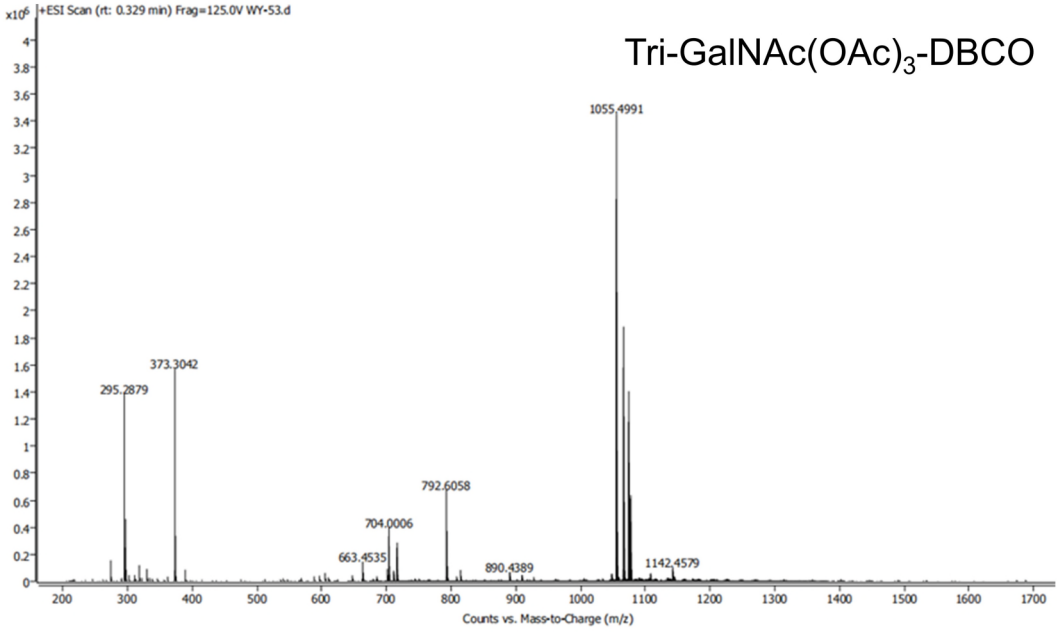
274.10
290.10
409.70
417.90
475.10
476.20
701.30
702.30
703.20
760.00
761.00
927.50
928.20
975.20
1056.30
1217.50
1289.80
1332.20
1338.50

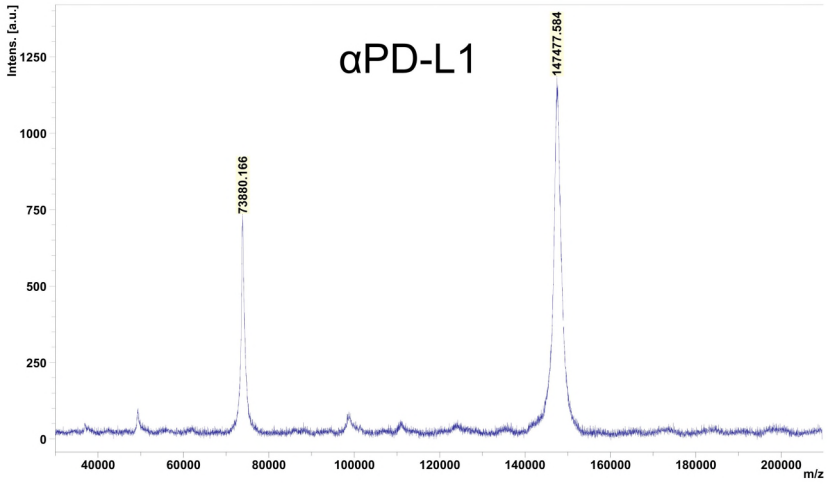


BMS-L1-RGD



Tri-GalNAc$(OAc)_3$-DBCO

MALDI-TOF-MS spectra



Target

Target type	0280784
Target serial number	1002475
Position	M4

Laser

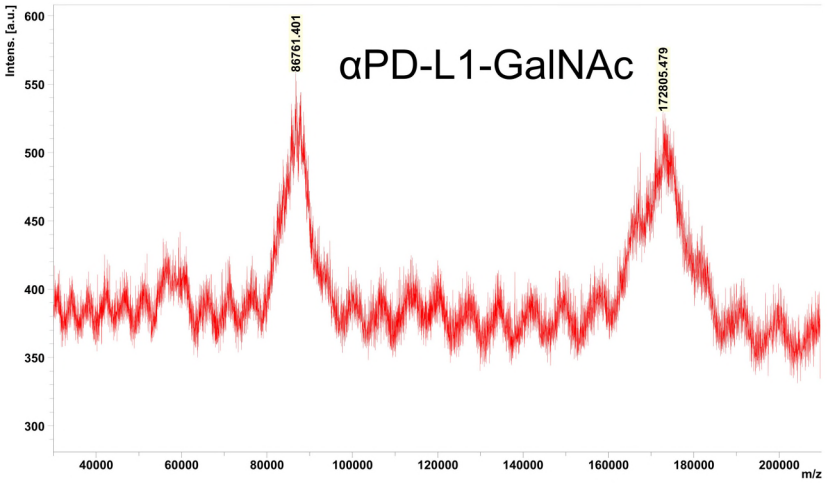
Laser beam attenuation	0
Laser beam focus	8
Laser repetition rate	2000 Hz
Number of shots	9000

Spectrometer

positive voltage polarity	POS
PIE delay	550 ns
Ion source voltage 1	20 kV
Ion source voltage 2	18.8 kV
Lens voltage	6.8 kV
Linear detector voltage	3.051 kV
Deflection on	
Deflection mass	
Reflector voltage 1	0 kV
Reflector voltage 2	0 kV
Reflector detector volt.	2.542 kV

Instrument

Instrument type	ultraflexTOF/TOF
Instrument serial	8276601.00598
Name of computer	UTX-00598
Operator ID or name	BDAL@DE
flexControl version	flexControl 3.4.135.0
flexAnalysis version	



Target

Target type	0280784
Target serial number	1002475
Position	N4

Laser

Laser beam attenuation	0
Laser beam focus	8
Laser repetition rate	2000 Hz
Number of shots	21000

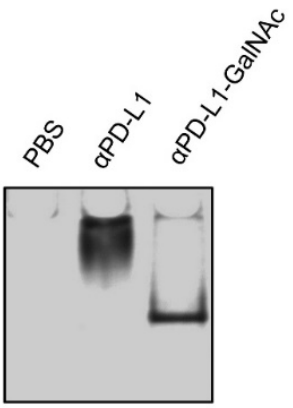
Spectrometer

positive voltage polarity	POS
PIE delay	550 ns
Ion source voltage 1	20 kV
Ion source voltage 2	18.8 kV
Lens voltage	6.8 kV
Linear detector voltage	3.051 kV
Deflection on	
Deflection mass	
Reflector voltage 1	0 kV
Reflector voltage 2	0 kV
Reflector detector volt.	2.542 kV

Instrument

Instrument type	ultraflexTOF/TOF
Instrument serial	8276601.00598
Name of computer	UTX-00598
Operator ID or name	BDAL@DE
flexControl version	flexControl 3.4.135.0
flexAnalysis version	

Native gel



Native gel electrophoresis showing the synthesis of αPD-L1-GalNAc.

References

1. Banik, S.M. et al. Lysosome-targeting chimaeras for degradation of extracellular proteins. *Nature* **584**, 291-297 (2020).
2. Ahn, G. et al. LYTACs that engage the asialoglycoprotein receptor for targeted protein degradation. *Nat Chem Biol* **17**, 937–946 (2021).
3. Huang, B. et al. Designed endocytosis-triggering proteins mediate targeted degradation. *bioRxiv* (2023) DOI: 10.1101/2023.1108.1119.553321.
4. Li, Y. et al. Covalent LYTAC enabled by DNA aptamers for immune checkpoint degradation therapy. *J Am Chem Soc* **145**, 24506–24521 (2023).
5. Pance, K. et al. Modular cytokine receptor-targeting chimeras for targeted degradation of cell surface and extracellular proteins. *Nat Biotechnol* **41**, 273–281 (2022).
6. Cotton, A.D., Nguyen, D.P., Gramespacher, J.A., Seiple, I.B. & Wells, J.A. Development of antibody-based PROTACs for the degradation of the cell-surface immune checkpoint protein PD-L1. *J Am Chem Soc* **143**, 593-598 (2021).
7. Zheng, J. et al. Bifunctional compounds as molecular degraders for Integrin-facilitated targeted protein degradation. *J Am Chem Soc* **144**, 21831–21836 (2022).
8. Marei, H. et al. Antibody targeting of E3 ubiquitin ligases for receptor degradation. *Nature* **610**, 182–189 (2022).
9. Zhang, H. et al. Covalently Engineered Nanobody Chimeras for Targeted Membrane Protein Degradation. *J Am Chem Soc* **143**, 16377–16382 (2021).
10. Yu, J. et al. Harnessing the lysosomal sorting signals of the cation-independent mannose-6-phosphate receptor for targeted degradation of membrane proteins. *J Am Chem Soc* **145**, 19107–19119 (2023).
11. Su, W. et al. Targeted degradation of PD-L1 and activation of the STING pathway by carbon-dot-based PROTACs for cancer immunotherapy. *Angew Chem Int Ed* **62**, e202218128 (2023).
12. Mikitiuk, M. et al. IGF2 peptide-based LYTACs for targeted degradation of extracellular and transmembrane proteins. *Molecules* **28**, 7519 (2023).
13. Sun, R., Meng, Z., Lee, H., Offringa, R. & Niehrs, C. ROTACs leverage signaling-incompetent R-spondin for targeted protein degradation. *Cell Chem Biol* **30**, 739-752 (2023).
14. Zhang, B. et al. Insulin-like growth factor 2 (IGF2)-fused lysosomal targeting chimeras for degradation of extracellular and membrane proteins. *J Am Chem Soc* **63**, e202316089 (2023).
15. Zhang, D. et al. Transferrin receptor targeting chimeras (TransTACs) for membrane protein degradation. *bioRxiv* (2023) DOI: 10.1101/2023.1108.1110.552782.
16. Zhu, L. et al. Conjugation with glucagon like peptide-1 enables targeted protein degradation. *Bioorg Chem* **141**, 106908 (2023).
17. Wang, Q. et al. A co-assembly platform engaging macrophage scavenger receptor A for lysosome-targeting protein degradation. *Nat Commun* **15**, 1663 (2024).