

Supplementary Information for

Biomolecular Condensates as Protein Degradation Tools for Intracellular Targets

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Materials

Epigallocatechin gallate (EGCG), iron(III) chloride hexahydrate ($\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$), 2-amino-2-(hydroxymethyl)-1,3-propanediol (Tris), dimethyl sulfoxide (DMSO), Dimethylformamide (DMF), nitric acid (HNO_3), hydrochloric acid (HCl), fluorescein isothiocyanate (FITC), Duolink® Proximity Ligation Assay kit (DUO92101) were purchased from MilliporeSigma. Minimum essential medium (MEM), Dulbecco's modified Eagle medium (DMEM), RPMI-1640 medium, Iscove's modified Dulbecco's Medium (IMDM), McCoy's 5A medium, Opti-MEM™ reduced serum medium, buffer saline (PBS), fetal bovine serum (FBS), penicillin/streptomycin, and 0.25% trypsin-ethylenediaminetetraacetic acid, Pierce Pro-Ject™ protein transfection reagent, Ni-NTA His bind resin, Texas Red™-X phalloidin (#T7471), QTracker 625 (#A10198) and QTracker 800 (#Q25071MP) were purchased from Thermo Fisher. Xfect™ protein transfection reagent was purchased from Takarabio. Oligopeptides of arginine and aspartic acid were purchased from GenScript. The Annexin V-FITC/PI apoptosis kit (#KA3805) was purchased from Abnova. Carboxyfluorescein succinimidyl ester (CFSE) was purchased from BD Bioscience (#565082). Cell Count Kit 8 (CCK8) and lactate dehydrogenase (LDH) cytotoxicity kit were purchased from Dojindo. Rabbit and mouse-specific HRP/DAB Detection IHC kit and AF488-TUNEL kit were purchased from Abcam. High-purity Milli-Q water with a resistivity of 18.2 MΩ cm was obtained from an inline Millipore water purification system.

UV-Vis absorption and fluorescence spectra measurements were conducted using a BioTek Cytation 3 cell imaging multi-mode reader. Dynamic light scattering (DLS) and ζ-potential measurements were performed on a Malvern Zetasizer Ultra. Phase contrast images were captured using an EVOS XL Core inverted microscope. Confocal laser scanning microscopy (CLSM) images were acquired using a Nikon SoRa Spinning Disk Microscope. TEM images and EDX profiles were obtained using a JEOL ARM200CF instrument operated at 200 kV. SEM images were acquired using a JEOL JSM-7900FLV operated at 10 kV; samples were coated with 10 nm osmium tetroxide using a Filgen OPC60A osmium coater. Atomic force microscopy (AFM) images were obtained using a Bruker ICON. Nanoparticle tracking analysis (NTA) was performed using NanoSight. Isothermal titration calorimetry (ITC) analysis was conducted using an Affinity ITC from TA Instruments. ICP-MS was performed on a Thermo iCAP Q. FACS was performed on a BD FACSymphony™ A5 Cell Analyzer.

Supplementary Table 1 Staining antibody lists used in the study.

Antibodies	Vendor	Catalog #	Application	Dilution
AF555 Anti-RAS	Abcam	ab308603	Flow	1:30
AF555 rabbit IgG, isotype ctrl	Abcam	ab208569	Flow	1:30
APC Anti-RAS	Abcam	ab310809	IF	1:50
AF 488 Anti-Alpha-synuclein	Abcam	ab195025	IF	1:100
Anti-Actin	CST	4970S	WB	1:1000
Anti-KRAS	CST	33197	WB	1:1000
Anti- α -Synuclein	CST	4179S	WB	1:1000
Anti-RAS (G12V mutant)	CST	51813	WB	1:1000
Anti-phospho-MEK 1/2	CST	9154	WB	1:1000
Anti-MEK 1/2	CST	9122	WB	1:1000
Anti-phospho-AKT	CST	4060S	WB	1:1000
Anti-AKT	CST	9272S	WB	1:1000
Anti-Rab5	CST	3547	WB	1:1000
Anti cRaf	Santa Cruz	sc-133	IP	1:500
Anti-IgG (Fc specific)	Invitrogen	31125	IP	1:1000
Goat anti-rabbit IgG, HRP	Invitrogen	31460	WB	1:10000
Goat anti-mouse IgG, HRP	Invitrogen	31430	WB	1:10000
Anti-Ki-67	CST	12202	IHC	1:100
Anti-RAS	CST	91054	IHC	1:100
Anti-p53	CST	2527	IHC	1:100
Anti-PSMD9	Abcam	ab181998	PLA	1:100
Anti-RAS	Abcam	Ab55391	PLA	1:200
Anti- α -Synuclein	Abcam	ab190376	PLA	1:500

Supplementary Table 2 Therapeutic antibody cargo lists used in the study.

Antibodies	Vendor	Catalog #	Concentration
Anti-RAS	Abcam	ab52939	0.5 mg/mL
Rabbit IgG, isotype ctrl	Abcam	ab172730	0.5 mg/mL
AF488 Anti-Ras	Abcam	ab308532	0.5 mg/mL
Anti-RAS (G12V mutant)	Abcam	ab313698	0.356 mg/mL
Anti- α -synuclein	Abcam	ab212184	0.645 mg/mL

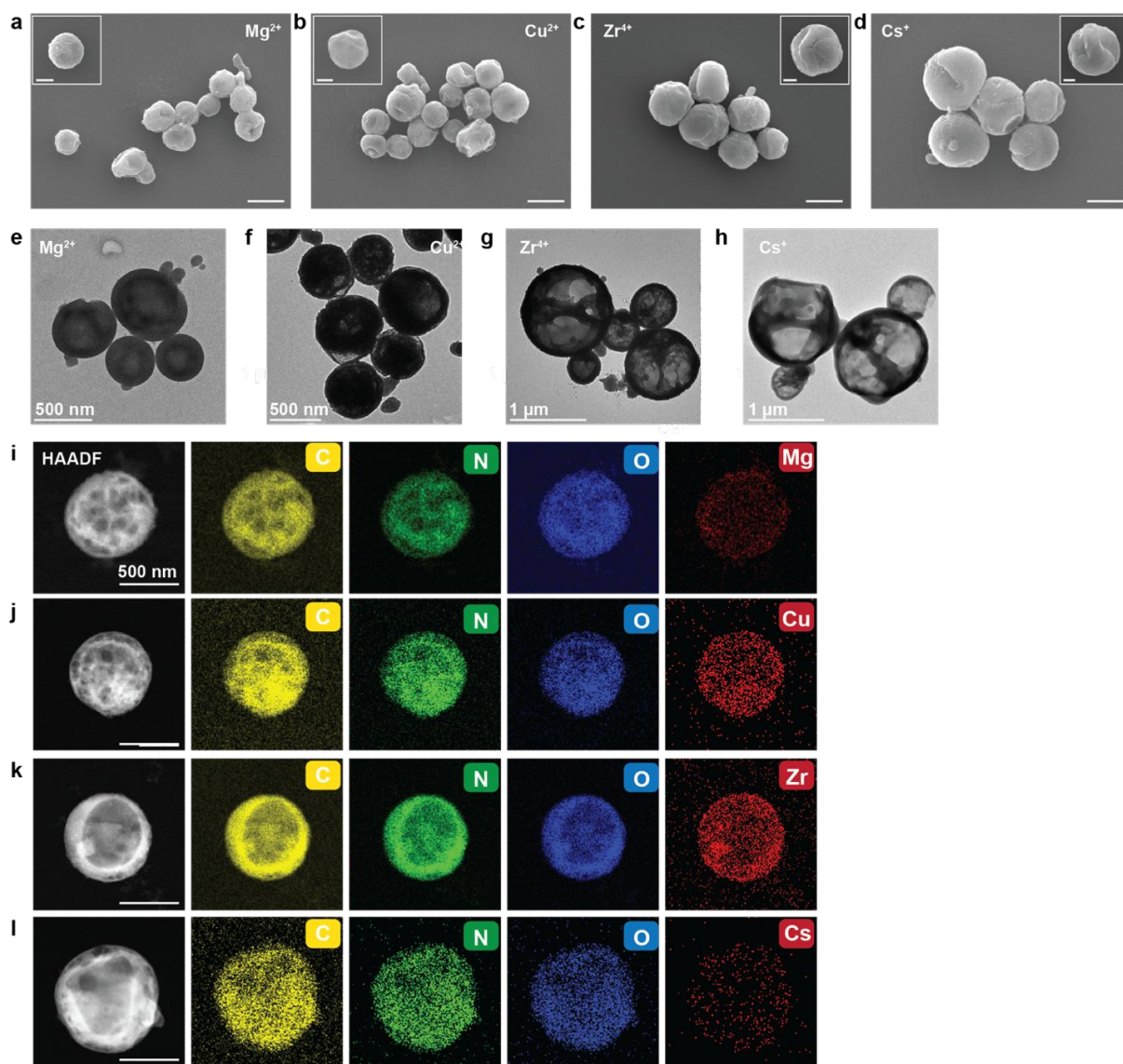
Supplementary Table 3 Molecular docking interaction analysis.

Docking pair	Binding Energy (kcal/mol)	Hydrogen Bonds	Hydrophobic Interaction	Salt Bridges	π -Cation Interaction
AAAG-19S	-4.0	Glu 65W (3.44 Å) Glu 65W (3.58 Å) Gln 68W (3.11 Å) Thr 78W (4.01 Å) Glu 110Y (3.03 Å) Lys 114Y (3.89 Å)	Arg 86W (3.69 Å) Phe 149Y (3.72 Å) Glu 159Y (3.99 Å)	Lys 65W (2.94 Å)	/
AAAG-26S	-4.3	Gln 885 (3.62 Å) Gln 885 (1.99 Å) Gln 885 (2.54 Å) Thr 895 (2.69 Å) Ser 1566 (3.08 Å) Ser 1566 (2.39 Å) Gln 1586 (2.25 Å)	Ile 1366 (3.70 Å)	/	/
AAAG- PSMD9	-4.6	Phe 304A (2.87 Å) Phe 304A (2.09 Å) Gly 491A (2.26 Å) Gly 491A (1.80 Å) Gly 491A (3.38 Å) Asn 493A (3.09 Å)	Glu 306A (3.54 Å) Asn 493A (3.76 Å)	His 301A (5.26 Å)	/
RRRG-19S	-7.1	Asn 30X (1.94 Å)	Phe 35X (3.58 Å) Arg 57X (3.70 Å)	Glu 77V (3.65 Å)	/

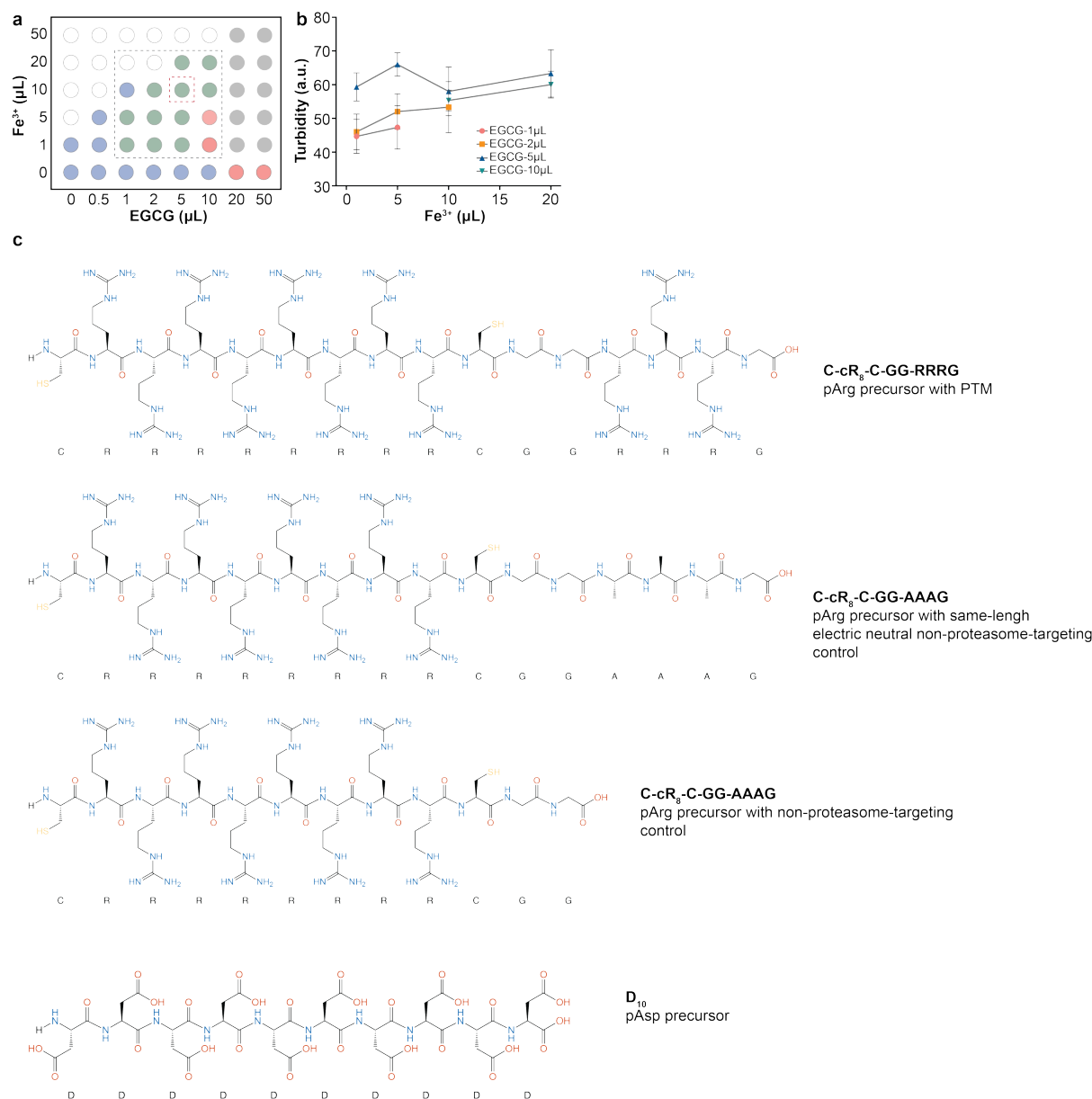
Asn 30X (2.16 Å)
 Arg 57X (2.75 Å)
 Arg 57X (2.53 Å)
 Arg 57X (2.79 Å)
 Glu 77X (2.37 Å)
 Thr 81V (2.76 Å)
 Lys 100W (2.65 Å)
 Ser 119V (2.52 Å)
 Ser 119V (2.74 Å)
 Glu 121V (1.95 Å)

RRRG-26S	-5.9	Thr 341 (3.53 Å)		
		Thr 341 (3.54 Å)		
		Thr 341 (2.29 Å)		
		Thr 341 (1.95 Å)		
		Thr 551 (2.40 Å)		
		Gly 561 (2.49 Å)		
		Ser 811 (2.92 Å)		
		Ser 811 (2.42 Å)		
		Ala 821 (1.96 Å)	Ala 821 (3.71 Å)	/
		Ala 831 (2.86 Å)	Tyr 1671 (3.71 Å)	/
		Asp 841 (2.73 Å)		
		Tyr 1671 (2.78 Å)		
		Ser 2021 (3.23 Å)		
		Ser 2021 (2.84 Å)		
		Ser 2021 (2.20 Å)		
		Ser 2031 (2.12 Å)		
		Ser 2031 (3.15 Å)		
		Ser 2031 (3.36 Å)		
Ser 2031 (2.18 Å)				

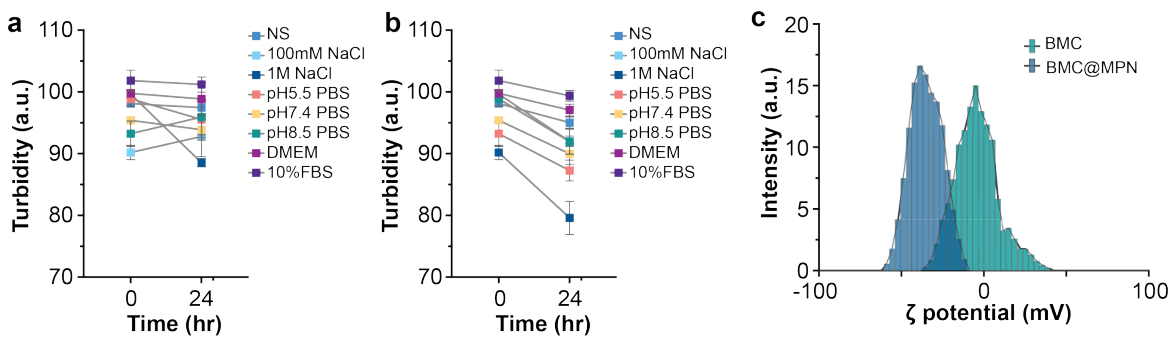
RRRG- PSMD9	-7.7	Asn 273A (2.47 Å)	Val 303A (3.69 Å)	His 301A (4.08 Å)	/
		His 301A (2.67 Å)			
		Phe 304A (2.08 Å)			
		Phe 304A (2.06 Å)			
		Gly 491A (2.50 Å)			
		Asn 493A (2.50 Å)			
		Asn 493A (2.53 Å)			
		Asn 493A (2.33 Å)			
		Gly 528A (2.48 Å)			
		Gly 528A (2.58 Å)			
		Ser 529A (2.04 Å)			
		Ser 529A (3.13 Å)			
		Lys 569A (3.07 Å)			
		Ser 789A (3.15 Å)			
		Gln 790A (2.80 Å)			
		Val 791A (2.72 Å)			



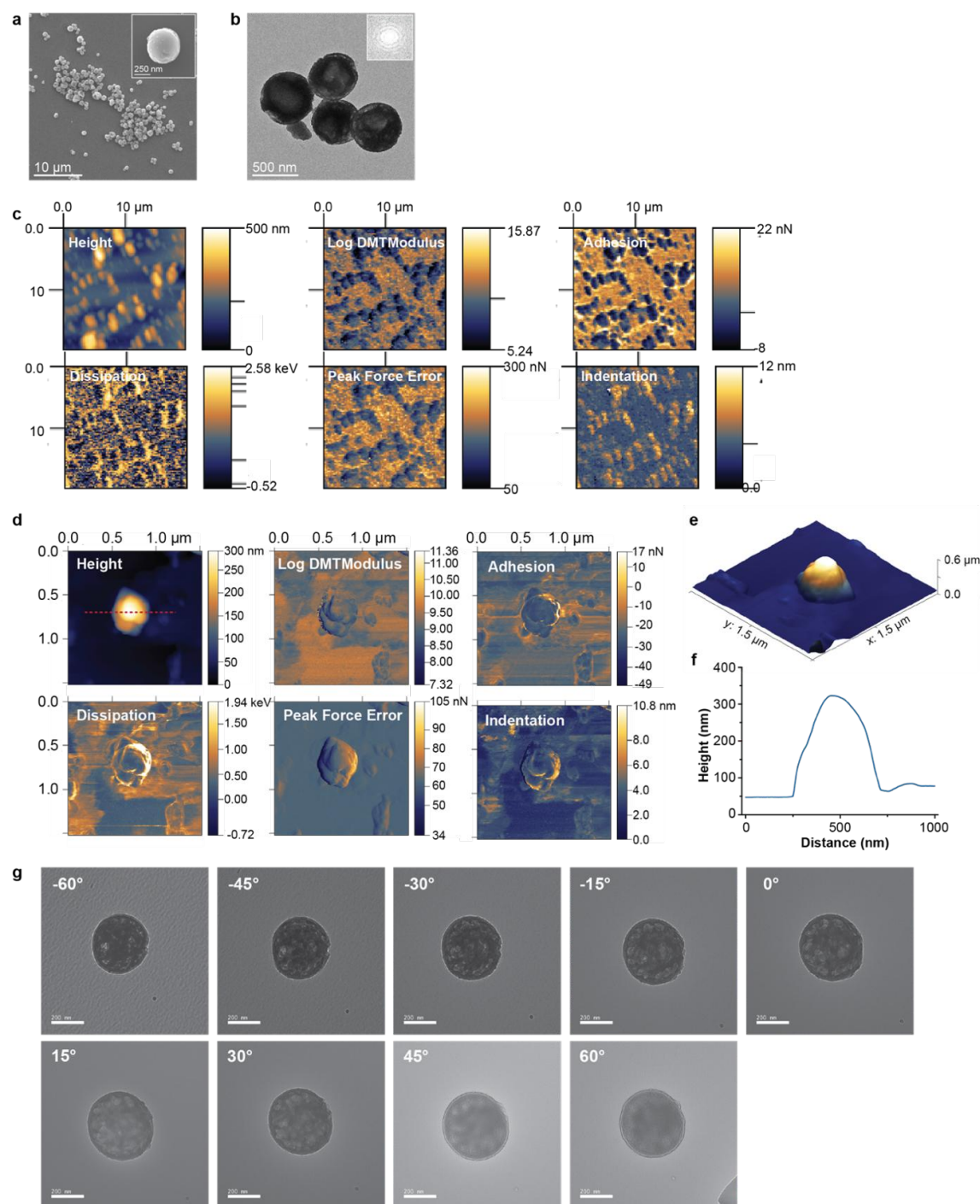
Supplementary Fig. 1 Optimization of MPN coating by tuning ion valence. SEM (a-d), scale bar = 500 nm), TEM (e-h), and EDX (i-l) images of IgG-BMC@MPN using metal ions of different valence (Mg^{2+} , Cu^{2+} , Zr^{4+} , Cs^{+}).



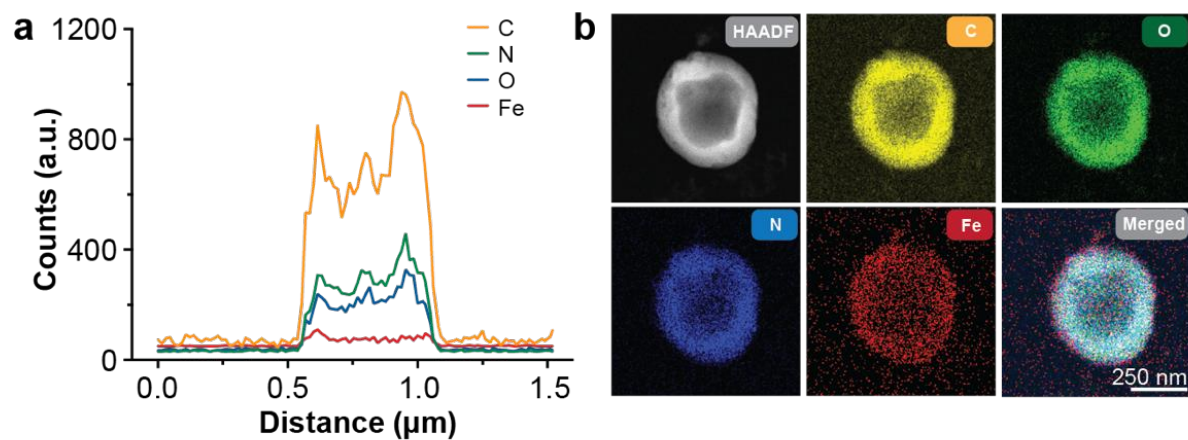
Supplementary Fig. 2 Optimization of MPN coating by tuning EGCG/Fe ratio. **a**, 1 mg/mL EGCG and 1 mg/mL FeCl₃, varying in volume, added to the 10 μL pArg condensate system are investigated for complex condensation. Empty dots: BMC disappeared after adding FeCl₃; red dots: aggregation occurred; blue dots: BMC remained after adding FeCl₃, but disappeared after being centrifuged; green dots: BMC remained after adding FeCl₃ and being centrifuged. Grey dots: data not collected due to aggregation induced by over-dose EGCG. Optimized BMC@MPN is highlighted in the dashed red box. A dashed grey box designates representative condensates for turbidity analysis. **b**, Turbidity analysis of representative condensates. Missing data points indicate aggregation or condensate disassembly. Data are shown as the mean ±SD (N=3). **c**, Full chemical structure of precursors used in this study.



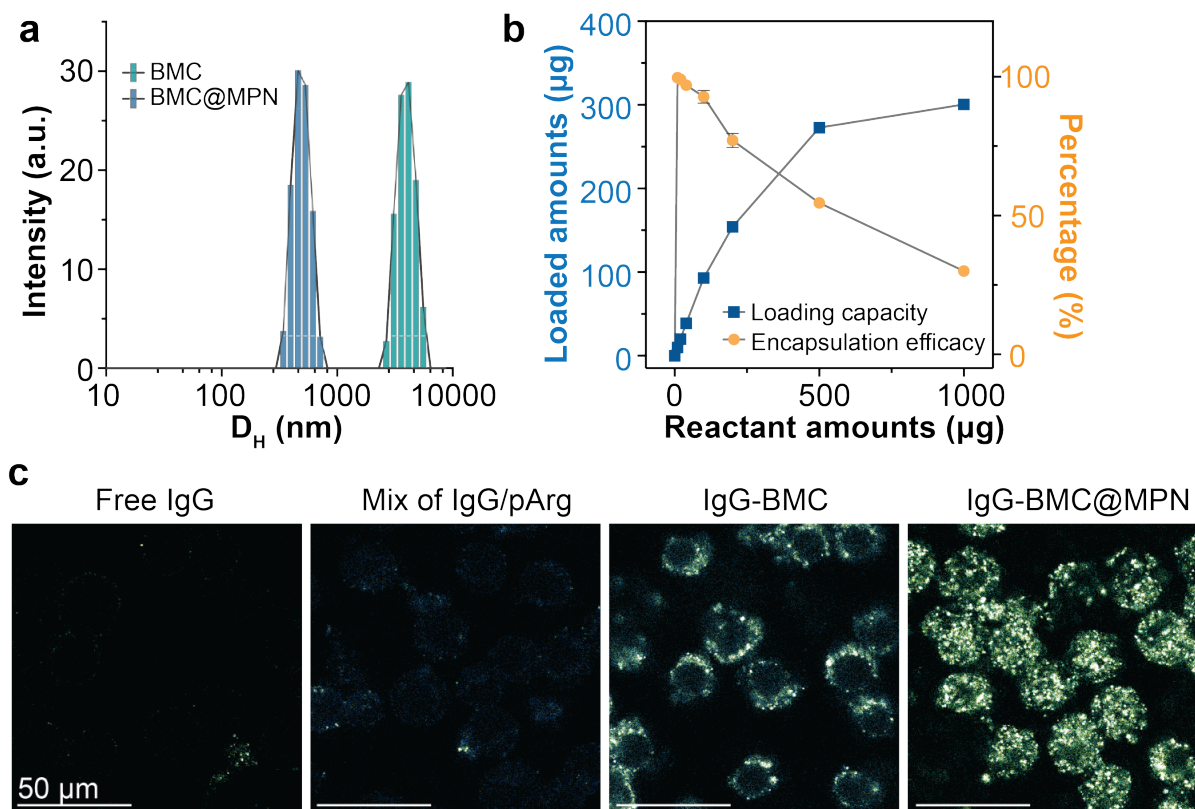
Supplementary Fig. 3 Improved colloidal stability of IgG-BMC@MPNs. **a**, Room temperature and **b**, 37°C turbidity change in solutions varying in osmotic pressure in 24 hours indicated improved osmotic stability of BMC@MPN compared to BMC. Data are shown as the mean $\pm$ SD (N=3). **c**, Zeta potential data of BMC and BMC@MPN indicating improved colloidal stability after MPN coating.



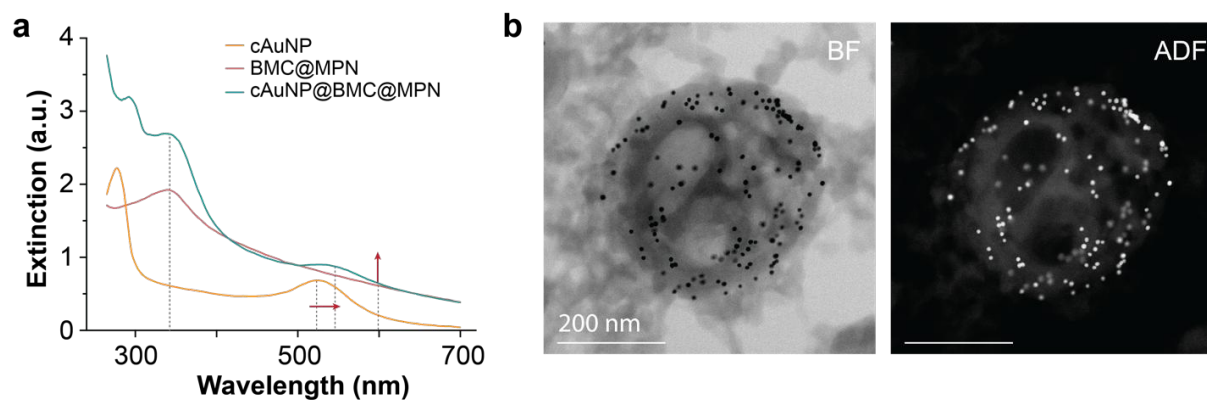
Supplementary Fig. 4 Characterization of IgG-BMC@MPNs. **a** SEM and **b** TEM images of IgG BMC@MPN. Nanomechanical properties mapping of BMC@MPN using AFM at **c** low and **d** high mag **e**, 3D height profile and **f**, line profile of BMC@MPN by AFM at indicated position (red dot line). **g**. TEM 3D images of BMC@MPN.



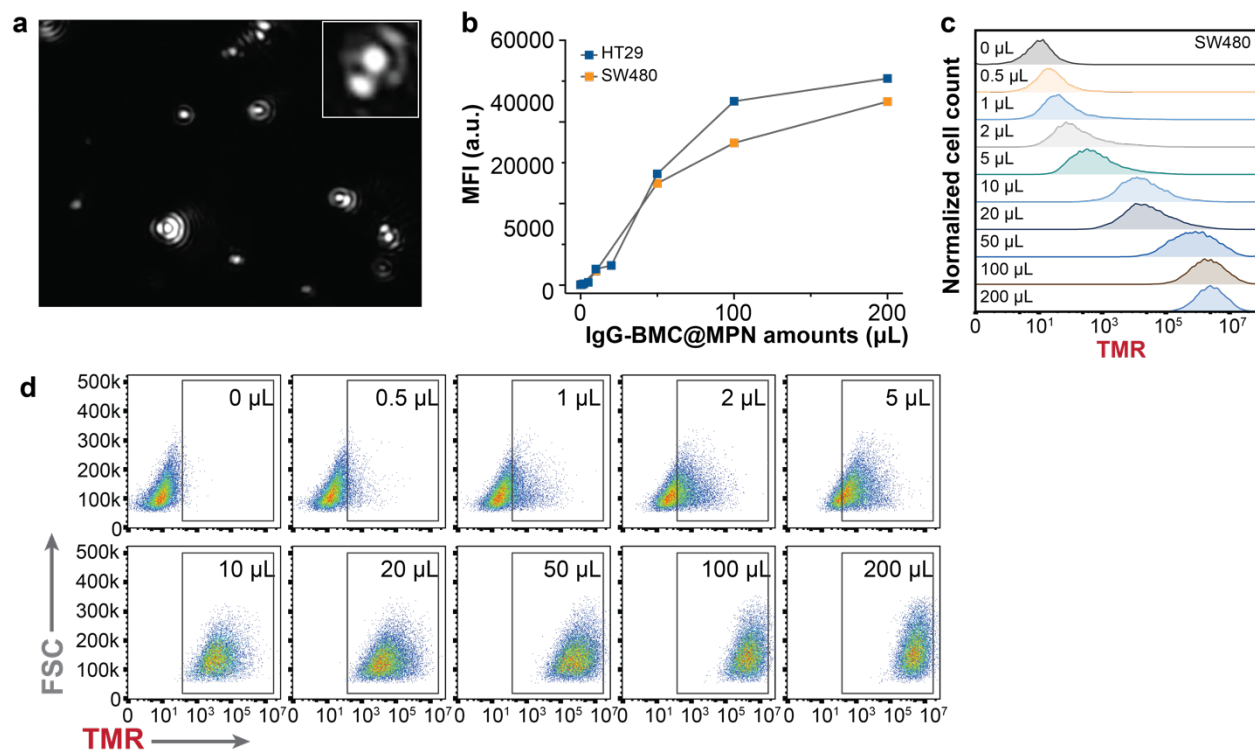
Supplementary Fig. 5 Cytoprotective function of MPN. **a**, EDX line profile of optimized IgG-BMC@MPN. **b**, EDS images of MPNs after core removal using dimethylformamide.



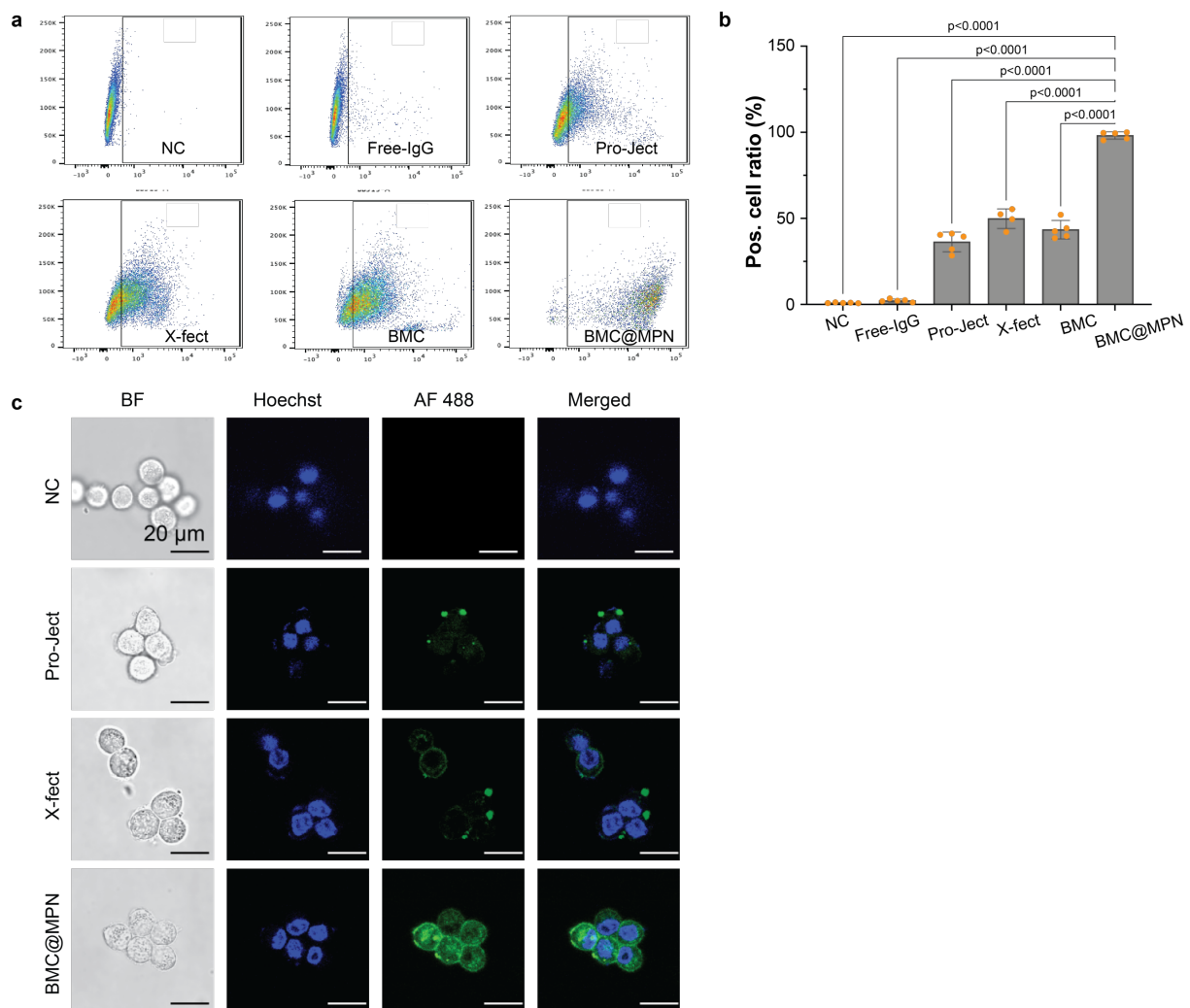
Supplementary Fig. 6 Size reduction effect of MPN coating. **a**, Droplet size was reduced after MPN coating, measured by DLS. **b**, IgG loading curve in 100 μ g IgG-BMC@MPNs. IgG-BMC@MPNs demonstrated a 3-time loading capability of IgG in weight. **c**, FITC-labeled IgG in its free form or mixed with pArg exhibited low-level cellular internalization, whereas delivery via IgG-BMCs and IgG-BMC@MPNs resulted in efficient uptake, with BMC@MPNs displaying stronger intracellular fluorescence, indicating enhanced IgG delivery compared to micro-BMCs.



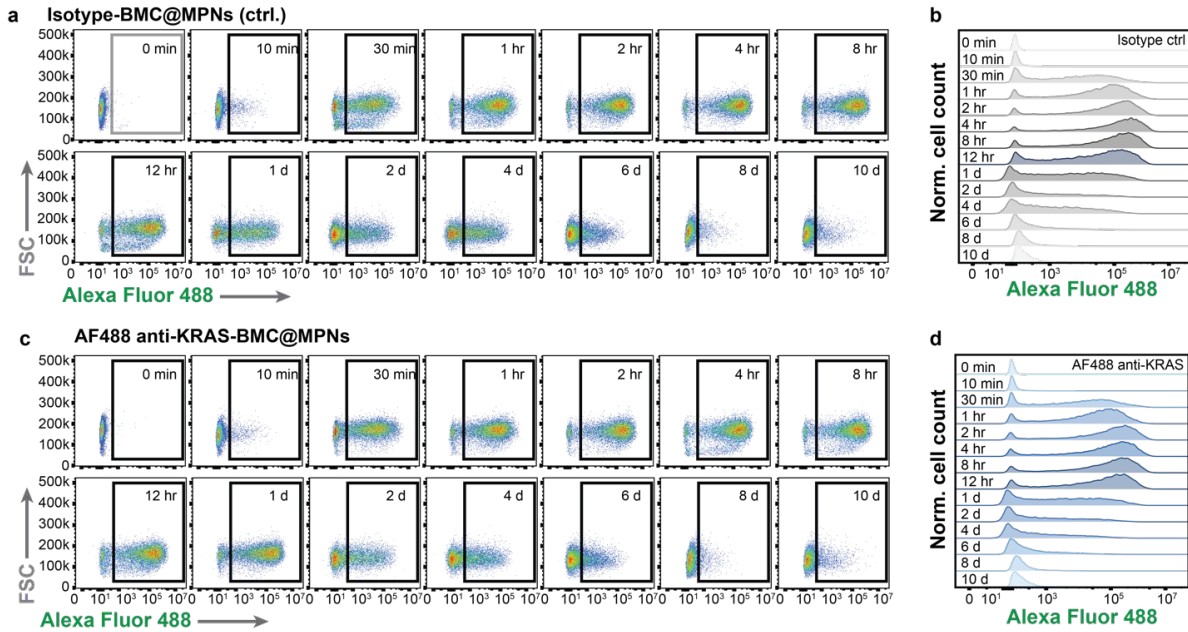
Supplementary Fig. 7 IgG release quantification. **a**, UV-vis spectra of 10 nm colloidal gold nanoparticles (cAuNP), BMC@MPN, and cAuNP IgG-loaded BMC@MPN. **b**, Bright field and annular dark field TEM images of cAuNP IgG-BMC@MPN. An average of 150 IgG–AuNPs were loaded per BMC, corresponding to approximately 1,500 IgG molecules based on the manufacturer’s specifications, to initiate KRAS degradation.



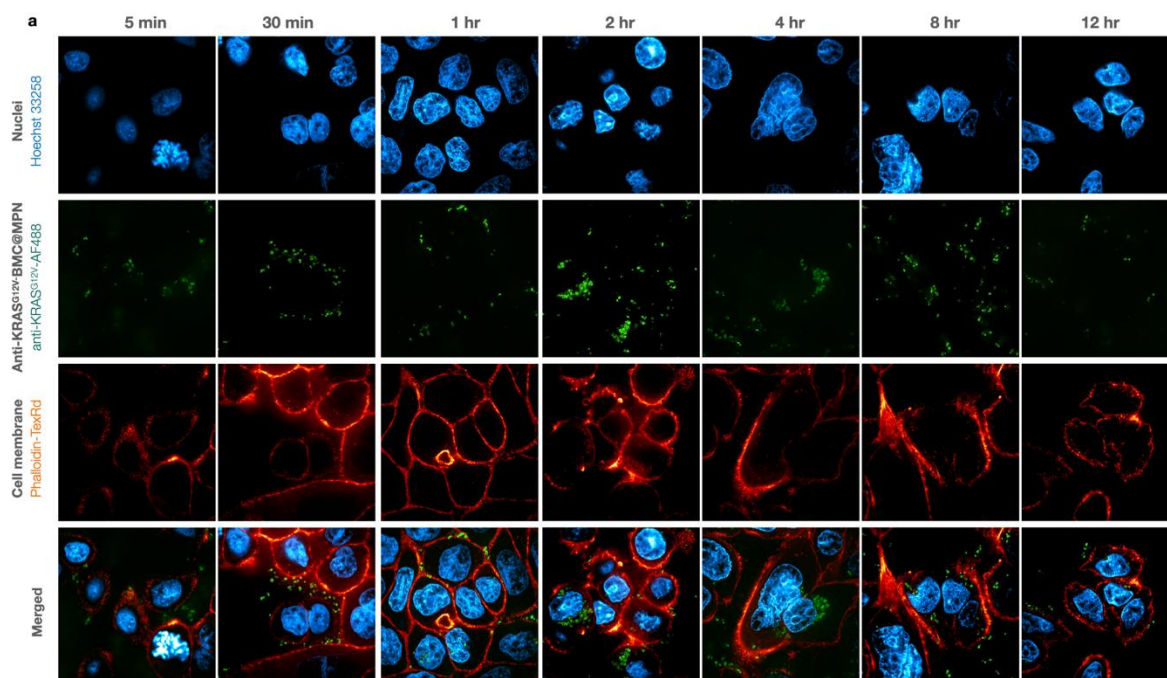
Supplementary Fig. 8 Saturation concentration for cellular uptake of IgG-BMC@MPNs. **a**, Representative NTA (nanoparticle tracking analysis) image of IgG-BMC@MPNs inflow tubing. **b**, **c**, and **d**, Uptake saturation curve quantified using FACS of TMR-IgG-BMC@MPNs in SW480 cells when incubated with different amounts of TMR-IgG-BMC@MPN.



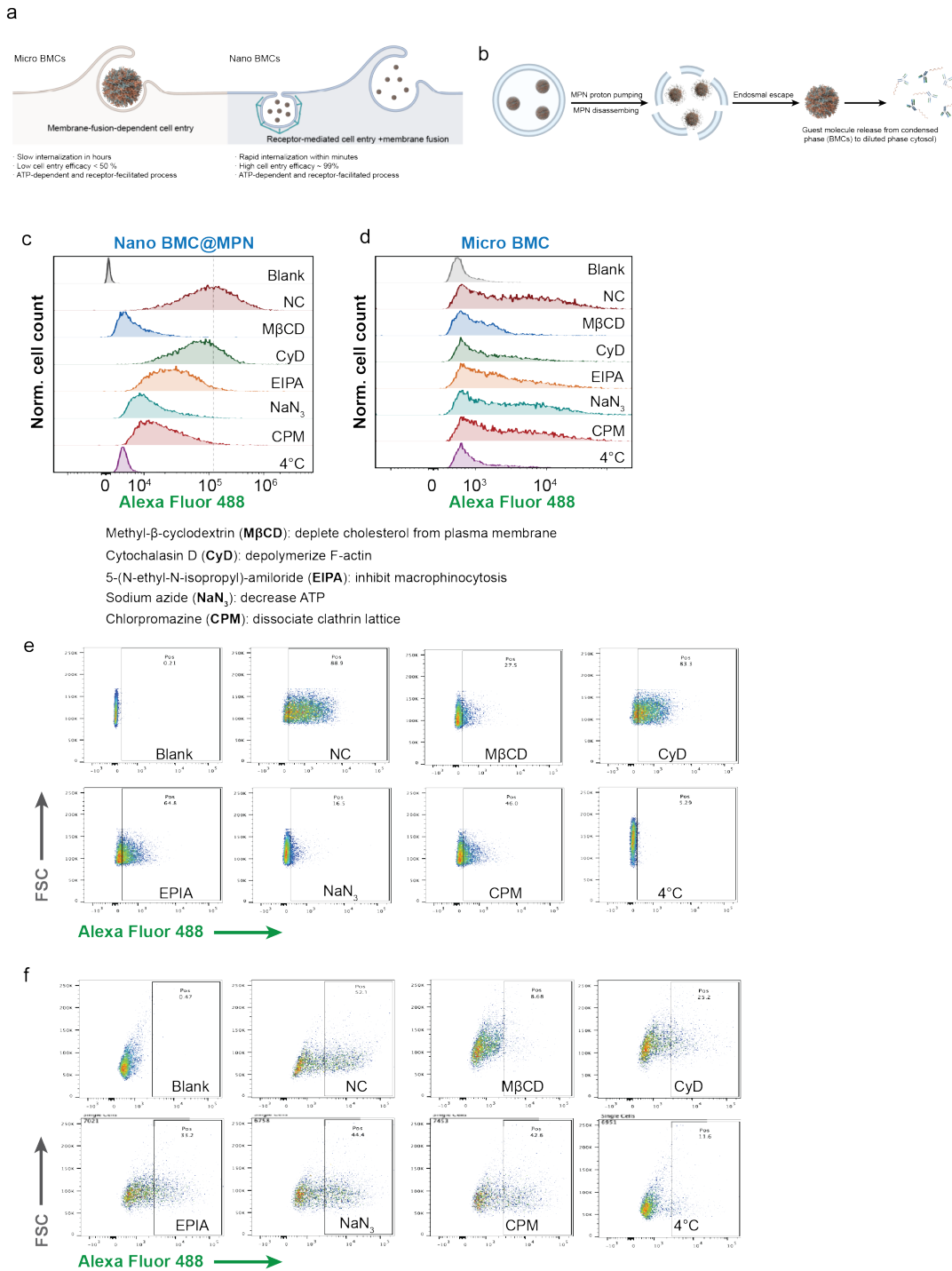
Supplementary Fig. 9 Cell uptake efficacy study of IgG-BMC@MPNs. **a**, FACS analysis, **b** quantification, and **c** microscopy images of cells transfected with Alexa Fluor 488 IgG in its free form, Pro-Ject, Xfect, or BMC@MPN in SW480 cells.



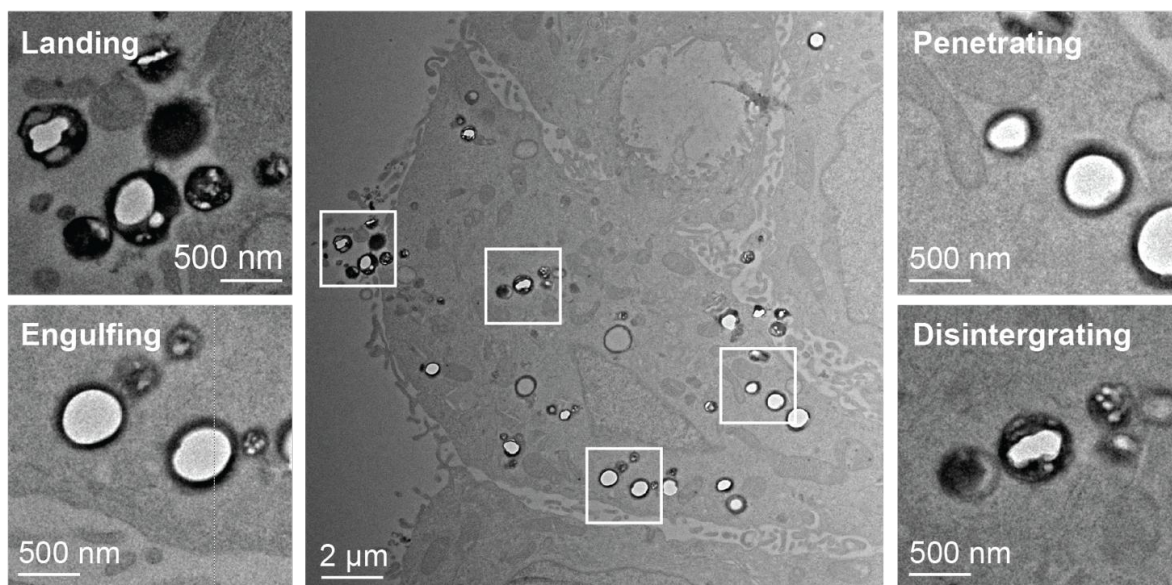
Supplementary Fig. 10 Time-dependent uptake curve of IgG-BMC@MPNs in SW480 cells. **a** and **b**, FACS analysis of AF488-conjugated non-specific IgG, denoted as isotype control, and **c** and **d**, AF488-conjugated anti-KRAS.



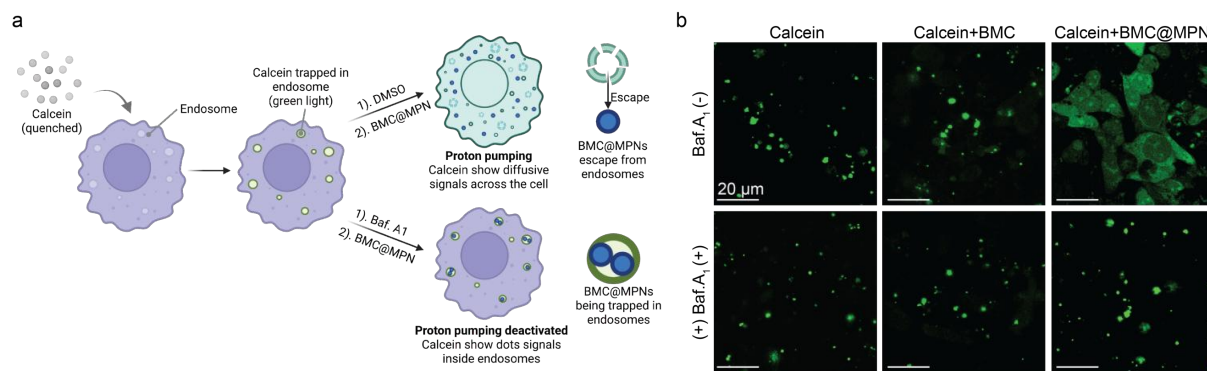
Supplementary Fig. 11 Microscopy study of bio-nano interaction. **a**, Fluorescence microscopy images of cell uptake of AF488-IgG-BMC@MPNs within 12 hr in SW480 cells.



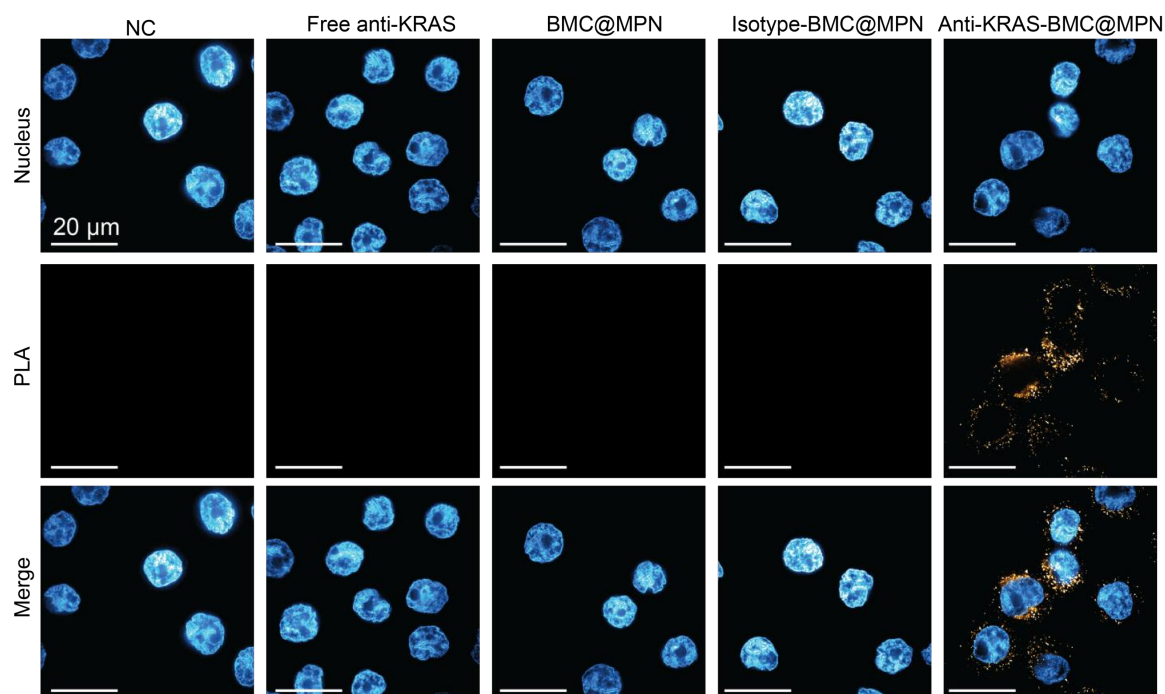
Supplementary Fig. 12 Cell internalization mechanism study. **a,b** Schematic of cell entry and endosomal escape process of BMCs. **c,e** FACS analysis of SW480 cells treated with nano AF488-IgG-BMC@MPNs or **d,f** IgG-BMCs. Two groups were included as controls: non-treated cells (blank) and cells treated with AF488-IgG-BMC@MPN without inhibitors (NC, non-treated control).



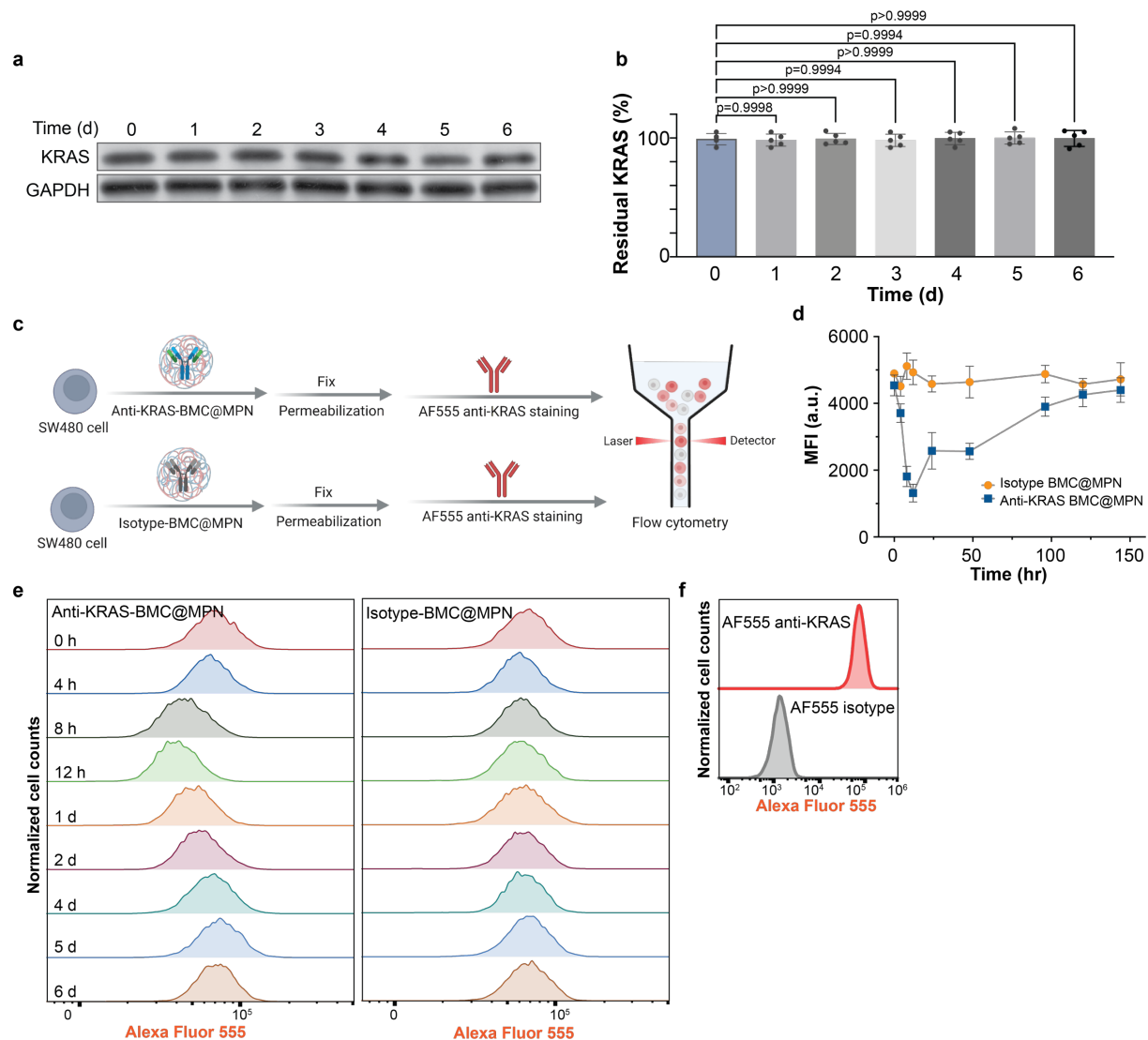
Supplementary Fig. 13 Cell internalization mechanism study by transmission electron microscope.



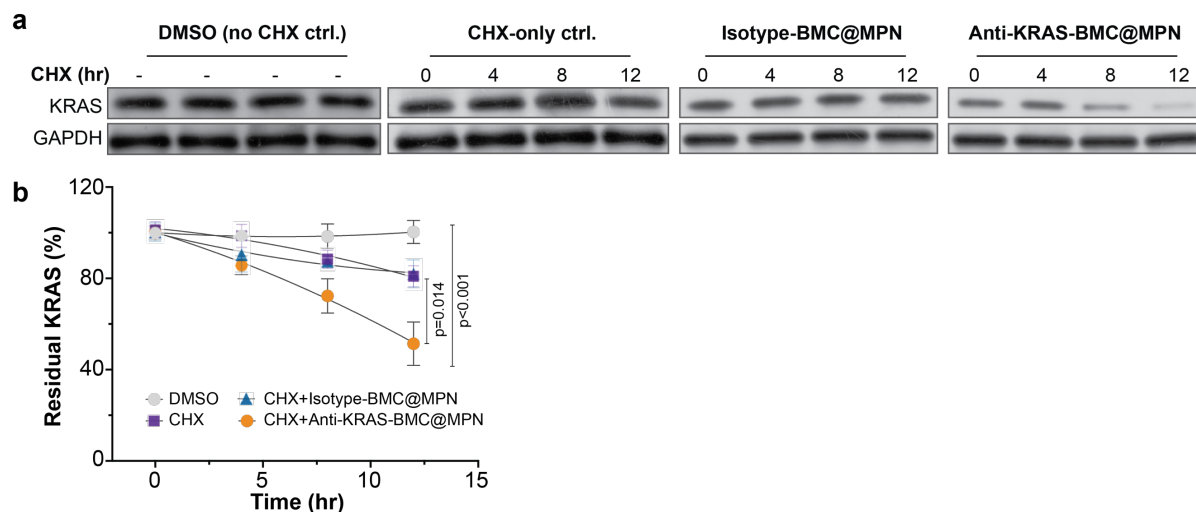
Supplementary Fig. 14 MPN facilitates endosomal escape of BMC@MPNs. **(a)**. Schematic of calcein leakage experiment upon non-treated condition or with bafilomycin A1 (an inhibitor of V-ATPase and termination of the inflow of H^+ and Cl^-). **(b)** CLSM images of SW480 cells incubated with calcein, calcein and BMCs, or calcein and BMC@MPNs in the absence (top row) and presence (bottom row) of inhibitor bafilomycin A1 for 4 h at a particle-to-cell ratio of 1000:1. BMCs or BMC@MPNs were not fluorescently labeled to avoid interference with the calcein signal.



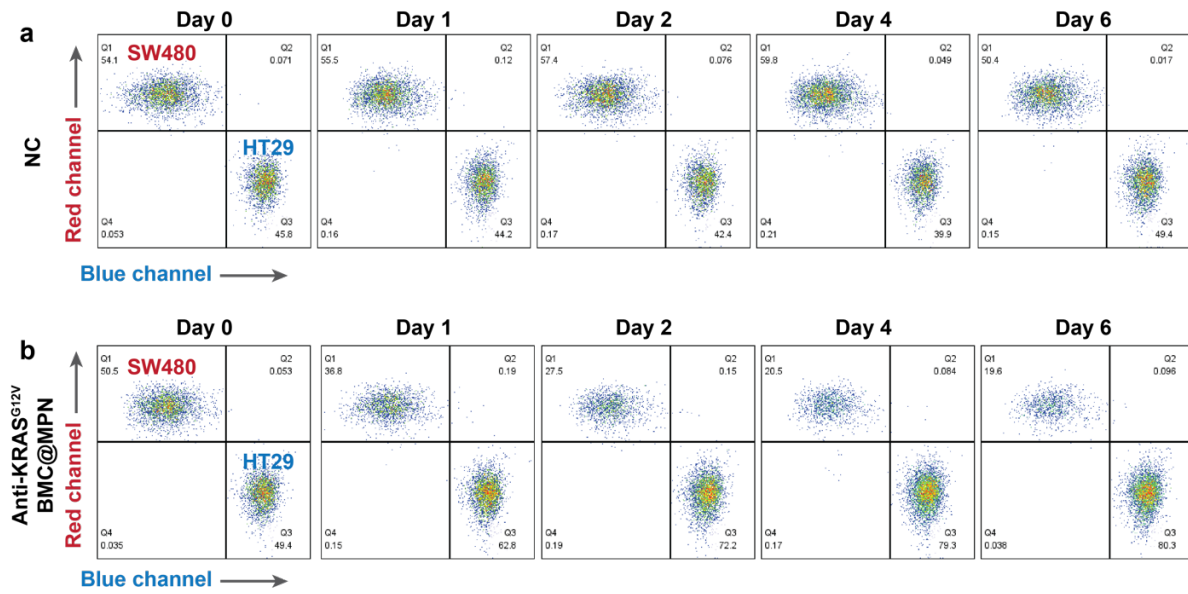
Supplementary Fig. 15 Validation of KRAS engagement by proximity ligation assay (PLA). SW480 cells were treated with free anti-KRAS, empty BMC@MPNs, isotype-IgG-BMC@MPNs, or anti-KRAS-BMC@MPNs for 12 hours. Intracellular KRAS engagement was assessed by PLA using anti-KRAS and anti-IgG antibodies to detect close proximity between the delivered antibody and endogenous KRAS. Green PLA puncta indicate successful target engagement; nuclei were counterstained with DAPI (blue). Robust PLA signals were observed only in cells treated with anti-KRAS-BMC@MPNs, confirming efficient intracellular KRAS binding. No PLA signals were detected in cells treated with free anti-KRAS, empty BMC@MPNs, or isotype-BMC@MPNs, demonstrating that both cytosolic delivery and target specificity are required for effective engagement.



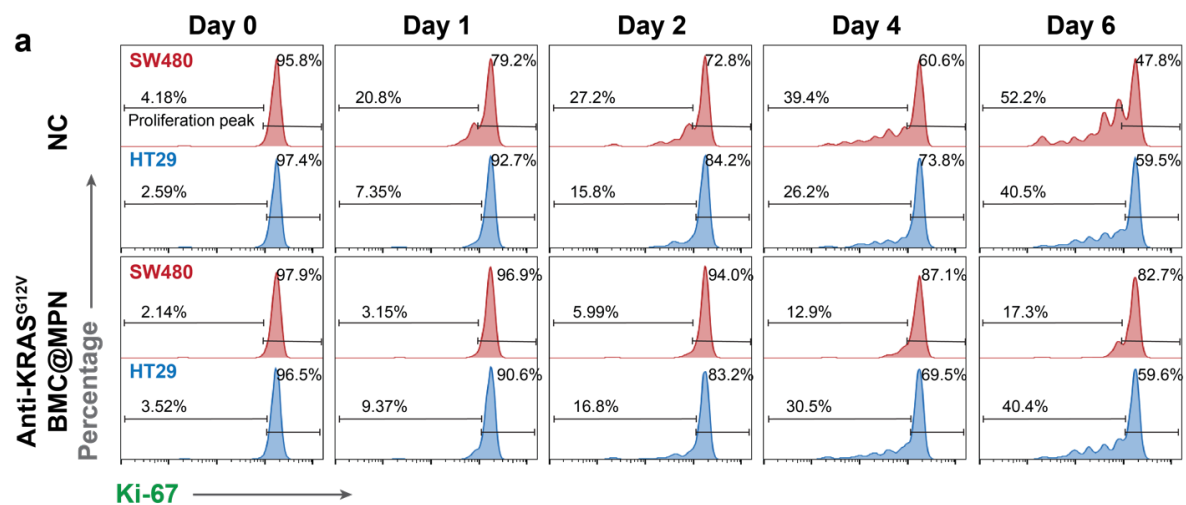
Supplementary Fig. 16 KRAS degradation kinetics induced by anti-KRAS-BMC@MPNs in SW480 cells. **a**, No degradation was observed in cells treated with non-specific isotype-BMC@MPNs, serving as the negative control. Greyscale quantification is shown in **(b)**. **c**, Schematic of 6-day FACS analysis of KRAS level, stained by AF555 anti-KRAS as detecting antibody, after being treated with anti-KRAS-BMC@MPN or isotype anti-KRAS-BMC@MPN in SW480 cells. **d-e**, flow cytometry data indicated KRAS degradation happened within 8 hours, reaching a maximal 72% reduction at 48 hours, followed by partial recovery to 85% at 6 days post-treatment, aligned with WB results. **f**, AF555 isotype staining was used as the baseline control.



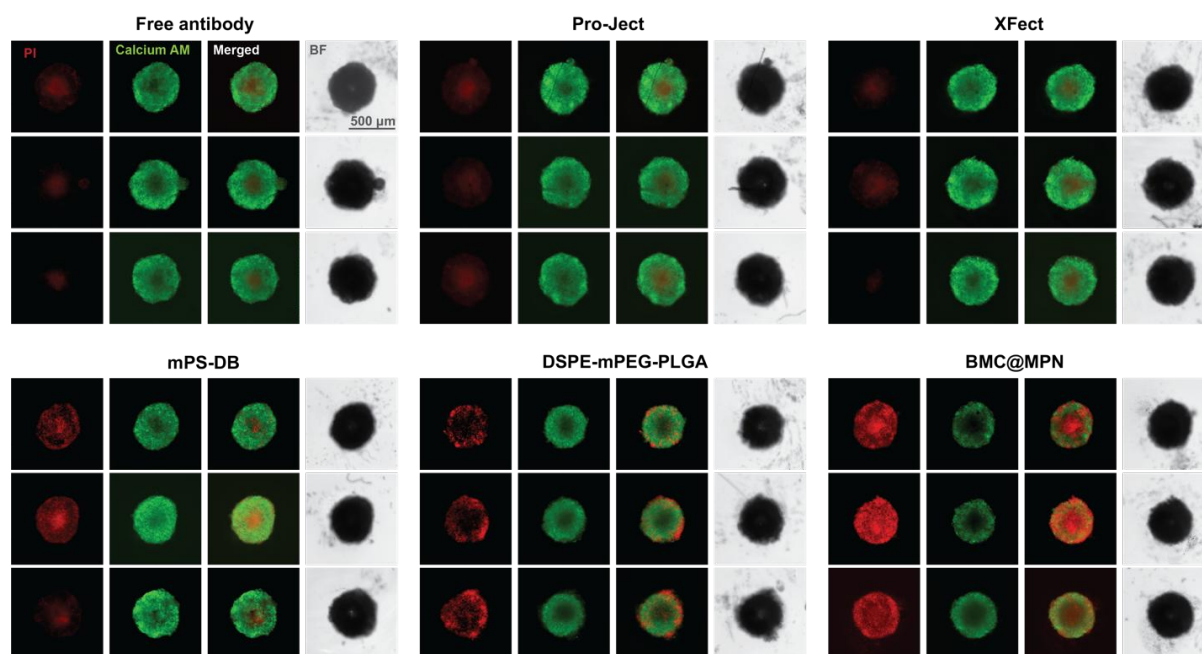
Supplementary Fig. 17 Evaluation of KRAS degradation kinetics by cycloheximide (CHX) chase assay. **a**, SW480 cells were pretreated with anti-KRAS-BMC@MPNs, isotype-BMC@MPNs, or DMSO for 4 hours, followed by the addition of cycloheximide (CHX, 50 μ g/mL) to halt new protein synthesis. Cells were harvested at 0,4,8,12hr time points, and KRAS levels were analyzed by western blot. KRAS degradation was significantly accelerated in cells treated with anti-KRAS-BMC@MPNs compared to isotype control and CHX-only groups. Quantification of KRAS levels (normalized to loading control) revealed a shortened KRAS half-life (<12 hours) in the anti-KRAS-BMC@MPN group, compared to its native turnover (~22–24 hours). Data represent mean $\pm$ SD from $n=3$ independent experiments. **b**, Quantification of KRAS degradation upon global protein synthesis inhibition.



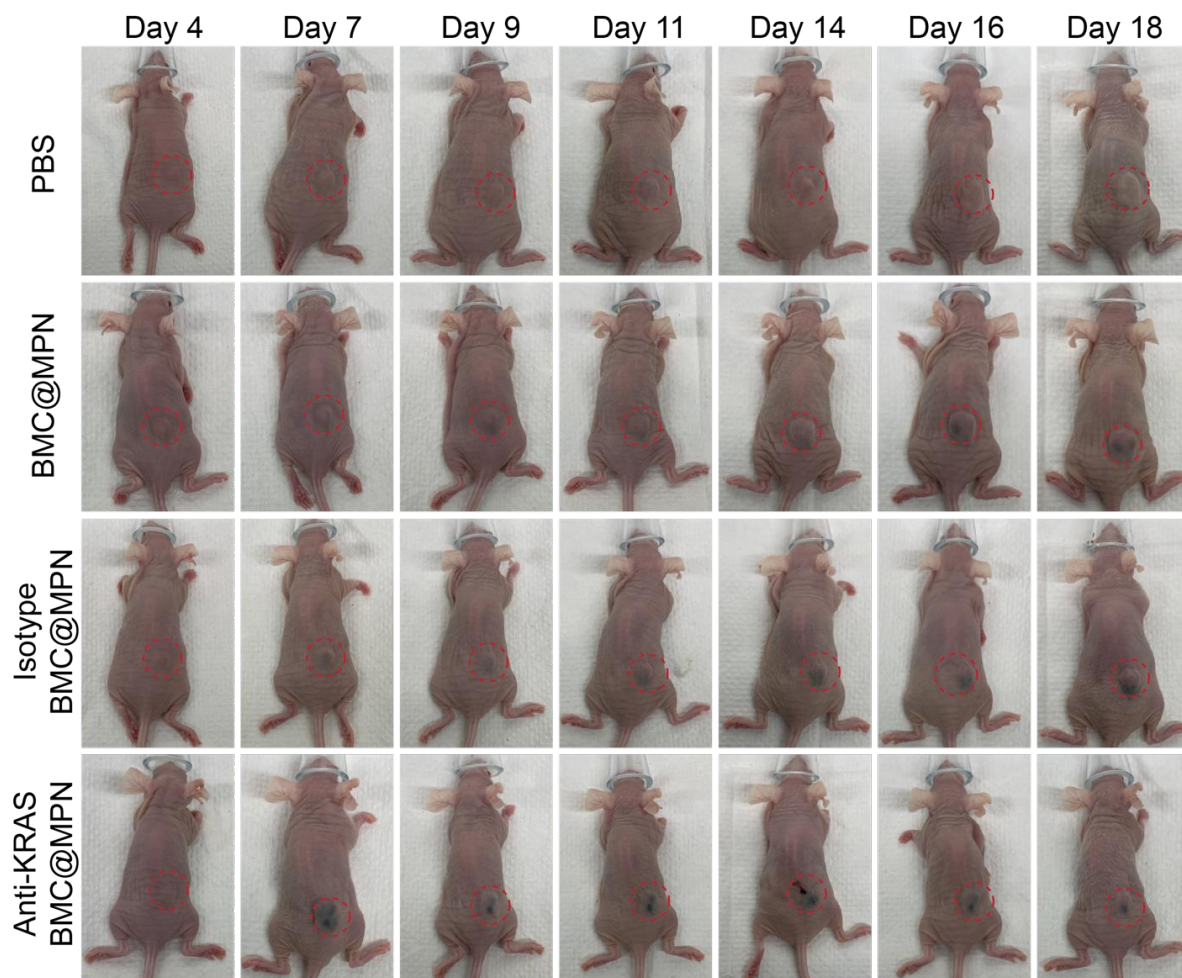
Supplementary Fig. 18 Six-day coculture cell growth assay. Flow cytometry results of representative SW480 and HT29 subpopulations in coculture, either untreated (**a**) or treated with anti-KRAS^{G12V} (**b**).



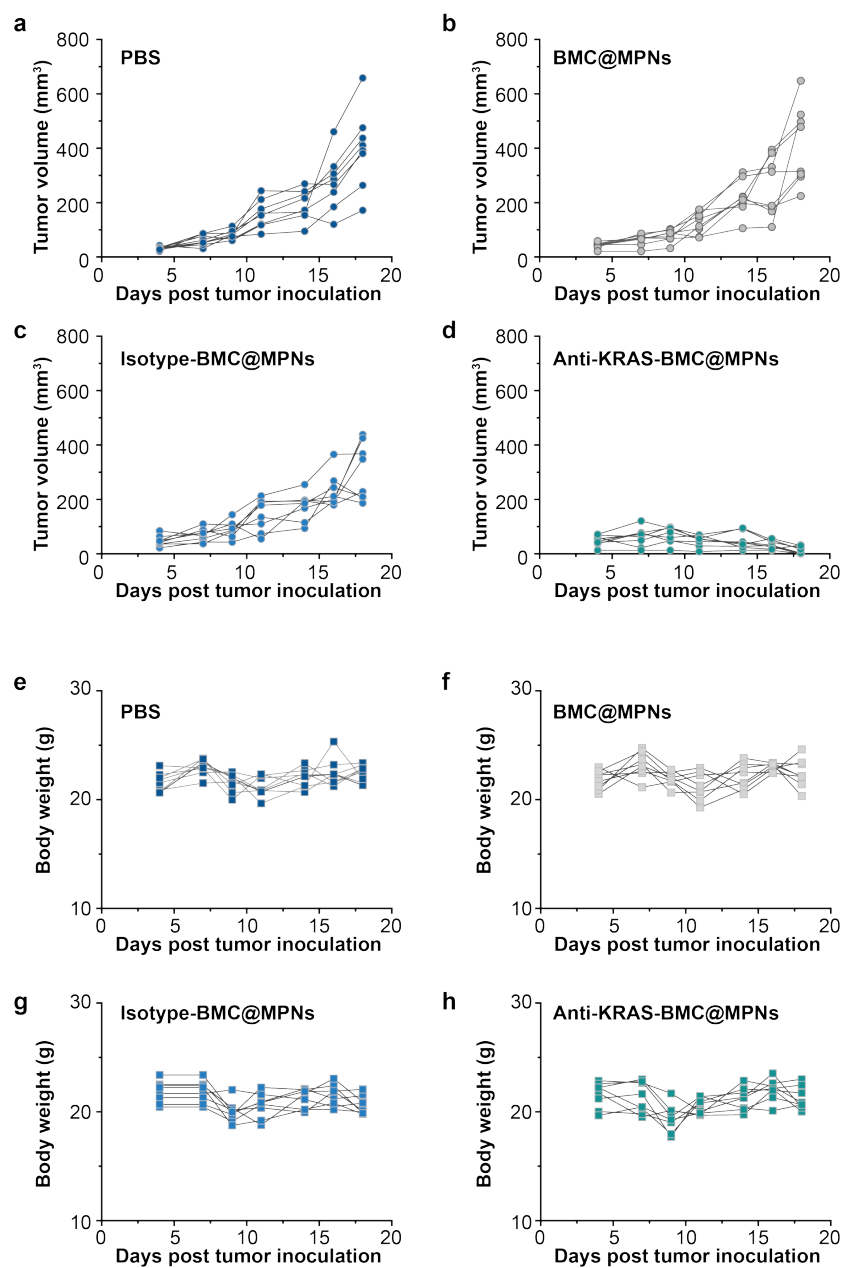
Supplementary Fig. 19 Six-day coculture proliferation assay. **a**, After 6 days of treatment with anti-KRAS^{G12V}-BMC@MPNs, the proliferative peak (Ki-67) of SW480 cells decreased compared to the non-treated group, while the proliferation level of HT29 cells was not significantly impacted.



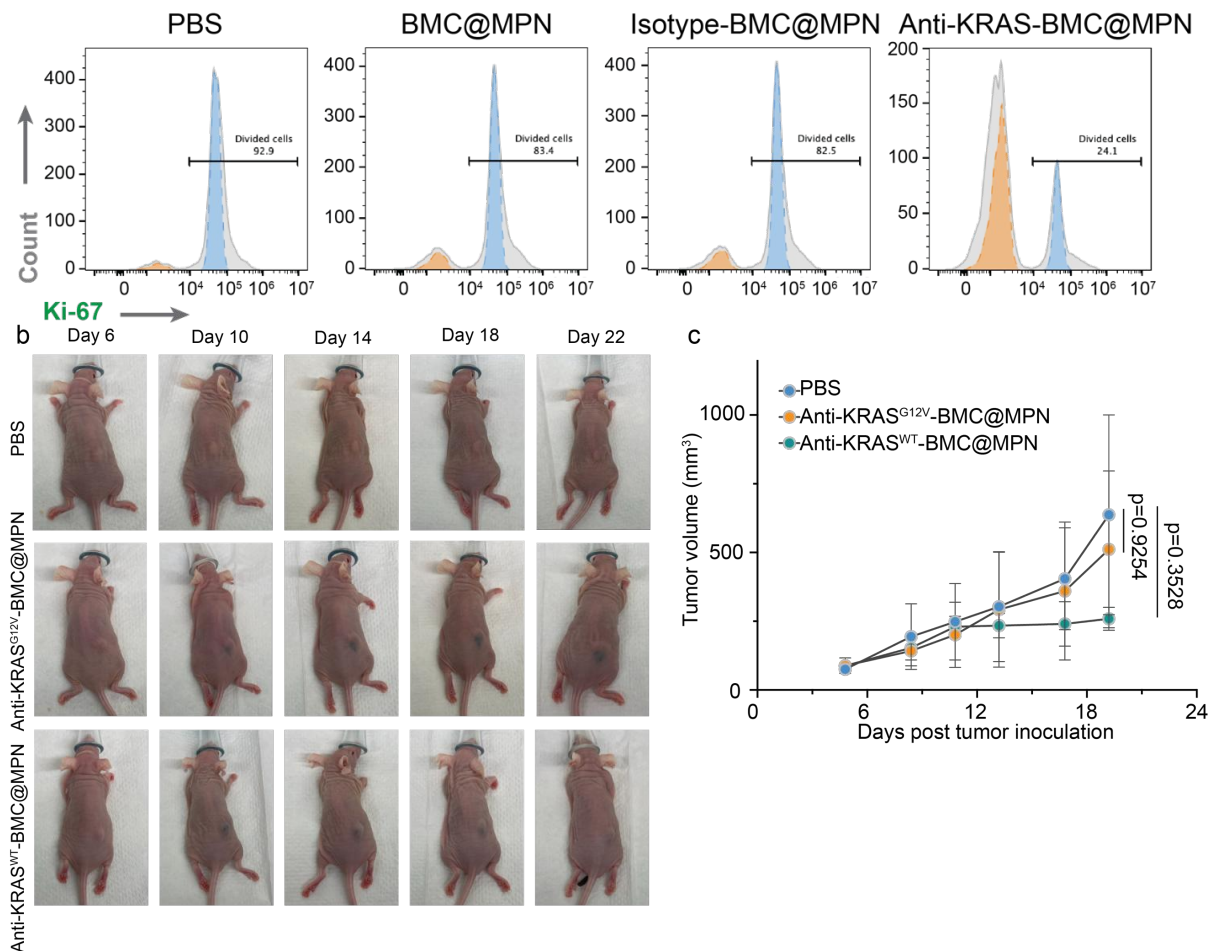
Supplementary Fig. 20 Penetration depth study of BMC@MPNs in spheroids. BMC@MPNs exhibited improved penetration depth and bioavailability in SW480 spheroid cells, compared to protein transfection reagents (Pro-Ject and XFect) and colloidal nanoparticles of comparable size and similar surface properties (mPS-DB, porous polystyrene-divinylbenzene; PLGA, polylactic-co-glycolic acid). Penetration depth was indicated by the ratio of dead cells stained by PI. Live cells were stained in Calcium AM.



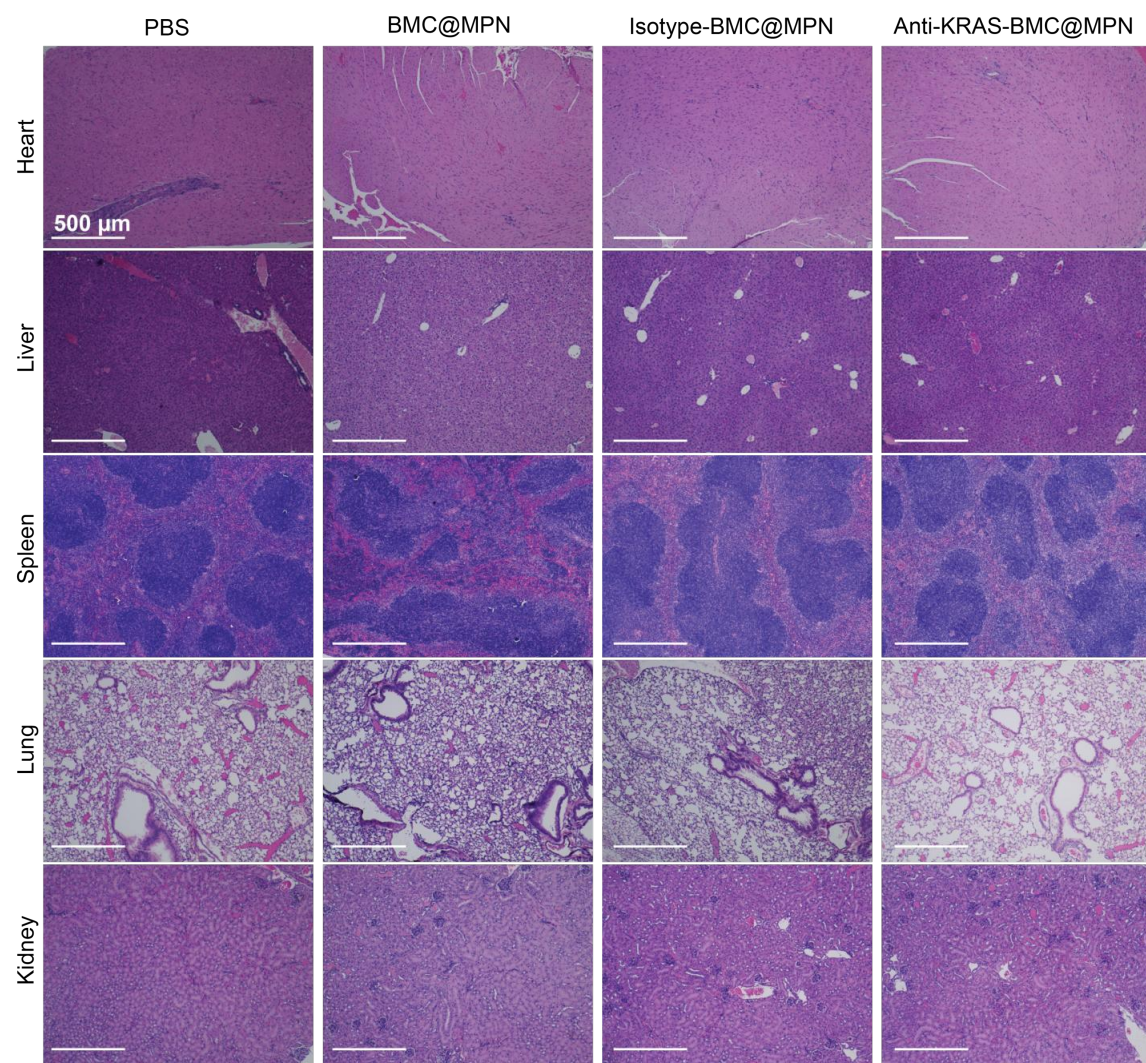
Supplementary Fig. 21 *In vivo* tumor growth images.



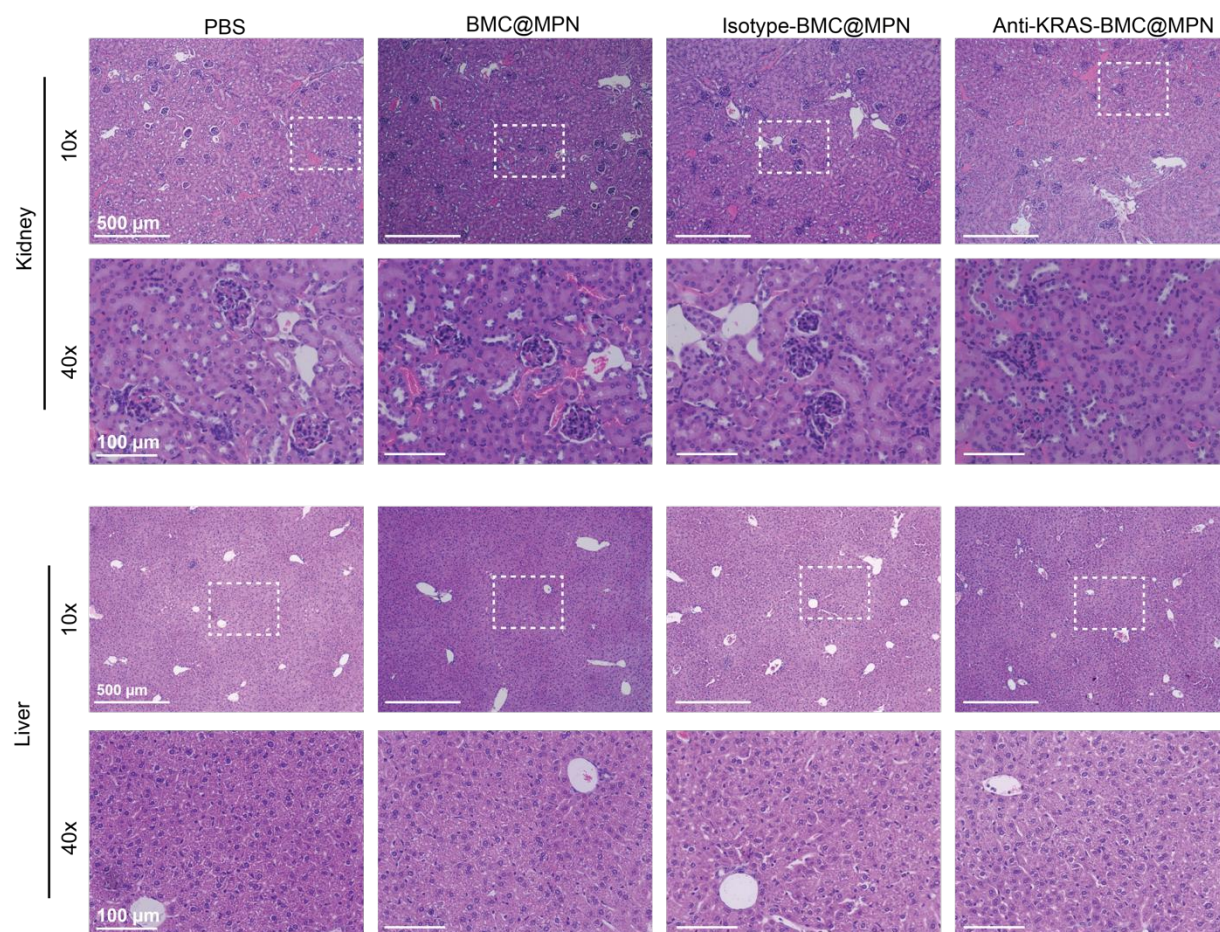
Supplementary Fig. 22 *In vivo* tumor growth and body change curve. (a-d), tumor growth curve. (e-h), body weight change curve.



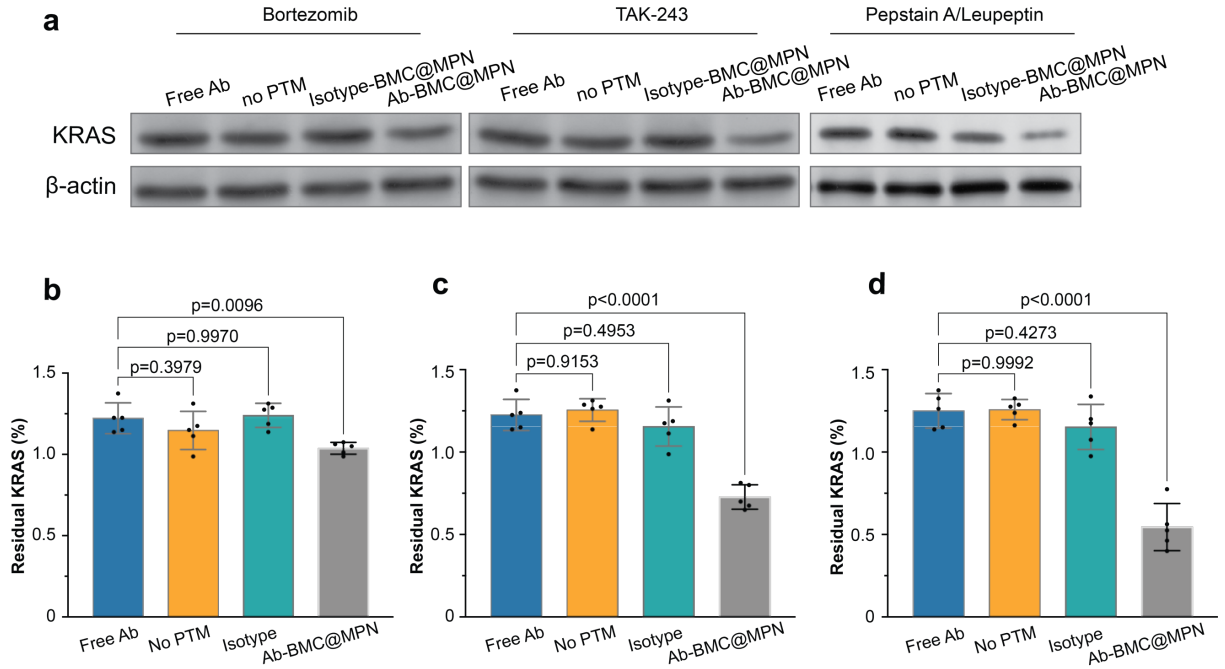
Supplementary Fig. 23 (a) Anti-proliferation assay of dissociated cells from tumors. (b). Representative images of HT29 (KRAS^{WT}) tumor-bearing mice (n=6) during the treatment procedure of PBS or anti-KRAS^{G12V}-BMC@MPN and anti-KRAS^{WT}-BMC@MPN. (c). Tumor growth curve.



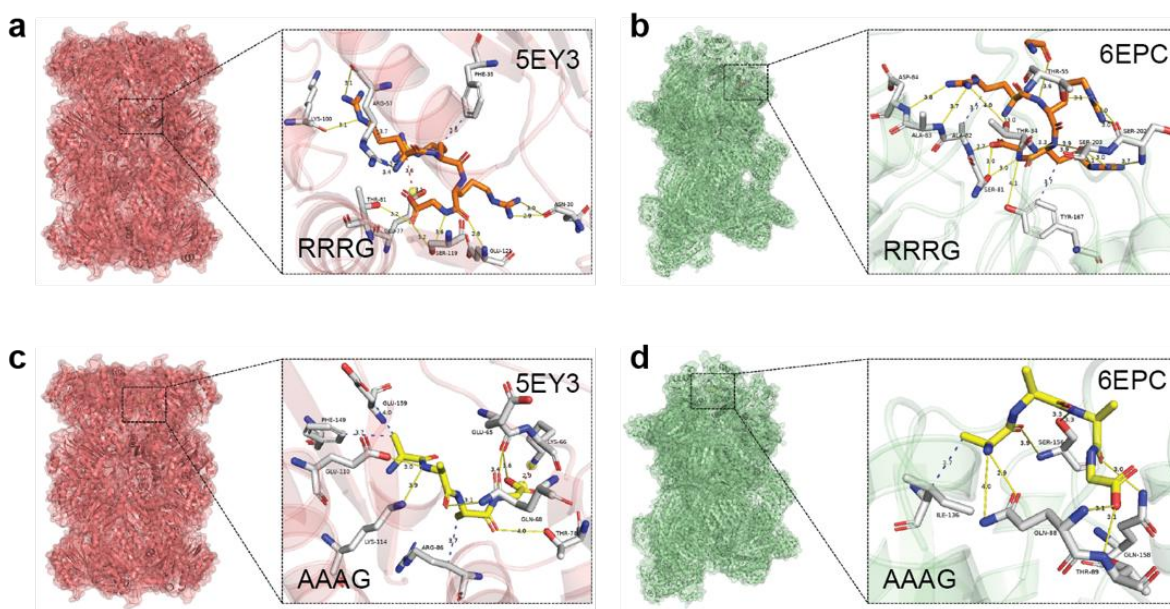
Supplementary Fig. 24 H&E staining of heart, liver, spleen, lung, and kidney. No abnormal histology was observed in any of the treated groups.



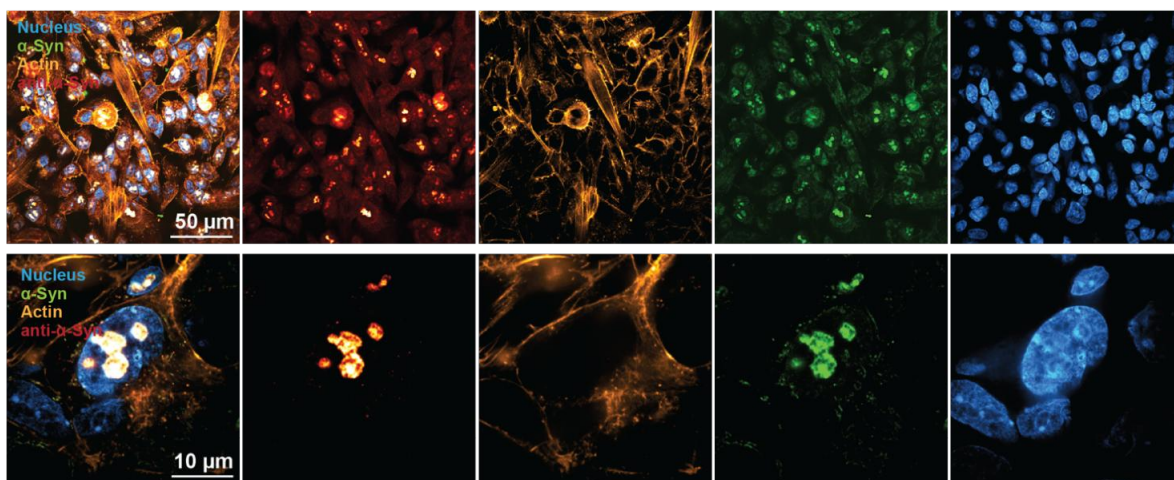
Supplementary Fig. 25 Hepatotoxicity and renal toxicity study. No abnormal histology was observed in any of the treated groups.



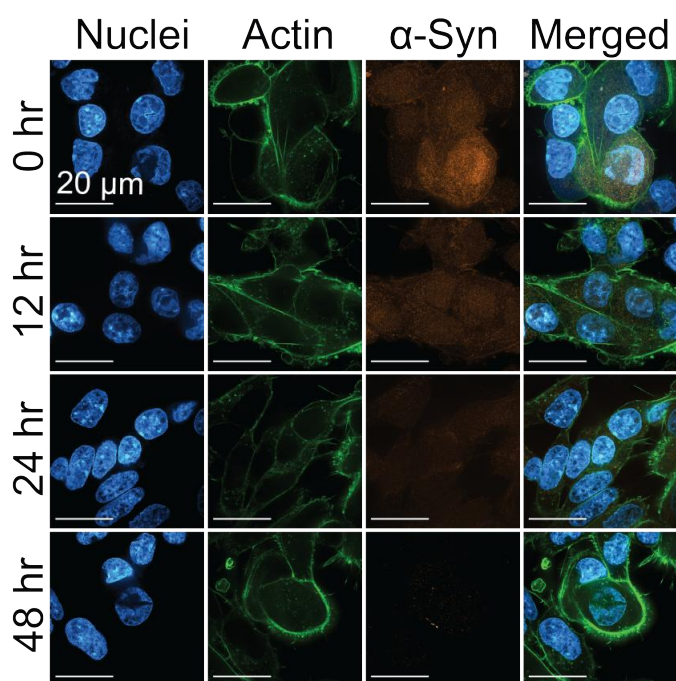
Supplementary Fig. 26 Degradation rescue effect of additional inhibitors. **a**, Immunoblot showed that when pretreated with bortezomib, an additional proteasome inhibitor, KRAS degradation was inhibited, while such an effect was not observed using TAK-243, a ubiquitin-activating enzyme inhibitor, or pepstain A/leupeptin, an additional autophagy-lysosome pathway inhibitor. **b**, Grayscale quantification of bortezomib (**b**), TAK-243 (**c**), and pepstain A/leupeptin (**d**) blots.



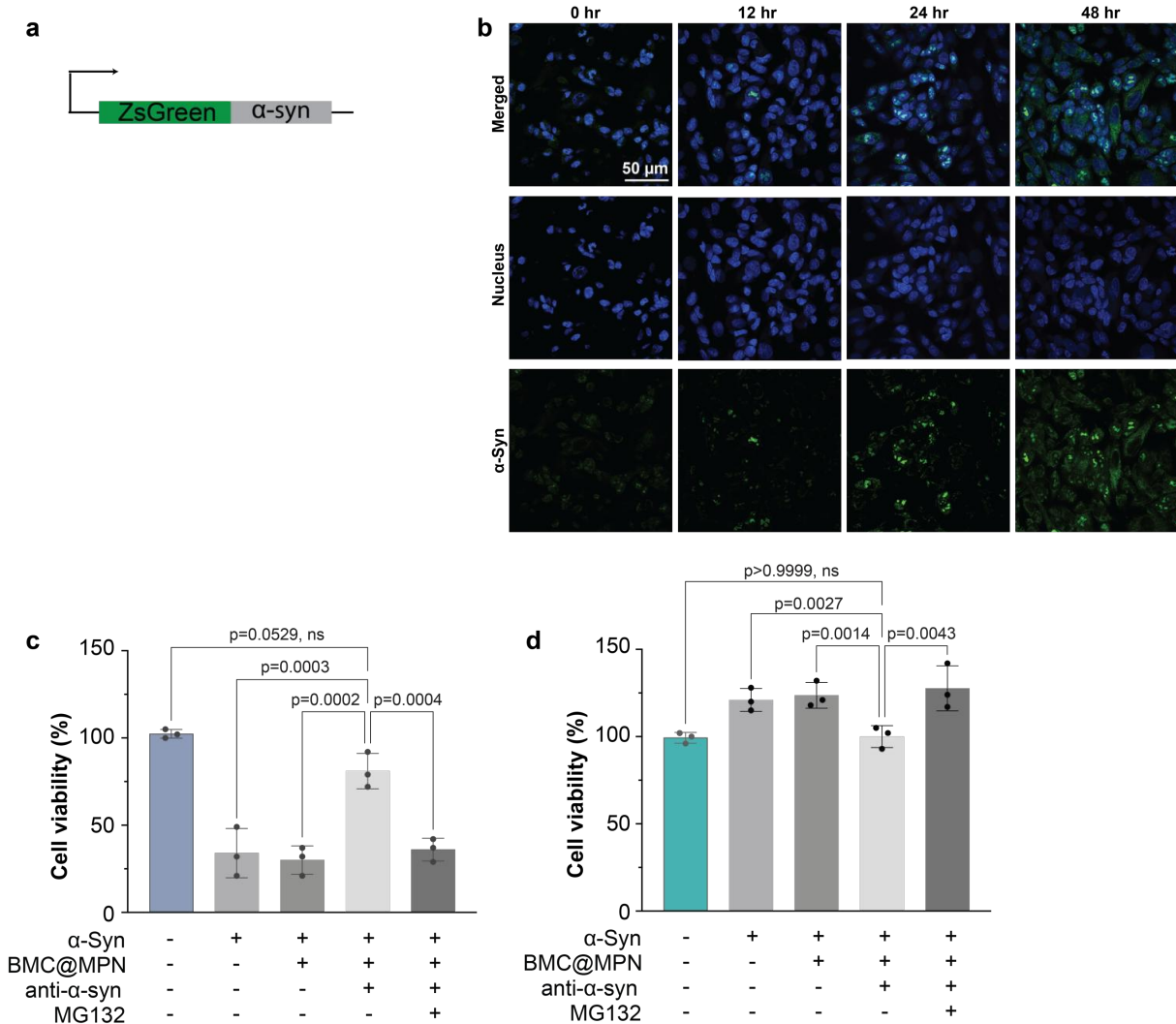
Supplementary Fig. 27 Molecular docking between proteasome-targeting motifs and proteasomes. **a.** RRRG and 26S. **b.** RRRG and 19S. **c.** AAAG and 26S. **d.** AAAG and 19S.



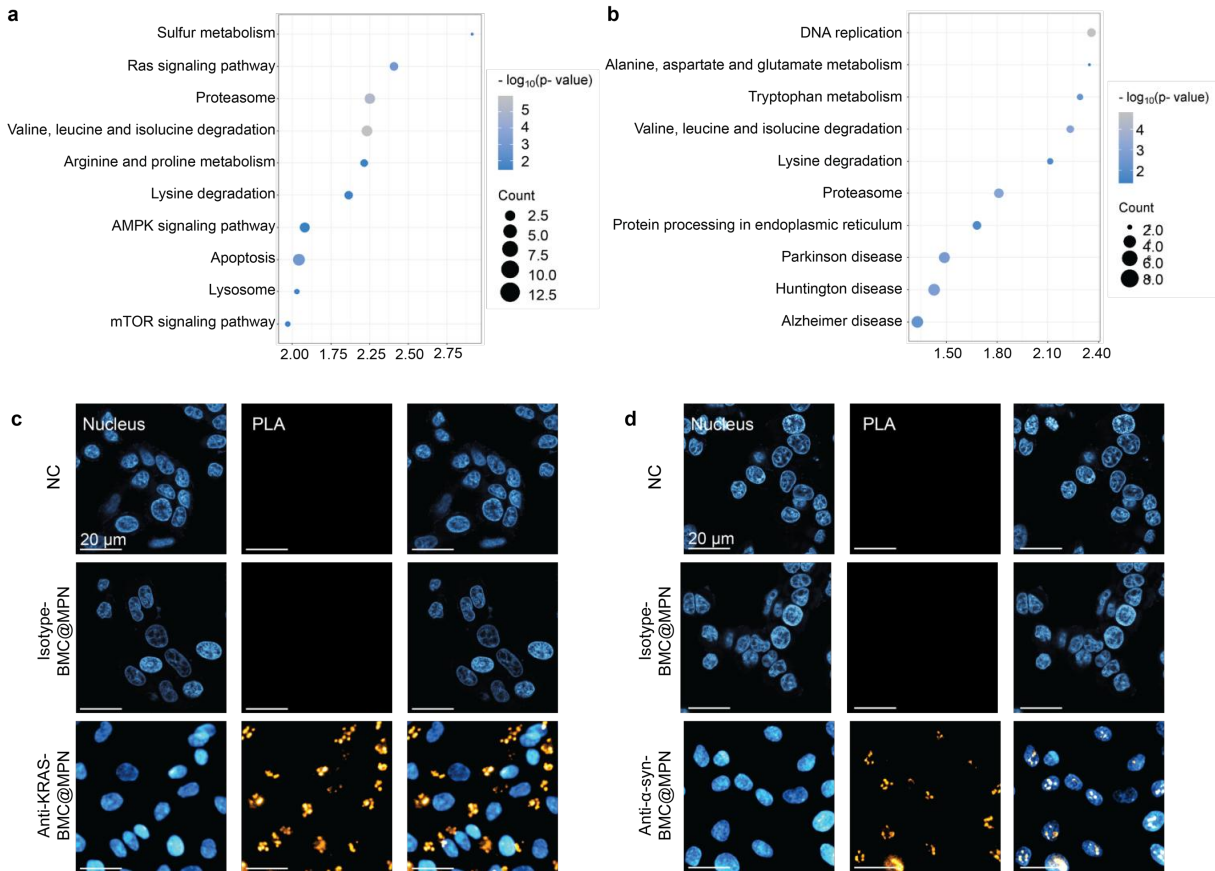
Supplementary Fig. 28 IgG-BMC@MPNs showed nuclear entry capability. Anti- α -syn-BMC@MPNs have high nucleus delivery efficacy in SK-N-SH cells.



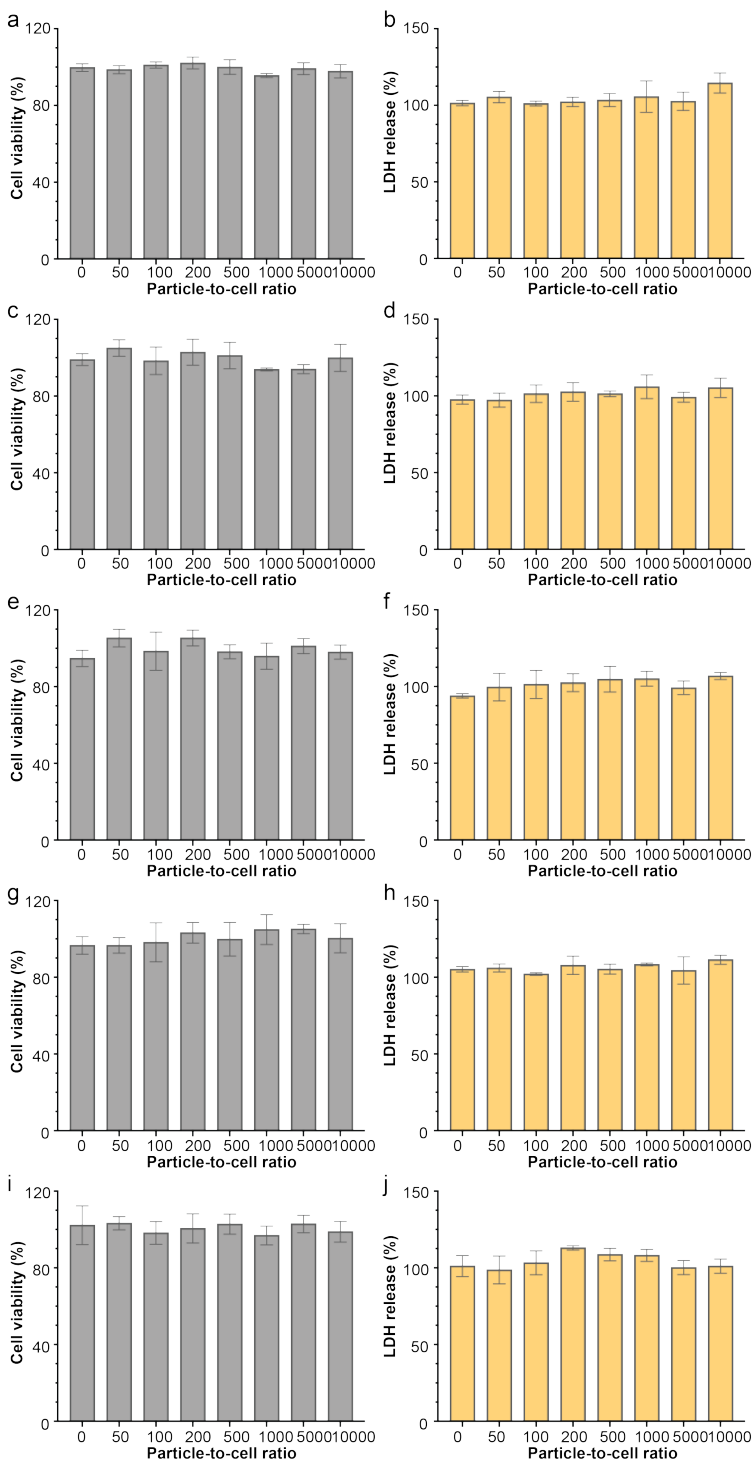
Supplementary Fig. 29 IgG-BMC@MPNs showed α -syn degradation capability. Anti- α -syn-BMC@MPNs showed α -syn degradation effect in SK-N-SH cells in 48 hr.



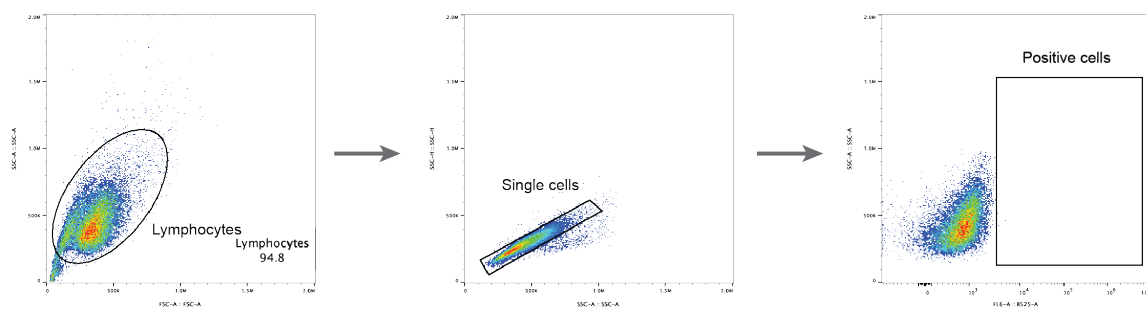
Supplementary Fig. 30 Rescue effect of BMC@MPNs for neurotoxicity in α -syn-overloaded cells. **a**, Viral construct for generating α -syn overload cell model. **b**, Establishment of α -syn overload cells. Post-transfection of SK-N-SH cells with pLVX-IRES-ZsGreen1/ α -syn. ZsGreen/ α -syn signal reaches the peak value around 48 hr after transfection. **c**, cell viability, and **(d)** LDH release study showing anti- α -syn-BMC@MPN treatment attenuated neurotoxicity due to α -syn overload.



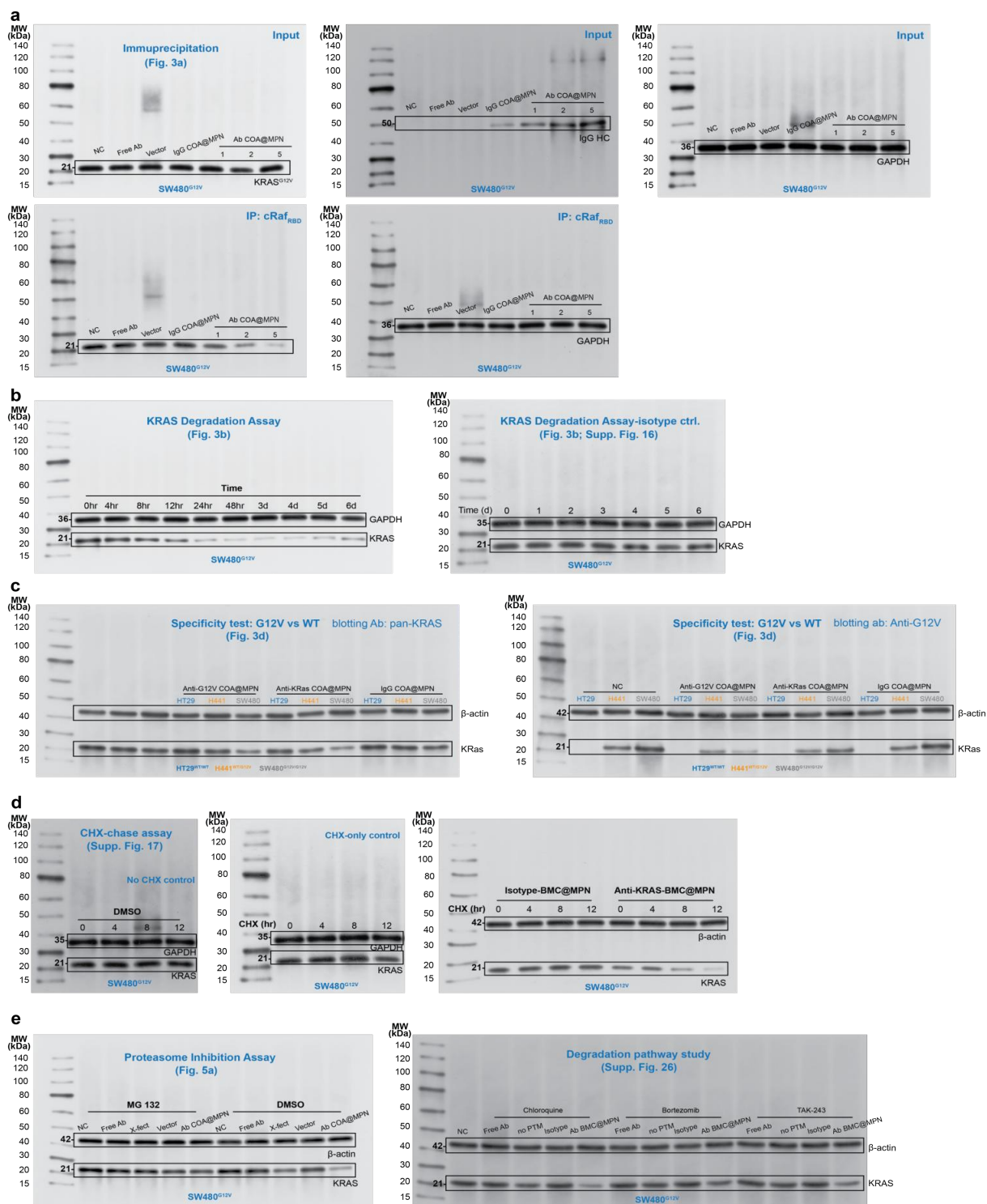
Supplementary Fig. 31 Proteomics analysis of KRAS and α -syn degradation. **a-b**, KEGG pathway enrichment analysis indicated the proteasome pathway as one of the top 10 hits for both cell types, along with the RAS signaling (**a**, SW480 cells) or neuro-disease pathways (PD, HD, AD) (**b**, SK-N-SH cells). **c-d**, PLA detecting PSMD9-target interactions during KRAS (**c**) and α -syn degradation (**d**). Distinct punctate PLA signals were observed in the cytosol of SW480 cells and the nucleus of SK-N-SH cells following treatment with anti-KRAS^{G12V}-BMC@MPNs and anti- α -syn-BMC@MPNs, respectively, indicating spatially resolved PSMD9 engagement and target degradation within their native subcellular compartments.



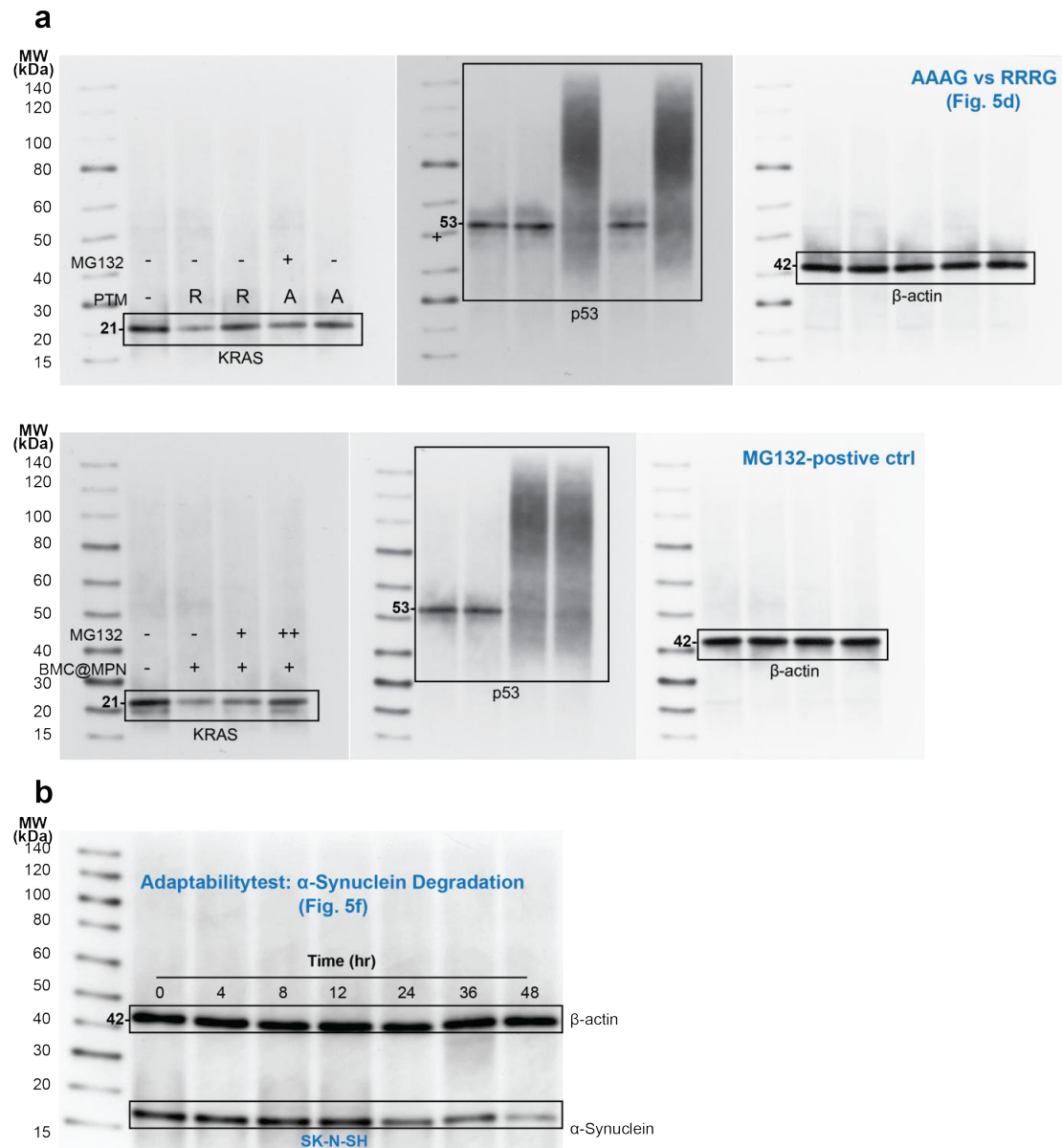
Supplementary Fig. 32 Cytotoxicity study of BMC@MPNs in different cell lines used in this study. CCK8 and LDH in HT29 (a,b), H441(c, d), SW480 (e,f), HEK293 (g, h), and SK-N-SH cells (i, j).



Supplementary Fig. 33 Flow cytometry gating strategy. A total of 20,000 events within the gates were acquired per replicate.



Supplementary Fig. 34 Uncropped western blots 1.



Supplementary Fig. 35 Uncropped western blots 2.

Supplementary References

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2. Jin, Z. et al. Endoproteolysis of oligopeptide-based coacervates for enzymatic modeling. *ACS Nano* **17**, 16980-16992 (2023).
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