

Biomolecular Condensates as Protein Degradation Tools for Intracellular Targets

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This manuscript has been previously reviewed at another journal. This document only contains information relating to versions considered at Nature Communications.

This file contains all reviewer reports in order by version, followed by all author rebuttals in order by version.

Version 1:

Reviewer comments:

Reviewer #1

(Remarks to the Author)

The authors have made comprehensive efforts to address the reviewers' comments and conducted additional experimental work to substantiate their claims. Thus, the manuscript has been improved, but before it is accepted for publication, there are a few claims that I believe should be toned down or clarified in the text:

1, This might be matter of semantics, but what do the authors mean by "passive entry" for macro-size BMCs (page 7, line 16; page 16, line 19 and 23, and Supp. Fig. 12a) ? Filipodia formation during BMCs engulfment involves active cytoskeleton remodeling, see for instance the review by Gleeson and co-workers (<https://www.mdpi.com/2077-0375/10/8/177>) or the study by Kress et al (<https://www.pnas.org/doi/full/10.1073/pnas.0702449104>). Thus, naming this process "passive" seems a bit misleading. Both entry mechanisms shown in Supp. Fig. 12 are active, although they involved different entities and length scales? I'd like the author to provide a more accurate differentiation here.

2. While in their system, nanoscale BMCs indeed appear to perform better than micro-scale BMC, the authors cannot make generalized statements that nano-BMCs are superior to micro-BMCs in terms of transfection efficiency and uptake kinetics. There are many recent studies showing that micro-BMCs can safely achieve above 90% efficiency and cargo release within minutes. For example, in Sun et al, (Nature Comms, 2024 <https://www.nature.com/articles/s41467-024-54463-z>) some peptide condensates achieved > 95% transfection rate with cargo release observed within a few minutes. In other words, while size can be a factor, there are many other variables that play an equally or even more critical role in mediating cell entry, including the viscoelastic properties of the condensates, their surface charge, specific peptide motifs, etc...The authors should make it clear that the superiority of the nano-scale BMCs over micro BMCs is valid for their system, but this is not generalized for all BMCs. Supp. Fig. 12 should be corrected accordingly.

3. In their reply, the authors state that the intracellular delivery of intact biomolecules (IgGs) is largely an unmet challenge that remains unexplored in the field of LLPS.

I believe that in this context, they are specifically referring to IgG antibody delivery, which they indeed demonstrate retain efficient intracellular binding activity? (although IgG delivery has been demonstrated for micro-BMCs by Bao et al JACS, 2025, <https://pubs.acs.org/doi/10.1021/jacs.4c17054>). However, in the broader context of biomacromolecules, there have been numerous recent examples that coacervates can very efficiently deliver large macromolecules cargos in cells, with unaffected biological activity, including large enzymes (e.g. 430 kDa beta-galactosidas); cytotoxic proteins (saporin), mRNA, pDNA, or CRISP-Cas modalities (see for instance Forenzo and Larsen, ACS Mol. Pharma. 2023, <https://pubs.acs.org/doi/10.1021/acs.molpharmaceut.3c00439>; Sun et al, ACS Nano 2023, <https://pubs.acs.org/doi/10.1021/acsnano.3c02669>). The authors may want to clarify this in the introduction or discussion.

On a different note, I have also read the comments of the other Reviewers, and in my view the criticism of Reviewer #3 "I am not sure these BMCs offer anything different than lipid nanoparticle formulations" is unwarranted. Peptide-based BMCs are entirely different from LNPs from many different aspects: they are made from completely different chemical building blocks, their materials (visco-elastic) properties are very distinct from LNPs, their fabrication process is fundamentally different and

much simpler and does not use of organic solvents, etc...In short, BMCs are indeed an entirely different class of delivery vehicles, with their own merits and advantages over other intracellular drug carriers and should be recognized as such.

Reviewer #2

(Remarks to the Author)

The authors have provided a detailed response to the previous reviews, with the addition of substantial new data.

An earlier concern was the lack of information about the mode of cellular uptake of the BMCs and their subsequent release into the cytoplasm. The inclusion of additional cell uptake inhibitors has allowed the authors to define the mechanism of uptake as clathrin-mediated endocytosis (Fig. 2j). They have now provided some evidence that the BCM@MPNs trigger endosomal release (Supp Fig. 14).

The addition of PLA to demonstrate direct interaction between the internalised antibody and its target (KRas) is a helpful addition (Supp Fig. 15). PLAs have now also been done to demonstrate interaction of the BMCs with PSMD9 (Fig. 31): in the case of the anti- α -syn-BMC@MPNs (Supp Fig 31d), the authors may want to speculate on the likely mechanism by which the antibodies reach the nucleus – given their large size relative to nuclear pore size?

Additional controls have been added as suggested in the previous reviews. One example is the new in vivo experiment shown in Supp. Fig. 23 – demonstrating the relative lack of efficacy of the anti-KRASG12V-BMC@MPN in a HT29 (KRASWT) xenograft model.

A minor point is that the authors were asked to justify their claim that CPPs have in the past been shown to be immunogenic. However, the papers they have cited concern ADCs rather than CPPs.

In the rebuttal the authors provide the calculation of the total IgG accumulation per cell: this is interesting and could be added to the Supp Materials.

Overall, the authors have more precisely stated the novelty of their delivery system, and their conclusions are now better supported by the addition of the new data.

Reviewer #3

(Remarks to the Author)

The authors have addressed some of the reviewer concerns from the prior round of review, but some gaps remain in the work that need to be clarified. In addition to the points below, further clarification of exactly how peptides remain bound to the antibodies after dissolution of the BMC in the cytoplasm and how this results in proteasome recruitment, and why free peptides do not compete with binding to the proteasome is needed.

Response to responses of initial comments:

Reviewer Comment: In the image in 2i, fluorescence is primarily in small, circular structures. What is the proposed reason for these structures and staining pattern? Do these co-stain with proteins in the endocytic pathway or lysosomal markers? It would be helpful to have a sense of what these represent, since they primarily appear to vesicular structures.

Response: These structures are our IgG-BMC@MPNs. There are no co-staining biomarkers F-actin (red) was stained to outline the cell membrane and indicate the relative intracellular localization of BMCs (green). We have used a larger font in the figure legend in the panel and added a description as well.

I do not see evidence that intracellular markers do not co-stain, was this experiment performed? If so, please include the data, if not, it seems like an important experiment to define where these complexes go inside cells.

Reviewer Comment: The substantial number of off-target degradation events in Fig. 5i,j is troubling for an approach that should be highly surgical in its ability to downregulate a specific target. I think this essential piece of data shows that there are some issues with the approach, with the targets chosen, or with the nature of the degradation which takes a long time and thus makes quantitative MS difficult.

Response: We would like to clarify that many of the proteins identified in our original Fig. 5i, j are not off-targets but instead functionally related effectors that naturally interact with the target protein in active signaling complexes, rather than direct degradation by our construct⁵⁴⁻⁵⁶. Importantly, the number of off-target hits we observed (83 downregulated and 117 upregulated out of >3500 signals for KRAS; 101 downregulated and 119 upregulated out of >3500 signals for α -syn) is comparable to or even lower than those reported in recent studies using intracellularly delivered antibodies or degrader constructs without genetic modification^{57,58}.

Response: To further validate the hits (i.e., proteasome subunits and target proteins, KRAS or alpha-

synuclein), we now included a proximity ligation assay (PLA) in our Supplementary Fig. 31 to evaluate the interaction between PSMD9—a 19S proteasomal subunit—and the target protein. The PLA signal supports a direct recruitment mechanism involving the proteasome, strengthening the specificity of our degradation strategy. We agree that long degradation windows can complicate MS quantification, and we are actively optimizing experimental timing to minimize such effects in future studies.

Whole cell-proteomics should be utilized to demonstrate selectivity of degradation.

Version 2:

Reviewer comments:

Reviewer #1

(Remarks to the Author)

The authors have addressed all Reviewers' comments. I recommend acceptance of the manuscript.

Reviewer #2

(Remarks to the Author)

The authors have satisfactorily responded to my questions/points in the last round of review.

Reviewer #3

(Remarks to the Author)

The authors have chosen not to perform the requested revision experiments, but to clarify their aims. While this is their prerogative, the original questions raised by the requested experiments remain unanswered.

"Response: The aim of Fig. 2i is to demonstrate the cytosolic accessibility of BMCs rather than to characterize their intracellular trafficking routes. In this experiment, we incubated a cell monolayer with Alexa Fluor 488-labeled BMCs, followed by staining of actin and nuclei to delineate cell boundaries and intracellular structures. There is no co-stain of intracellular markers other than actin and nuclei. The green channel denotes BMCs, the blue channel marks the nucleus, and the red channel outlines the cell boundaries. As shown in Fig. 2i, BMCs are clearly internalized and distributed within the cytosolic region of the cells."

Without some intracellular co-stain, this experiment could just be showing endosomes which is not particularly informative or useful. The original comment understood the aim of the figure, but request further information to provide additional value to the experiment.

"Response: The aim of Fig. 5i-j is to demonstrate that the target protein is degraded through engagement with the proteasome subunit. Therefore, we believe that pull-down proteomics and the proximity ligation assay (PLA) performed represent more appropriate experimental approaches for probing such interactions.

In contrast, whole-cell proteomics will capture global upstream and downstream protein changes besides targets, which makes the fold change of the target less interpretable. Moreover, changes in overall proteasome subunit abundance may not be detectable, as the system is designed to degrade a specific target protein rather than alter the global levels of proteasome components."

While the aim of the figure is as stated, it shows that there a substantial number of off targets and interactions that would complicate selective degradation. The aim of the requested experiment is to clarify how selective this approach can be, as without selectivity there is very little reason to use a complicated delivery vehicle for an antibody.

Version 3:

Reviewer comments:

Reviewer #3

(Remarks to the Author)

Thanks to the authors for attempting to address the requests. The only additional request prior to publication would be that the proteomics data from the last revision be included in the manuscript, as I do not see it referenced in the main text or supplementary information, and discussed appropriately, with all targets more substantially degraded labeled. After that is done, there are no additional concerns. In general, experiments for revisions should be included in manuscripts.

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We sincerely thank the Reviewers and Editors for their thoughtful feedback and constructive suggestions. In response, we have added new data and expanded our discussion to address the points raised. We have carefully revised the manuscript and provide a detailed, point-by-point response below. For clarity, reviewer comments are shown in blue italics, followed by our responses in black, with key points highlighted in bold. We would be grateful for the opportunity to resubmit our work for further consideration at *Nature Communications*.

Reviewer #1

The authors are using biomolecular condensates (BMCs) as a tool to deliver targeting protein degradation (TPD) modalities. Specifically, they are using rather simple BMCs by complexing two oppositely-charged peptides: one positively-charged peptide containing an Arg-rich domain cyclized with a disulfide bond using termini Cys residues, and a negatively-charged peptide made of poly-Aspartic acid. The peptides assemble into BMCs via liquid-liquid phase separation (LLPS), a process during which an immunoglobulin-G (IgG) antibody can be recruited within the condensates.

The specific IgG is selected for its ability to induce degradation of the intracellular pathogenic KRAS protein, and the rationale behind the approach is that IgG antibody cannot cross the cell membrane and therefore must be packaged into a delivery vector. Since recent studies have demonstrated that condensates are able to efficiently cross the cell membrane to deliver therapeutics inside cells, they hypothesized that their IgG-loaded condensates would be able to overcome this key issue of the non-permeability of IgG.

The manuscript includes condensates design and recruitment of IgG, cell studies with tumor cells including co-culture assays, and in vivo work. The approach of using condensates to deliver IgG antibody therapeutics is interesting but the paper has key flaws, and the claimed novelty is inaccurate.

Response: We thank the Reviewer for taking the time to review our manuscript and provide thoughtful feedback, which helped us clarify and improve several aspects of our work.

We want to highlight our design as a nanoscale biomolecular condensate (BMC) platform for intracellular target degradation. Our approach: **(i) uses biomimetic liquid–liquid phase separation to condense full-length IgG for KRAS G12V recognition; (ii) forms nano-scale BMCs that minimize the membrane bending/remodeling limitation typical of micron-scale condensates; and (iii) integrates a proteasome-recruiting motif, eliminating the need for genetic modification of degradation adaptors or target proteins.**

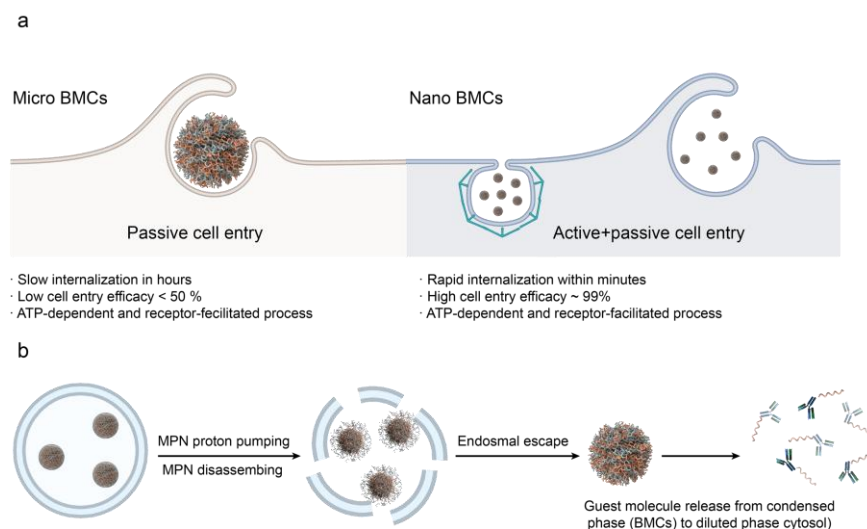
Additionally, the biomedical application of LLPS-based material has been an emerging field, and we agree that prior studies have explored condensates as delivery vehicles^{1,2}. However, our study presents a distinct advance by using minimalistic peptide components to (1) enable condensing of full-length IgGs and (2) capable of driving proteasome-mediated degradation of intracellular targets without the need for gene editing or exogenous enzymatic machinery. These features are not commonly achieved in existing systems.

We have revised the manuscript to more clearly highlight these advantages and distinguish our approach from previous work.

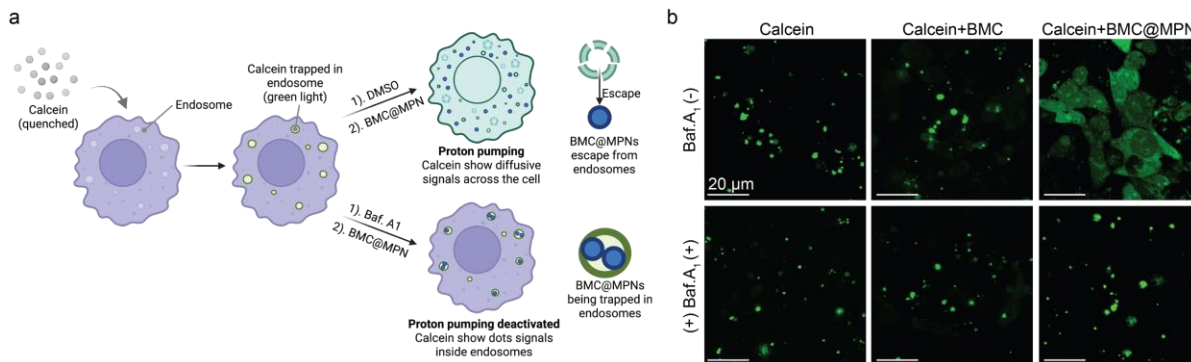
Comments

1. A central aspect of the study is the ability of the condensates to cross the cell membrane since the target of IgG are intracellular. Thus, understanding the cell uptake mechanism and IgG is of critical. However, this aspect of the study has of flaws, with data that contradict each other and that are incorrectly interpreted.

Response: We have now added a schematic illustration (Supplementary Fig. 12) depicting the proposed cell entry process of nano BMC@MPNs. Our results highlight **the new finding of active endocytosis** due to size reduction over passive mechanisms such as direct membrane fusion or passive membrane bending^{3,4}, which are commonly reported for current microcondensate systems^{2,5,6}. These passive pathways have been debated for potentially causing membrane rupture and inducing morphological changes in cells or vesicles upon condensate contact⁷⁻⁹. We have



Supplementary Fig. 12 | BMC@MPNs enter the cytosol through a three-step process (1) membrane interaction and cellular uptake, (2) endosomal escape, and (3) controlled release of guest molecules into the cytosol.



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.

included related discussion in our conclusion section. We here highlight the cell entry process in 3 steps: (1) membrane interaction, (2) endosomal escape, and (3) cytosol release of guest molecules. We have included additional data in our revised manuscript to support this hypothesis and to compare the cellular uptake behaviors between nano-BMC@MPNs and micro-BMCs (Supplementary Fig. 12) and added new data for endosomal escape (Supplementary Fig. 14)

i) The authors used the amiloride inhibitor amiloride, which did not inhibit uptake and are thus concluding that the uptake does not occur through pinocytosis, which is an energy-dependent pathway. There are more potent inhibitors to pinocytosis, in particular EIPA which may well inhibit uptake.

Response: In our revised experiments, we treated cells with 50 μ M 5-(N-ethyl-N-isopropyl)amiloride (EIPA) to inhibit pinocytosis as suggested (Fig. 2j and Supplementary Fig. 12; relevant figures are shown below). The results show that micro BMC uptake decreased to **36%**, while nano BMC@MPN retained **62%** residual uptake, suggesting that **nano BMCs rely less on pinocytosis**. Complementary studies using a panel of endocytotic inhibitors revealed that nano BMC@MPNs utilize additional endocytic pathways (i.e., energy-dependent and clathrin-mediated endocytosis, with mean fluorescence intensity, MFI, reduced to **15%** and **23%**, respectively). These pathways are not observed in micro BMCs or reported in existing micro-condensate designs^{1,2}. We believe this shift in cell entry preference helps mitigate the risk of membrane rupture and/or cell or vesicle morphological changes upon contact with BMCs^{4,7,8}, an issue that remains under debate in the context of using molecule-enriched condensates as drug delivery vehicles^{9,10}.

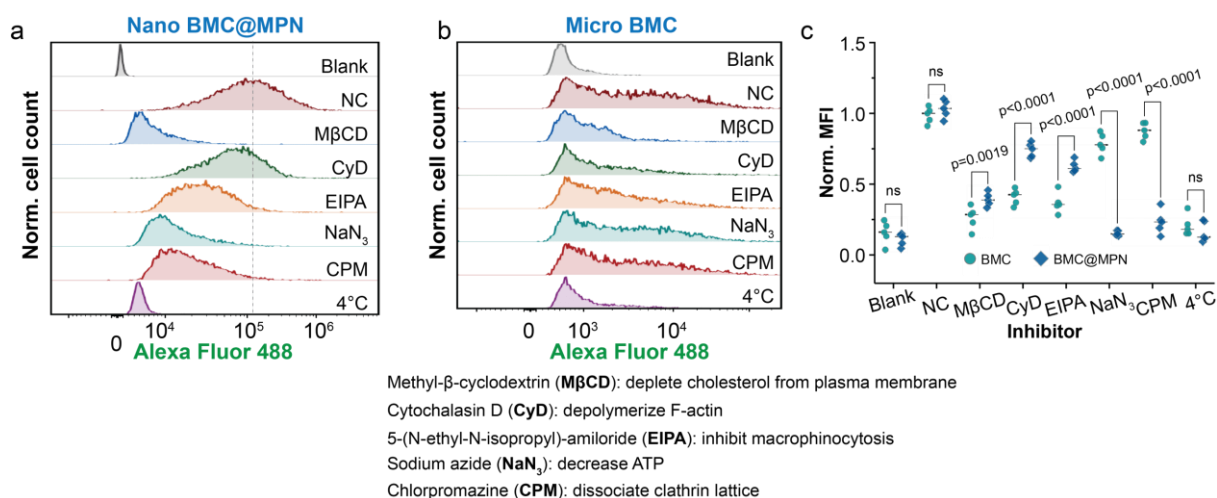


Fig. 2j and Supplementary Fig. 12 | Flow cytometry raw data comparing cell internalization preference of nano BMC@MPN (a) and micro BMC (b) under the same inhibiting conditions. (c), Statistical comparison of MFI (median fluorescence intensity, data were normalized to NC, respectively) indicated nano BMC@MPNs exploit energy-dependent (inhibited by NaN₃) and clathrin-mediated endocytosis (inhibited by CPM), and are less dependent on pinocytosis (inhibited by EIPA), actin polymerization (inhibited by CyD).

In summary, our extended our data to compare the cellular entry preferences of micro BMCs and nano BMC@MPNs. **Micro BMCs primarily rely on passive membrane fusion and bending, whereas nano BMC@MPNs also utilize ATP-dependent and receptor-mediated**

internalization. This distinction accounts for their significantly higher cytosolic delivery efficiency (**99%** for nano BMC@MPNs vs. **42%** for micro BMCs, Fig. 2h, relevant figures are shown below). These findings highlight the critical role of size reduction, as nano BMC@MPNs can access additional uptake pathways, providing a key functional advantage in BMC design.

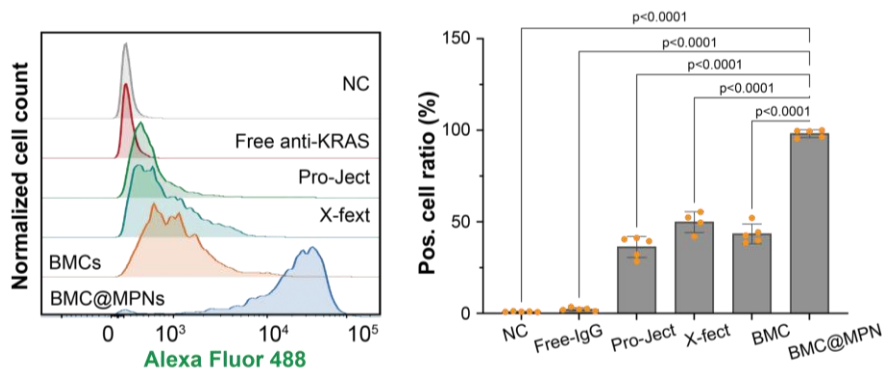


Fig. 2h and Supplementary Fig. 9 | Flow cytometry raw data comparing cell internalization efficacy among free-IgG, commercial transfection reagents (Pro-Ject and X-ject), BMCs, and BMC@MPN. BMC@MPNs have 99% transfection efficacy (quantified by positive cell ratio), outperforming BMCs (42%), X-ject (51%), Pro-Ject (39%), and free-IgGs (2%).

ii) Then, at 4°C, the uptake is prevented, while the authors stated that “uptake did occur at 4°C” (P7, L9-11). On this basis, the authors are stating that their BMCs enter the cells through both passive (through the CPP domain) and active (energy-dependent) uptake mechanism. This does not make sense since uptake is clearly inhibited at 4°C. Furthermore, SEM images in Fig. 2K (which are of rather poor resolution, higher quality images should be obtained) show the presence of ruffles/filipodia, which is a direct evidence of cytoskeleton, energy-dependent remodeling involving actin repolymerization.

Response: The residual fluorescence signal in the no-BMC control group may result from strong membrane adsorption of BMC@MPNs rather than true cellular uptake. To address this, we optimized the washing protocol by using 0.1% Tween 20 in PBS instead of PBS alone to remove uninternalized material. The revised data (Fig. 2j; Supplementary Fig. 12, relevant figures are attached below) show **no statistically significant difference** in normalized median fluorescence intensity (MFI) between the **4 °C group** and the **Blank group** ($p = 0.2912$, MFI was normalized to the non-inhibitor-treated group, denoted as NC), supporting that no uptake occurred under conditions where receptor activity and membrane fluidity are theoretically inhibited. Additionally, we have now updated TEM images for cell internalization study, which better reflect cell-BMC spatial distribution and interaction (Fig. 2k; Supplementary Fig. 13)

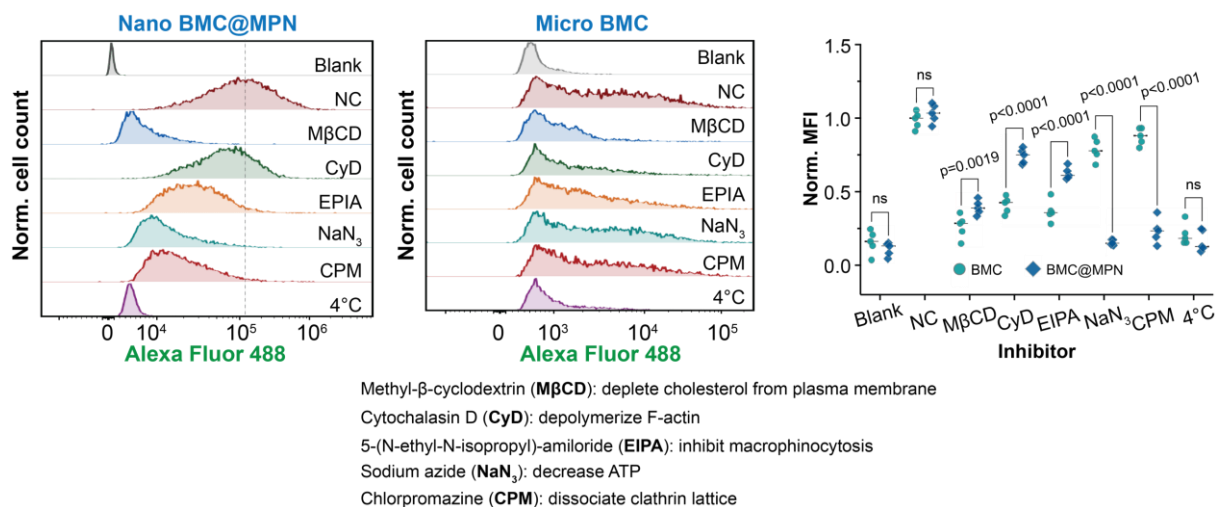


Fig 2j and Supplementary Fig. 12 | Flow cytometry raw data comparing cell internalization preference of nano BMC@MPN (a) and micro BMC (b) under the same inhibiting conditions. (c), Statistical comparison of MFI (data were normalized to NC, respectively) indicated that the cell internalization of nano BMC@MPNs and micro BMCs was inhibited at 4°C.

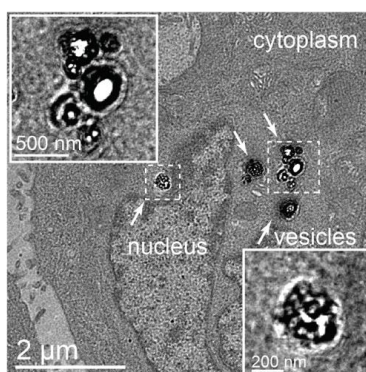
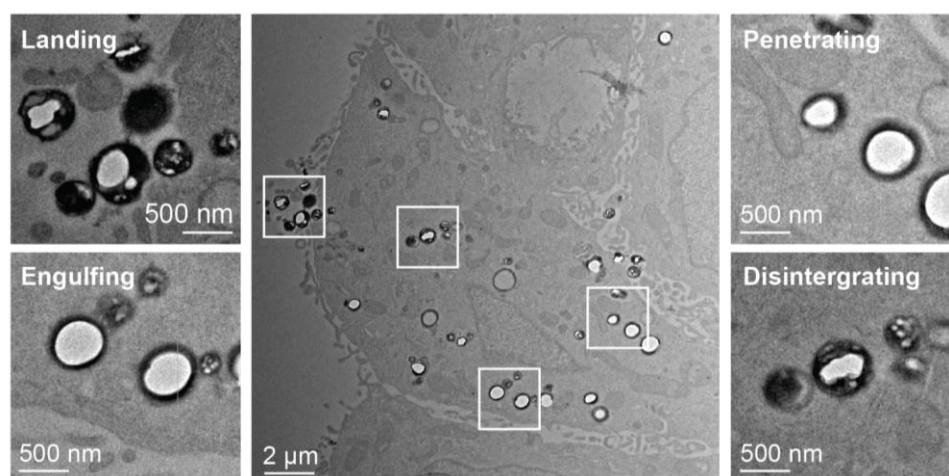


Fig. 2k, TEM images of BMC@MPNs after being internalized by SW480 cells. BMC@MPNs were enclosed within intracellular vesicles. The electron-sparse inner core corresponds to the BMC, while the electron-dense outer shell, rich in iron, represents the MPN coating.



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

In summary, the cell uptake mechanism, a critical aspect of BMC, has fundamental flaws, and the authors do not have conclusive evidence of how BMC crosses the cell membrane. Stating that uptake occurs through both passive and active pathways essentially means that any uptake pathway is possible, and the authors thus don't have a clear understanding of how BMC cross the cell membrane.

Response: In the revised manuscript, we have now provided additional experimental evidence using specific chemical inhibitors (Fig. 2h, j; Supplementary Fig. 12) and temperature-dependent uptake studies (4 °C vs. 37 °C) to distinguish active from passive pathways. These data collectively show that micro BMCs primarily rely on passive membrane fusion and deformation, while nano BMC@MPNs utilize receptor-mediated and energy-dependent endocytosis. Rather than suggesting that “any” pathway is possible, our findings reveal a size-dependent shift in internalization mechanisms, **highlighting passive uptake for larger condensates and active, pathway-specific uptake for nanoscale BMC@MPNs. This mechanistic distinction clarifies the uptake process and supports the rationale for engineering BMCs at the nanoscale to improve intracellular delivery and biosafety^{8,11}.**

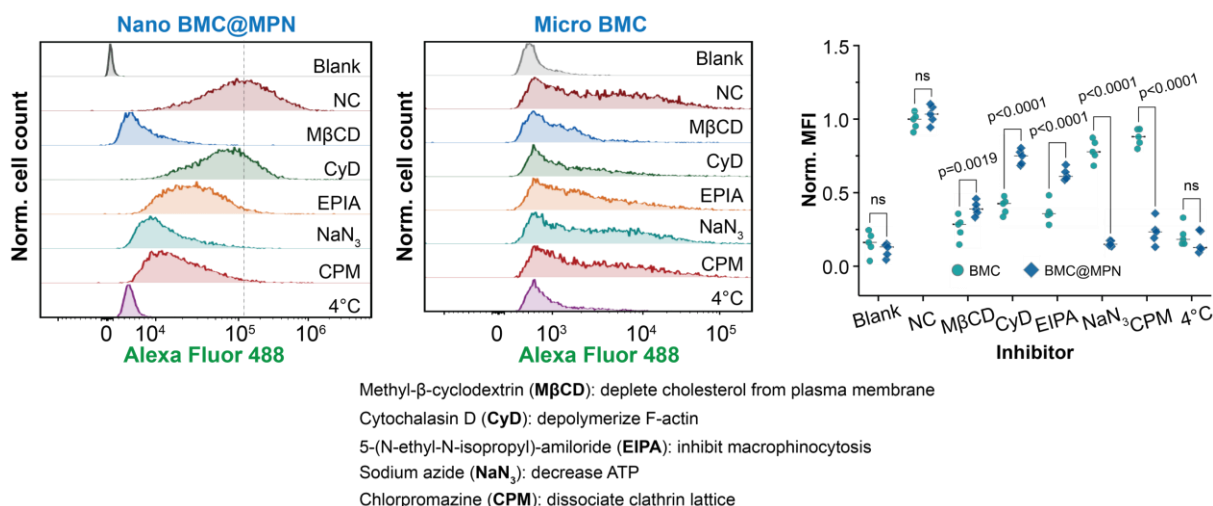


Fig 2j and Supplementary Fig. 12 | Flow cytometry raw data comparing cell internalization preference of nano BMC@MPN (a) and micro BMC (b) under the same inhibiting conditions. (c), Statistical comparison of MFI (data were normalized to NC, respectively) indicated that the cell interlunation of nano BMC@MPNs and micro BMCs was inhibited at 4°C.

2. It is not clear how the proteasome is recruited. Figure 1b suggests that inside cells, some of the cationic peptides remain bound to the antibody and these recruit the proteasome. Why/how do they remain bound to the antibody? Have the authors incubated positively-charged peptides only with antibody to see if it binds? If it does (as the proposed mechanism suggests), then given the cell penetrating properties of polyArg, is there actually a need to add the negatively-charged peptide? Control of complete coacervates (positive + negative peptides + antibody) versus antibody and cationic peptide only is missing.

Response: In our study, the antibody–peptide complexes were formed via electrostatic interactions. This approach, also reported in other protein–peptide systems^{12,13}, helps to preserve antibody bioactivity while enabling efficient peptide incorporation. In our case, the antibody–pArg complex

mediates specific recognition of KRAS G12V variants, followed by proteasome recruitment via the PTM domain to enable targeted protein degradation.

To demonstrate the complexation between positively charged pArg and negatively charged IgG, we incubated a fix amount of IgG (100 μ L 1mg/mL) with increasing amounts of pArg precursor (0, 0.5, 1, 5, 10, 25, 50, 100, 200, 500, 900 μ L, 1mM), adjusting the total volume to 1000 μ L before measuring zeta potential. Under these conditions, the number of particles (i.e., IgG molecules) remained constant across samples, allowing the zeta potential changes to reflect surface charge neutralization via electrostatic interactions with pArg. As shown in Fig. 2b, the zeta potential shifted from -9.4 ± 0.8 mV to -0.33 ± 1.2 mV and eventually to $+19.8 \pm 1.4$ mV. **This dose-dependent trend indicates that electrostatic interactions occur between IgG and pArg, with a fitted apparent K_D of 4.34μ M, which is a value comparable to reported non-covalent peptide–antibody complexes that retain biological activity in functional studies^{12,13}.**

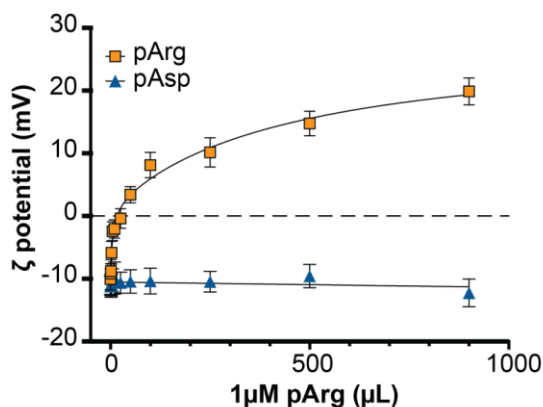
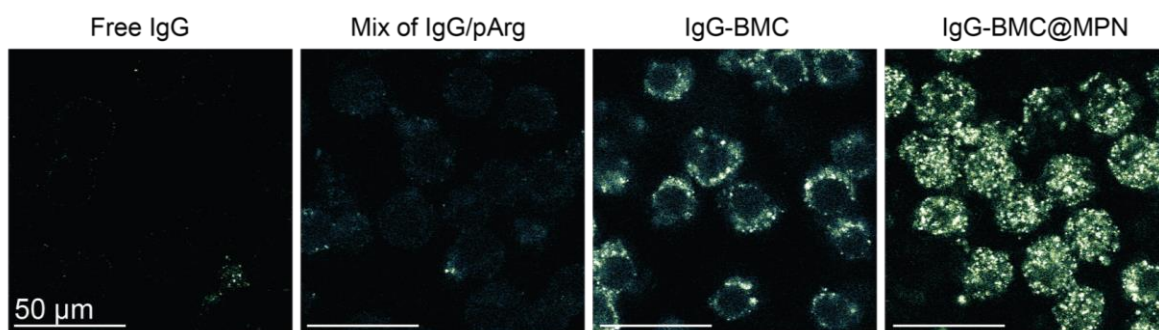


Fig. 2b | Zeta potential increased when 100 μ L of 1 mg/mL IgG was incubated with increasing concentrations of pArg precursor. pAsp precursor was used as a negative control, confirming that electrostatic interactions occur between IgG and positively charged pArg, but not with negatively charged pAsp.

To assess the role of BMC and BMC@MPN formation in IgG internalization and proteasome recruitment, we incubated SW480 cells overnight with free IgG, IgG–pArg mixture, IgG-BMC, or IgG-BMC@MPNs, and analyzed the intracellular distribution of FITC-labeled IgG by fluorescence microscopy (Supplementary Fig. 6, relevant figures are shown below). Free IgG showed no detectable internalization, while the IgG–pArg mixture produced only weak intracellular signals. In contrast, both IgG-BMC and IgG-BMC@MPNs exhibited markedly enhanced intracellular fluorescence, with BMC@MPNs showing the strongest signal. **These results demonstrate that BMC formation is required for efficient cellular uptake, and that the formation of BMC@MPNs further improves internalization.** This also supports the necessity of including pAsp (D₁₀ peptide) to facilitate condensate formation, as pArg/IgG mixtures alone do not form BMCs without the pAsp polyanionic counterpart.



Supplementary Fig. 6 | BMC formation is essential for cellular uptake. FITC-labeled IgGs were incubated with SW480 cells in four forms: free IgG, IgG mixed with pArg, micro BMCs, and nano BMC@MPNs. Only the BMC and BMC@MPN groups showed internalized IgG signals, indicating that condensate formation is required for effective IgG internalization.

3. How do the condensates disassemble inside the cell to release their IgG payload? This is critical to the overall design of the carriers, but the study does not have any data addressing this central question. Before in vivo studies are undertaken, it is important to understand the release mechanism of the condensates once it reaches the cytosol.

Response: We agree that understanding the intracellular release mechanism is critical for the design of effective delivery systems. While our current study focuses on demonstrating intracellular delivery efficiency, **we propose that once the MPN coating is disrupted upon endosomal escape^{14,15}, and the underlying condensates become susceptible to osmotic dilution. This shift from a condensed to a dilute phase promotes the release of encapsulated IgG into the cytosol.** This mechanism is supported by the inherent physical properties of synthetic LLPS-based systems, which are sensitive to changes in local concentration and ionic strength^{9,16,17}. In our Fig. 2f, we compared turbidity change of BMC and BMC@MPN in complete medium (10% FBS DMEM) as the diluted phase. **Our result indicated that without the MPN membrane, which will be disrupted during endosomal escape¹⁵ (Supplementary Fig. 14), BMCs (condensed phase) will spontaneously release guest molecules to the medium (diluted phase).**

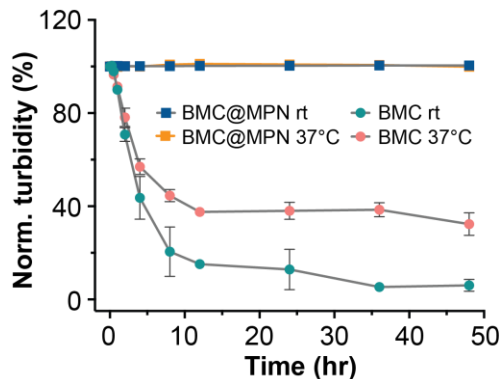
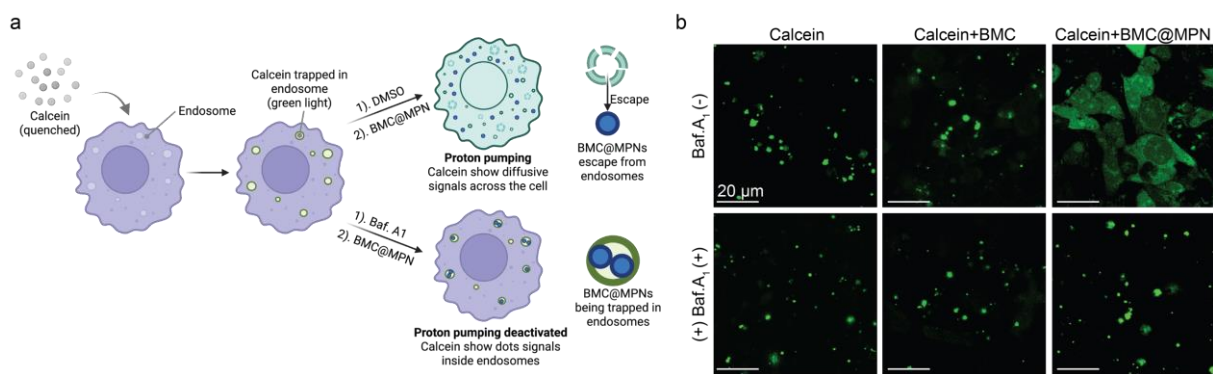


Fig. 2f | Turbidity of BMCs and BMC@MPNs at r.t. or 37°C in DMEM. 48-hour turbidity change showed improved colloidal and thermostability of BMCs after MPN coating.



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.

4. Another key question linked to the previous comment: once coacervates dissociate inside cell, wouldn't this release a vast excess of the positively charged peptides that would compete for proteasome binding?

Response: While coacervate dissociation may release free positively charged peptides, we believe this does not interfere significantly with proteasome binding or function for the following reasons. Firstly, our control experiments using the AAAG mutant, which retains charge but lacks the active proteasome-recruiting sequence, did not induce KRAS degradation despite similar overall peptide content. **This suggests that simple charge abundance is insufficient to drive degradation.**

Secondly, intracellular concentrations of **released peptides are diluted upon condensate disassembly**, further reducing the likelihood of nonspecific competition. Finally, given 100 μg pArg can condense 300 μg IgG, free pArg is expected to show weak influence on the overall degradation event. **These points together support that degradation is driven by sequence-specific, structured recruitment rather than nonspecific competition from excess peptides.**

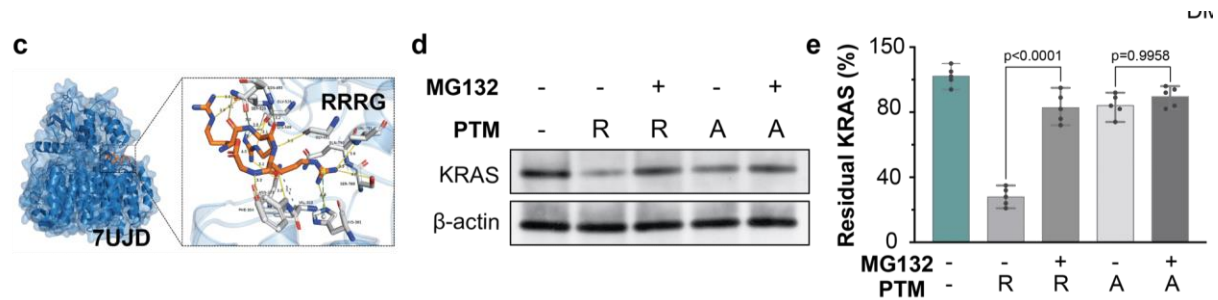


Fig. 5 | Proteasome-mediated degradation enables the disruption of intracellular targets. **c**, Representative pose of the interaction between PTM and the 7UJD proteasome regulatory subunits, shown in a space-filling representation. **d**, WB analysis of KRAS degradation using RRRG (R) or AAAG (A) negative control. Only the RRRG motif induced KRAS degradation, demonstrating that electrostatic interaction with PSMD9 is essential for proteasome recruitment. **e**, grayscale quantification histogram. **f**, 48-hr quantitative α -syn degradation analysis using SDS-PAGE and greyscale analysis.

5. The correct control to validate the proposed mechanism would be the CPD region fused to GG-AAAG (the authors use this as control in their simulations in Supp. Fig. 23). This should not lead to any target degradation (although KRAS would still be inhibited).

Response: This is a valid point. In response, we have now conducted the recommended control experiment using the CPD region fused to GG-AAAG, as proposed in our simulation (Supplementary Fig. 27). This construct did not result in KRAS degradation (residual KRAS: **24%** w PTM vs **82%** w/o PTM), although minor KRAS inhibition was still observed (residual KRAS: **87%**). These findings support our proposed mechanism and confirm that the presence of the full degradation module is required to induce targeted protein degradation. The new experimental data have been included in the revised manuscript (Fig. 5d-e).

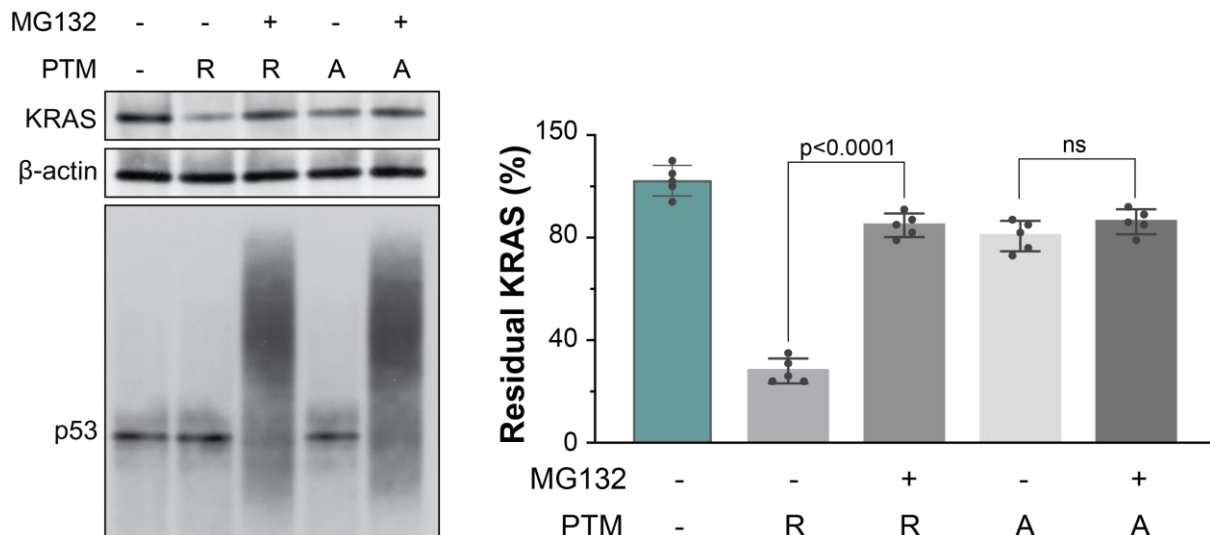
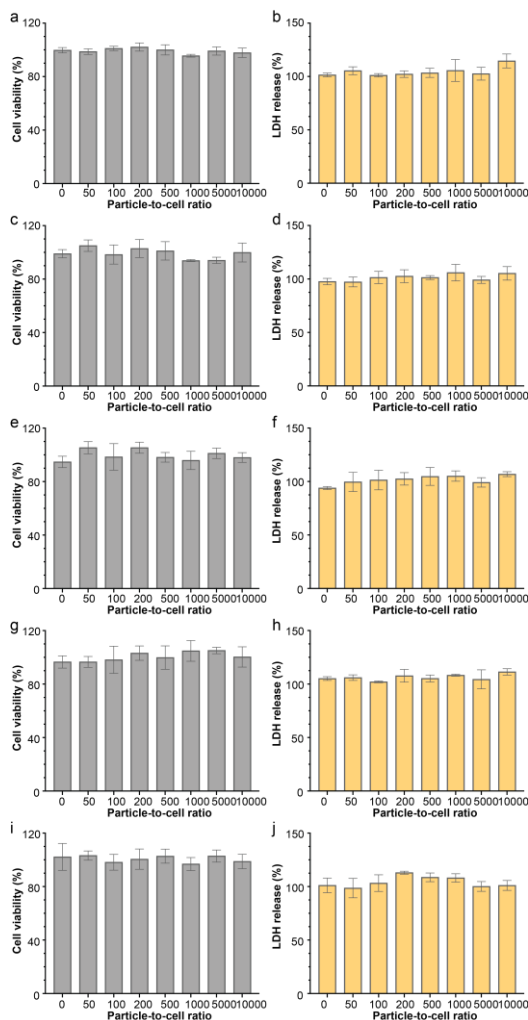


Fig. 5d,e | PTM is essential for KRAS degradation. Western blot (left) and quantification (right) show that KRAS degradation occurs only when the PTM motif (RRRG) is present and proteasome activity is intact (i.e., without MG132). In contrast, the AAAG control, which lacks proteasome recruitment capability, showed no significant reduction in KRAS levels.

6. Fig. 5g: it seems the dose titrations to evaluate LDH release was done only at one concentration? Typically, LDH release studies are carried out at different doses.

Response: In the original submission, LDH release was evaluated at a single concentration to assess potential cytotoxicity under standard treatment conditions. The experimental goal of our LDH assay differs from LDH release titration, which aims to evaluate the concentration-dependent cytotoxicity of the input. Our goal is to use LDH and CCK8 at fixed input concentrations as an orthogonal test to evaluate cell viability when exposed to different treatments. The optimized input concentration was defined as the saturated cellular uptake of BMC@MPN (cell-to-particle ratio of 1:1000) in our previous experimental sections.

In response to the reviewer's suggestion, we have now included a dose-response study assessing LDH release alongside the CCK8 assay at multiple concentrations in cell lines used in this study (Supplementary Fig. 32, revised). The results confirm that BMC@MPNs do not induce significant cytotoxicity across the tested dose range, further supporting the safety of our system.



Supplementary Fig. 27 | 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). BMC@MPNs have no cytotoxicity at particle-to-cell ratio up to 1:10000.

7. In terms of novelty, the authors are claiming in numerous instances that they are deploying condensates “for the first time” for *in vivo* studies. This claim is incorrect as they have been several studies demonstrating *in vivo* application of condensates with therapeutic efficacy, see in particular:

- i) Tian et al, *Small*, 2024 (<https://onlinelibrary.wiley.com/doi/10.1002/sml.202311890>) have developed complex condensates which that have used as oral delivery for *in vivo* study for colon cancer.
- ii) Bao et al, *JACS* 2025 (<https://pubs.acs.org/doi/full/10.1021/jacs.4c17054>) designed a condensate that delivered an antibody targeting degradation of EGFR, an approach that shares many parallels with the present study since it involves the delivery of an antibody that exploits the proteasome degradation pathway.
- iii) Bao et al, *JACS* 2025 (<https://pubs.acs.org/doi/full/10.1021/jacs.4c17054>) designed a condensate that delivered an antibody targeting degradation of EGFR, an approach that shares many parallels with the present study since it involves the delivery of an antibody that exploits the proteasome degradation pathway.

The authors have overlooked these recent studies, and thus the key novelty claim – “first *in vivo* demonstration of condensates-mediated delivery of therapeutics” – cannot be made.

Response: We have carefully reviewed the referenced studies and agree that they demonstrate important examples of *in vivo* application of condensate systems. However, we respectfully note that our manuscript differs in several fundamental aspects that contribute to its novelty. Specifically, our study introduces a proteasome-targeted condensate system designed for selective intracellular degradation of endogenous proteins—an approach not explored in the cited work, either in the precursor design or the guest molecules they used. In contrast to previous studies that primarily focus on condensate-mediated delivery or retention, our system integrates molecular design for cytosolic delivery, proteasome recruitment, and target degradation.

Furthermore, we demonstrate *in vivo* efficacy using engineered nano-scale BMC@MPNs, which **outperformed the current micro-sized condensate design in terms of cell entry**, which is a key barrier to intracellular delivery and degradation in a physiologically relevant model, while remaining understudied in the LLPS field. We have now revised the manuscript to clarify these distinctions and avoid generalized claims of “first-time” application, while still emphasizing the unique mechanistic and functional innovations of our system.

We also point out the fundamental difference that distinguishes our research from the cited work below.

- iv) Tian et al, *Small*, 2024 (<https://onlinelibrary.wiley.com/doi/10.1002/sml.202311890>) have developed complex condensates which that have used as oral delivery for *in vivo* study for colon cancer.

Response: We believe our research distinguishes from this work in the following points: A key strength of our work compared to Tian et al. lies in the **fundamental difference between the guest molecules**. Their system was demonstrated using Emodin, a small molecule (270.2 Da) with a rigid, planar structure and well-established chemical stability. Emodin can be readily encapsulated by a wide range of carriers, including liposomes, polymeric nanoparticles, and micelles. In contrast, our system is designed to encapsulate and deliver full-length IgG, a large (~150 kDa), Y-shaped glycoprotein

with high molecular weight, complex tertiary and quaternary structure, and sensitivity to pH, temperature, and enzymatic degradation. Unlike small molecules, IgG delivery requires preventing aggregation, and avoiding premature degradation or clearance, making its intracellular delivery particularly challenging. Successfully achieving this with our platform highlights its robustness and suitability for delivering structurally delicate biologics—challenges not addressed in Tian et al.'s small-molecule model.

- v) *Bao et al, JACS 2025 (<https://pubs.acs.org/doi/full/10.1021/jacs.4c17054>) designed a condensate that delivered an antibody targeting degradation of EGFR, an approach that shares many parallels with the present study since it involves the delivery of an antibody that exploits the proteasome degradation pathway.*

Response: We believe our research distinguishes from this work in the following points:

1. A key strength of our approach from Bao et al's work is its complete reliance on native cellular components: we target endogenous proteins using the unmodified proteasome, without introducing exogenous degradation machinery. This design more faithfully mimics physiological conditions and enables a more accurate investigation of BMC behavior in the native tumor microenvironment, significantly enhancing translational relevance. Bao et al.'s work relies on the co-expression of TRIM21, an exogenous E3 ubiquitin ligase, to degrade EGFR, an exogenously introduced protein. In a separate demonstration, they targeted endogenous EZH2, a histone-lysine N-methyltransferase, which represents a distinct therapeutic context from our work. In contrast, our study focuses on endogenous KRAS and α -synuclein, clinically validated targets in cancer and neurodegenerative diseases, respectively. **This highlights a fundamentally different therapeutic strategy.**
2. Furthermore, **our work reported comprehensive and informative *in vivo* data.** Beyond demonstrating antitumor efficacy, we include a detailed evaluation of biosafety, including serum chemistry, body weight tracking, and organ-level histopathology—elements that are absent from Bao et al.'s study.
3. Finally, it is important to note that the proteins targeted by Bao et al., EGFR (~180 kDa) and EZH2 (~86 kDa), are considerably larger than KRAS (~21 kDa) and α -synuclein (~15 kDa). Larger proteins offer more surface area and conformational flexibility, making them more susceptible to binding by a wide range of degraders beyond antibodies. **In contrast, small, compact proteins like KRAS present a greater challenge due to limited accessible epitopes and high structural stability.** Successfully targeting such proteins highlights the precision and robustness of our strategy.

- vi) *Wen et al, Nature Chemistry 2024 (<https://www.nature.com/articles/s41557-024-01705-8>) have developed ssDNA/histone condensates with the ability to recruit different type of therapeutics. They also conducted in vivo studies on tumor-bearing mice, in that case by treating the tumor with a condensate packaged with the oncolytic adenovirus serotype 11 (ad11), which they demonstrated induced a strong anti-tumor immune response. The target is different from the present study, but the authors carried out in vivo studies using BMCs as a carrier.*

Response: We believe our research distinguishes itself from this work in the following points:

Wen et al's work focused more on extracellular interactions (i.e., oncolytic virus residence and immune response), while our study focused more on intracellular events. Besides our protein of interest, we also provided insights regarding how other intracellular activity was influenced (e.g., proliferation, apoptosis). Lastly, we incorporated a proteasome-targeting motif into our precursor, making it a bifunctional element in addition to its ability to induce LLPS.

Moreover, **we wish to reinforce the novelty of our work as the first *in vivo* demonstration of nano-sized condensates (i.e., nanoBMCs).** To support this, we (1) highlight the advantages of nanoBMCs over microBMCs, and (2) we believe that the references discussed here substantiated the growing interest and potential of using LLPS materials *in vivo*; however, **none** involve nanoscale systems with the uptake benefits shown in our design, which is a key advantage of our approach of (1) increasing overall cytosolic delivery efficacy and (2) avoiding cell membrane disruption. Additionally, two out of the three micro LLPS material references (*Nat. Chem.* and *JACS*) were published the same time as we submitted our manuscript, which further indicated our research in the leading place in the area.

*8. P5, L9-11: The “dogma” that delivery vehicles should be in the 100’s of nm size range may be established for more solid-like nanocarriers (e.g. LNPs or polymer NPs) but there is no evidence that it remains true with viscoelastic entities such as condensates. As a matter of fact, the studies mentioned above achieved good efficacy despite the condensates being in the micron-scale range. For example, in Bao et al (JACS, 2025), the droplets were 1-2 μm . In Tian et al (Small, 2024), efficient oral administration was achieved with complex coacervates $\sim 3 \mu\text{m}$ in size. And in Wen et al (Nature Chemistry 2024), the droplets were about 2 μm . In all these recent examples, *in vivo* efficacy was achieved with condensates above 1 μm in size. Thus, the notion that nano-scale carriers are better for *in vivo* studies has not been properly tested for BMCs and the claim that *in vivo* efficacy was achieved because the condensates were sub-micron is not experimentally substantiated.*

Response: We agree that the optimal size range for BMCs *in vivo* remains an open question, especially given their viscoelastic nature, which may distinguish them from conventional solid-like nanocarriers. These three studies indeed demonstrate that micron-scale condensates can achieve therapeutic efficacy *in vivo*, and we have revised the manuscript to acknowledge these recent findings.

However, our study provides a distinct perspective by **comparing micro- and nano-scale BMCs of the identical chemical composition and functional architecture.** Our results show that nano-scale BMC@MPNs achieve significantly higher cytosolic delivery efficiency and target degradation, which we attribute to improved cellular uptake and access to energy-dependent internalization pathways that are less accessible to larger, micron-scale condensates. **While we do not claim that nano-scale size is universally superior for all BMC applications, our data suggest that reducing size enhances functional delivery efficiency in our system.**

Additionally, in contrast to current micro-scale BMC systems, which often rely on passive mechanisms such as membrane wetting, scission, bending, or fusion, and have been reported to induce membrane rupture or morphological changes in cells and vesicles^{4,18}, our nanoBMC@MPNs exhibit a distinct active endocytic uptake behavior. **We believe this shift toward active endocytosis contributes to enhanced intracellular delivery efficiency and helps**

to reduce the risk of membrane disruption, addressing key safety and mechanistic concerns previously associated with microBMC-based delivery platforms.

We have now revised the manuscript to emphasize this distinction and its implications for safe and effective intracellular delivery, while acknowledging that other studies have achieved efficacy with micron-scale condensates. **We believe our work complements existing literature by systematically evaluating how condensate size impacts delivery behavior and degradation efficacy in a proteasome-targeted context.**

9. There are several concerns regarding the animal experiments:

i) The plot of Fig. 4b indicates a tumor about 30 mm³ 4 days after injection. Assuming a spherical tumor, its diameter is around 3-4 mm, which is very small. It is very difficult to conduct intratumoral injection for very small tumors. What was the volume of injection? What would be the effect of the treatment on bigger tumor? An initial tumor size of about 100 mm³ would be more representative of a human tumor.

Response: The injection volume used in our study was **10 μ L**, which we found feasible for intratumoral administration even in small tumors with diameters of approximately 3–4 mm. We agree that larger tumor models are more commonly used in preclinical studies to better mimic human tumor conditions. However, given the lack of references for *in vivo* delivery of nano BMCs in the LLPS field (i.e., dosing consideration, injection volume, time duration), we opted for early-stage tumors to minimize variability and facilitate initial evaluation of delivery efficiency and therapeutic response.

We agree that further optimization, particularly in larger, more clinically relevant tumors, is necessary to fully assess translational potential. We have noted this limitation in the revised manuscript. Our follow-up studies will aim at addressing delivery performance in more advanced tumor models.

ii) Why some tumors after treatment have some blue/dark part to compare with PBS?

Response: The blue/dark coloration observed in some treated tumors is due to the presence of the metal–phenolic network (MPN) coating, which imparts a characteristic color to the BMC@MPNs (indicated in our Fig. 2d). We have clarified this point in the revised figure legend of Fig. 4c.

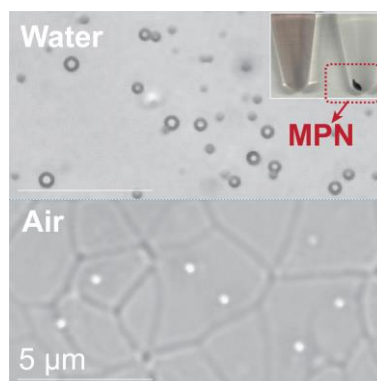


Fig. 2d | In Fig. 2d, we show a photograph of BMC@MPNs after centrifugation, displaying a characteristic dark blue color attributed to the MPN coating.

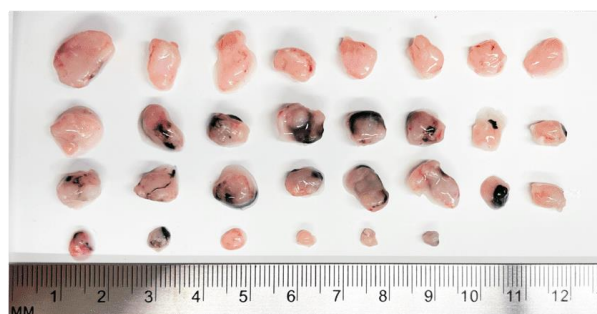


Fig. 4c | **c**, Representative tumor images at the endpoint. From top to bottom: PBS, BMC@MPN, isotype-BMC@MPN, and anti-KRAS-BMC@MPNs-treated groups. Dark blue color in the tumor is the color of MPN coatings.

iii) What is the biodistribution after injection? Do BMCs stay in tumor or some escape and accumulate in the body, especially considering the high concentration and multiple injections (3 times a week for 2 weeks)?

Response: Our study was designed to assess therapeutic efficacy after intratumoral dosing, and although we initiated exploratory biodistribution tests, the metal–phenolic network (MPN) shell markedly quenches conventional fluorophores. Consequently, standard fluorescence-based in vivo imaging at our administered doses did not yield stable signals¹⁹; accordingly, we cannot provide conclusive biodistribution data at this stage. We fully agree that defining the in vivo fate of BMC@MPNs, particularly under repeated dosing, is essential. We are actively optimizing MPN-compatible readouts and will pursue follow-up studies using elemental tracking and related approaches to quantify biodistribution, retention, clearance, and potential off-target accumulation.

iv) P11, L16-17. The authors are stating that “IgG-BMC@MPNs is safe and effective TPD modalities for in vivo anti-tumor therapy”. This is only true for the specific condition they have carried out, but a proper toxicology study needs to be performed to validate such affirmation and intratumoral injections does not fully demonstrate in vivo activity or safety. What is the fate of the BMCs after intravenous (IV) injection?

Response: agree that comprehensive toxicological evaluation, particularly under systemic administration, is essential for fully validating the safety profile of IgG-BMC@MPNs. **To support this, we conducted serum biochemistry analysis of 15 biomarkers covering osmotic balance** (PO_4^{3-} , Na^+ , K^+ , Ca^{2+}), **chemical homeostasis** (alkaline phosphatase, alanine transaminase, amylase, albumin, total bilirubin, total protein, blood urea nitrogen, glucose), **and immune response** (creatinine, globulin), all of which showed no significant abnormalities following repeated treatment, suggesting no acute systemic toxicity under the tested conditions²⁰.

We agree that intravenous (IV) administration represents an important direction for future development; however, it falls outside the scope of the current study of investigating the variant-specific degradation capability of BMC@MPNs. We are actively planning follow-up investigations to assess the biodistribution, pharmacokinetics, and safety profile of BMC@MPNs under systemic delivery.

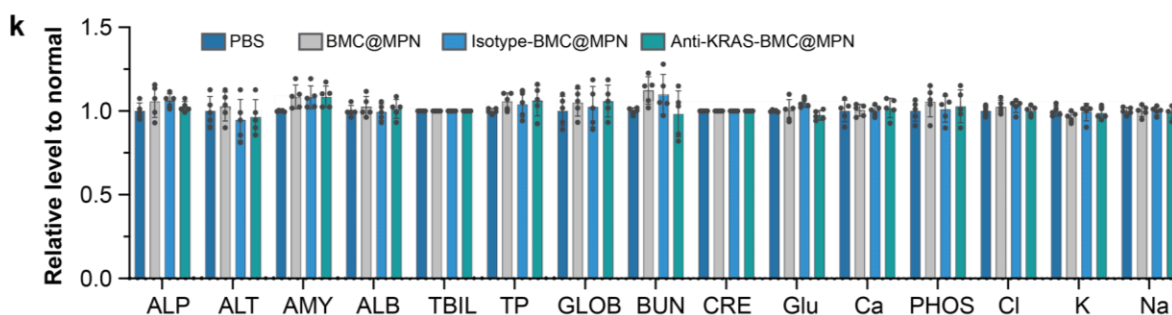


Fig. 4k | Serum biochemistry analysis of nude mice at the endpoint. ALP, alkaline phosphatase; ALT, alanine transaminase; AMY, amylase; ALB, albumin; TBIL, total bilirubin; TP, total protein; BUN, blood urea nitrogen; CRE, creatinine; GLOB, globulin (calculated); Glu, glucose; Ca, calcium; PHOS, phosphorus; Cl, chloride; K, potassium; Na, sodium.

v) PolyArg is a very generic cell-penetrating module and works well on many cell types. It will be very hard to introduce cell-specificity using this system, so utility for specific cell/organ targeting would be limited. Also, despite statements often made in the literature, there are studies showing PolyArg can be toxic inside cells (see for example <https://pmc.ncbi.nlm.nih.gov/articles/PMC10571751/>).

Response: We agree that polyarginine is a broadly acting cell-penetrating peptide and does not inherently confer cell- or organ-specific targeting. In our system, **we introduce metal-phenolic network (MPN) coating that could serve as a modular and multifunctional interface that allows further surface engineering.** MPNs have been shown to readily incorporate various functional ligands, including targeting peptides, antibodies, and aptamers, enabling programmable cell-specific delivery^{14,21,22}. While we mainly focus on developing BMC-category material in this study, we envision leveraging the advantage of MPN coating as well as other coating strategies in future studies to achieve targeted delivery without relying on pArg.

Additionally, in our platform, the pArg as a minimalist LLPS precursor is not confined to its cell-penetrating properties. Rather, it plays a dual role: (1) facilitating phase separation to form biomolecular condensates with IgG, and (2) enabling selective proteasome recruitment *via* the

proteasome-targeting motif (PTM). **This functional integration distinguishes our system from conventional polyArg-based delivery tools.**

Finally, the goal of the current study is to establish proof-of-concept for the *in vivo* application of nano-scale condensates and their ability to mediate intracellular KRAS degradation. We have now revised the manuscript to clarify these points and acknowledged future opportunities to incorporate targeting elements through MPN surface functionalization.

Reviewer #2 (Comments for the Author):

This article describes the synthesis and in vitro and in vivo characterization of a novel system for intracellular delivery of antibodies which is also designed to engage the proteasome for target protein degradation. The system consists of biomolecular condensates between cargo antibodies and peptides that have cell-penetrating and proteasome engagement functions. The BMCs are covered in a semipermeable metal-phenolic network to enhance their stability.

In vitro studies are very comprehensive and include demonstration of the biophysical properties of the BMCs, the extent and mode of their cell uptake, and their ability to degrade a specific intracellular mutant protein (KRASG12V).

An in vivo study is also presented. This is limited to administration of epitope-specific BMCs (by intratumoral injection) in a single murine model (SW480 xenograft). The reported data indicate that intratumoral targeting of KRAS has been achieved.

The authors also show some more preliminary data concerning the use of the delivery system to downregulate α -synuclein, as a demonstration of the generalizability of the approach.

*The main claim to novelty is that BMCs which are designed to target specific disease-related molecules have not previously been shown to perform as intended in vivo before. This claim seems generally well-founded. There is a published report of a synthesised BMC being tested in mice – although the intent was to control gene expression rather than to degrade protein as in the current study. Nevertheless, the authors may think it appropriate to cite that work i.e. Fischer AAM et al. *Small* 2024, 20:2311834.*

The methodology is in general robust and, except for a few minor flaws (see below), the quality of presentation is very good. In general, the writing style of the paper is clear and easy to follow. The abstract/introduction and conclusions are appropriate and contain relevant information. The paper is appropriately referenced without any obvious omissions in the list of references. Overall the conclusions are justified but some discussion of possible limitations of this delivery system should be included in the conclusions section.

Response: We appreciate the recognition of the novelty of our manuscript, as well as the constructive feedback provided, which helped us strengthen the manuscript.

In response to the Reviewer's suggestion, we have now included the work by Fischer AAM et al. (*Small* 2024, 20:2311834) in the revised manuscript and acknowledged its relevance as an important example of in vivo application of synthetic BMCs. We have expanded our Introduction regarding frontier in vivo applications of BMCs, alongside how our work distinguishes from these in regards to precursor design, guest molecule component, and therapeutic scope.

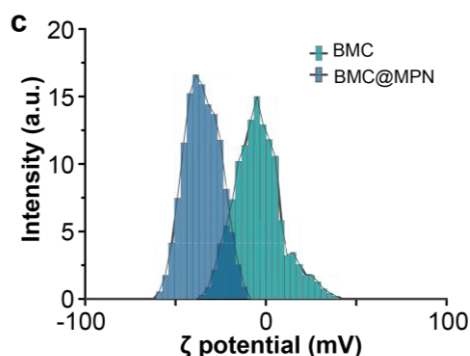
In addition to citing Fischer AAM et al's work as an important example in the synthetic biology field, we have also discussed recent *in vivo* studies by Tian et al. (*Small* 2024, 20(33), 2311890), Bao et al. (*JACS* 2025, 4c17054), and Wen et al. (*Nat. Chem.* 2025, 17, 279–288) as representative applications of BMCs in our revised Introduction section. We have revised the manuscript to highlight how our work distinguishes from these studies by using **nano-scale BMCs** for the intracellular delivery of intact macromolecules (IgGs) and enabling targeted protein degradation. This represents a critical and largely unmet challenge in biomedical engineering that remains underexplored in the field of LLPS. To the best of our knowledge, this specific application has not yet been demonstrated in the existing literature.

Additionally, we have expanded the discussion section to discuss potential limitations of our delivery system, including the need for further evaluation of biodistribution, safety under systemic administration, and opportunities for achieving cell-specific targeting in future studies.

Specific points for the authors to consider:

It is the positive charge of arginine-rich peptides that confers their cell-penetrating properties. However, in the BMCs described in this paper the positive charge is deliberately neutralized through mixing with D10. How, therefore, is internalization effected?

Response: While it is true that the positive charge of arginine-rich peptides contributes to their cell-penetrating properties, our system highlights the cell entry benefits associated with nano condensates. Upon formation of BMCs with D₁₀, the net surface charge overturned to -33 ± 0.8 mV; Moreover, the resulting condensates present a multivalent and dynamic interface that facilitates membrane interaction and internalization, which outperformed commercial transfection reagents, Pro-Ject (a lipid nanoparticle-based reagent) and X-fect (a CPP-based reagent) by roughly two-fold (Fig. 2h). Our revised data (Fig. 2j) show that nano-scale BMC@MPNs utilize energy-dependent endocytic pathways—including clathrin-mediated uptake—rather than relying solely on electrostatic-driven membrane penetration. **This is further supported by uptake inhibition studies and temperature-dependent assays, demonstrating that internalization is actively mediated, not only dependent on charge.**



Supplementary Fig. 3c, Zeta potential data of BMC and BMC@MPN indicating improved colloidal stability after MPN coating.

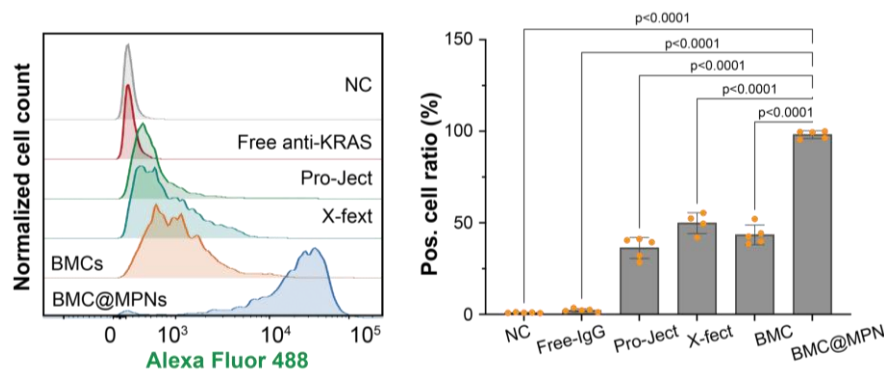


Fig. 2h | Flow cytometry raw data comparing cell internalization efficacy among free-IgG, commercial transfection reagents (Pro-Ject and X-ject), BMCs, and BMC@MPN. BMC@MPNs have 99% transfection efficacy (quantified by positive cell ratio), outperforming BMCs (42%), X-ject (51%), Pro-Ject (39%), and free-IgGs (2%).

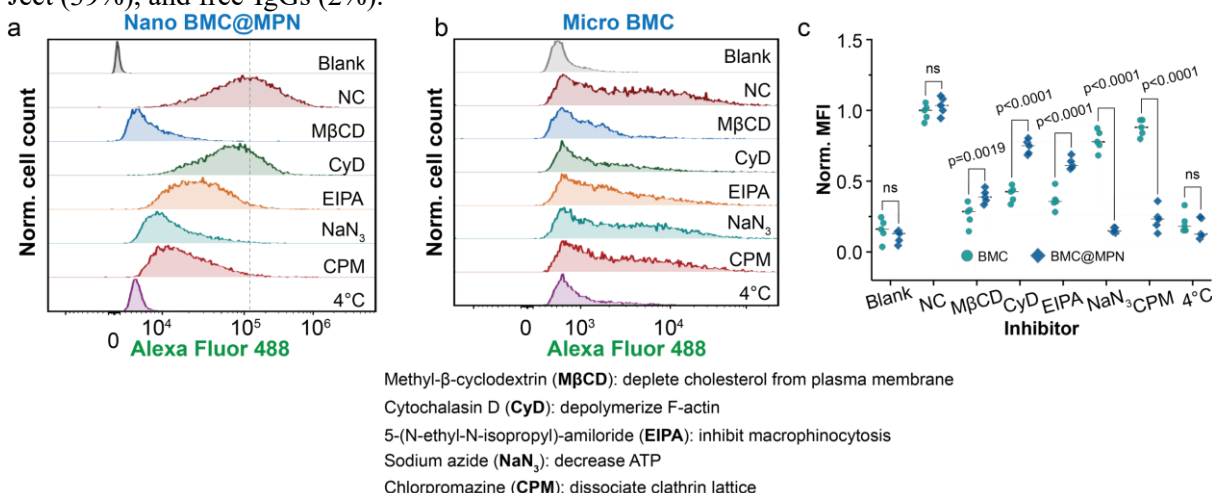


Fig. 2j and Supplementary Fig. 12 | Flow cytometry raw data comparing cell internalization preference of nano BMC@MPN (a) and micro BMC (b) under the same inhibiting conditions. (c), Statistical comparison of MFI (data were normalized to NC, respectively) indicated nano BMC@MPNs exploit energy-dependent (inhibited by NaN₃) and clathrin-mediated endocytosis (inhibited by CPM), and are less dependent on pinocytosis (inhibited by EIPA), actin polymerization (inhibited by CyD).

Please explain the relevance of cyclization of the R8 peptide.

Response: Cyclic CPPs have been shown to exhibit improved stability against proteolytic degradation and enhanced cellular uptake compared to their linear counterparts^{23,24}.

In the introduction the authors write that “naked antibodies with cell-penetrating peptides, can provoke an unwanted immune response”. What evidence is there for this? No references to justify this statement are provided.

Response: We have now cited the following references in the revised manuscript to support this statement:

Ref 23: Beck et al., *Nat. Rev. Drug. Discov.* 16, 315–337 (2017)

Ref 24: Fu et al, *Signal Transduct. Target. Ther.* 7, 93 (2022)

While the immunogenicity of antibody–drug conjugates (ADCs) has been improved in current-generation designs, it remains a critical challenge due to the complexity of these constructs. These concerns highlight the importance of controlled antibody presentation, such as through encapsulation or condensation strategies like our BMC@MPN platform, to mitigate immune responses and improve therapeutic performance^{25,26}.

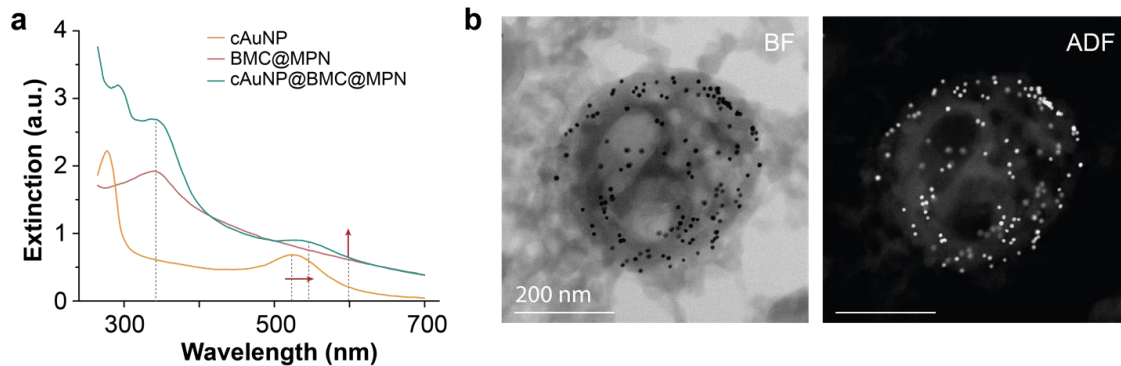
Is it possible to calculate the number of copies of IgG per BMC? This information together with the number of particles uptaken into an individual cell – would provide the total number of IgG that accumulate on average in a cell. How does this compare to the number of copies of the intracellular target? Is it necessary to engage every copy of KRAS for the cytotoxic effect that has been shown?

Response: To address this point, we designed quantification experiments to study (1) the number of IgG molecules loaded per BMC particle, (2) the total intracellular IgG accumulation per cell, and (3) the relationship between delivered IgG and intracellular KRAS copy number. The experimental design, rationale, and results are summarized below:

1. Quantification of IgG loading per BMC using ICP-MS and TEM:

To determine the number of IgG molecules encapsulated per BMC particle, we labelled anti-KRAS IgG with colloidal gold nanoparticles (cAuNPs, 10 nm). The total gold content within the purified IgG-BMC@MPNs was quantified using **inductively coupled plasma mass spectrometry (ICP-MS)**. The amount of encapsulated IgG was calculated based on the known stoichiometry of cAuNPs per IgG molecule according to the manufacturer's [data sheet](#) below (the highlighted line corresponds to the particle used in this study). The total number of IgG molecules loaded was divided by the total BMC particle count (**measured by nanoparticle tracking analysis, NTA**) to determine the average number of IgG molecules per BMC particle. Using this approach, we calculated that each BMC encapsulates approximately **1500** copies of IgG per particle. **This number is aligned with our TEM images of cAuNP-IgG-BMC@MPNs (Supplementary Fig. 7), and 150 cAuNPs on average were condensed per BMC@MPNs, which equals 1050-1800 IgG molecules per particle.**

Particle Diameter	#Au atoms	MWt. (daltons)	#particles/ml	#Ab/(part.)
6 nm	6500	$1.3 \cdot 10^6$	$2.4 \cdot 10^{13}$	1-2
10 nm	$30 \cdot 10^3$	$6 \cdot 10^6$	$5 \cdot 10^{12}$	7-12



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.

2. Quantification of BMC uptake per cell using confocal microscope (supported by our current data, Fig. 2i):

To determine the number of BMC particles internalized per cell, SW480 cells were incubated with fluorescently labeled BMC@MPNs for 4 hrs, followed by confocal microscope analysis. Single-particle segmentation using ImageJ allows us to estimate that, on average, **60 BMC@MPN particles are internalized per cell under experimental conditions.**

3. Calculation of total intracellular IgG accumulation per cell:

The total number of intracellular IgG molecules per cell was calculated by multiplying the number of internalized BMC particles (**60**) by the average number of IgG molecules loaded per particle (**1500**), resulting in approximately **60*1500=90,000** IgG molecules per cell.

4. Stoichiometric calculation between delivered IgG and target KRAS:

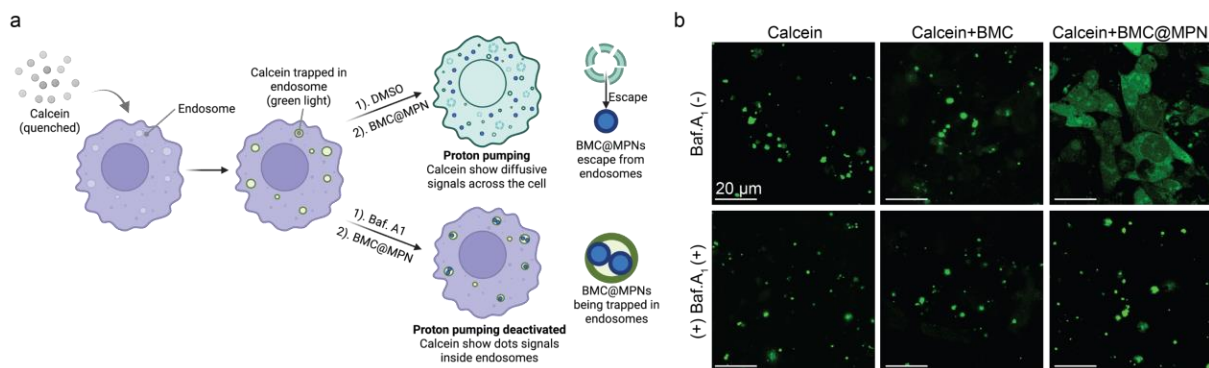
Total KRAS depends on species and cell size, which can vary from a range of ~50,000–550,000 total Ras copies per cell, with KRAS representing 50–75% of total Ras, reported by a PSAQ (protein-standard absolute quantitation) study²⁷. **Based on these estimates, the intracellular IgG delivered by BMC@MPNs is sufficient to engage approximately 45–100% of endogenous KRAS molecules per cell.** It is worth noting that complete engagement of all KRAS copies is not required for functional degradation, given the cell survival dependence on KRAS mutants²⁸. Prior studies have demonstrated that partial suppression of KRAS signaling is sufficient to impair downstream pathways and elicit phenotypic effects²⁹⁻³². This is consistent with our observations that ~60–70% reduction in

KRAS protein levels produces measurable cytotoxic effects without requiring full occupancy of all target molecules.

The experiment with endocytic inhibitors suggests cholesterol dependent-endocytosis as the main mode of cell entry. But how then do the BMCs escape endosomes? There is a paucity of data in the paper about the intracellular fate of the BMCs or how they are trafficked. Studies using endosome/lysosome markers may illuminate this. For example, in to Fig. 2i, are the BMCs (AF488) free in the cytosol or trapped in an organelle?

Response: We agree that understanding the intracellular fate of BMCs is critical to fully elucidating their mechanism of action.

The MPN coating in our system has been well-studied for its capacity to facilitate endosomal escape via the proton sponge effect, as demonstrated in previous studies. This property contributes to the efficient cytosolic delivery observed in our system. In response to this comment, we have now included the calcein leakage assay to confirm the endosomal escape capability of BMC@MPNs in our revised manuscript (Supplementary Fig. 14). Briefly, the **calcein leakage assay** assesses endosomal escape by detecting increased fluorescence when self-quenched calcein is released from endosomes into the cytosol, where dilution relieves quenching. A diffuse fluorescence signal indicates successful endosomal disruption, while a punctate fluorescence suggests entrapment.

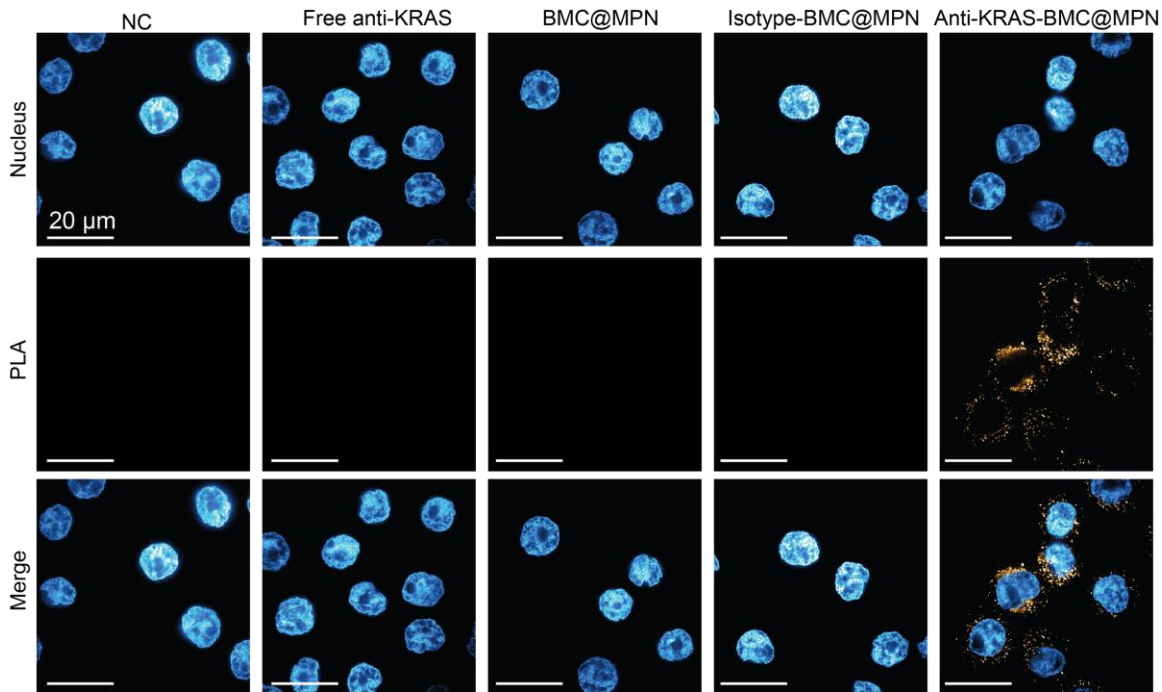


Supplementary Fig. 14 | MPN facilitates endosomal escape of BMC@MPNs. **(a).** Schematic of calcein leakage experiment upon non-treated condition or with bafilomycin A1 (an inhibitor of V-ATPase and termination of the inflow of H^+ and Cl^-). **(b)** CLSM images of SW480 cells incubated with calcein, 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.

Supp Fig. 13 doesn't really support the claim "that the cargo antibody binds KRAS", as it simply shows that both the antibody and KRAS are located mainly in the cytosol. The authors could consider, for example, proximity ligation assays to address this point.

Response: We fully agree that direct evidence of intracellular antibody-KRAS engagement would strengthen our conclusion. In response, we have now performed additional proximity ligation assays (PLA) to assess the interaction between the delivered antibody and endogenous KRAS within cells. As shown in the revised Supplementary Fig.15, puncta PLA signals were observed only in cells treated with anti-KRAS-BMC@MPNs, while minimal or no dot signals were detected

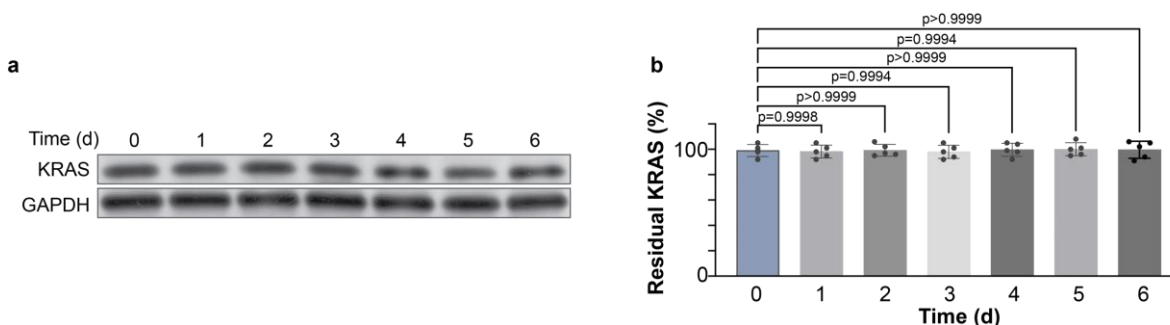
in cells treated with free anti-KRAS, empty BMC@MPNs, or isotype-BMC@MPNs. These results directly demonstrate that the cytosolically delivered antibody engages KRAS, further supporting our original claim. The new data and corresponding descriptions have been included in the revised manuscript.



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.

Ideally the WB in Fig. 3b should show KRAS expression following incubation with a non-specific isotype antibody-BMC

Response: In the revised manuscript, we have now included the additional control group using isotype-IgG-BMC@MPNs in the western blot analysis shown in the updated Supplementary Fig. 16. The result showed that treatment with isotype-BMC@MPNs did not alter KRAS expression levels, further confirming that **KRAS degradation is dependent on specific antibody-target engagement rather than nonspecific effects of BMC@MPN delivery**. The revised data and corresponding description have been incorporated into the manuscript.



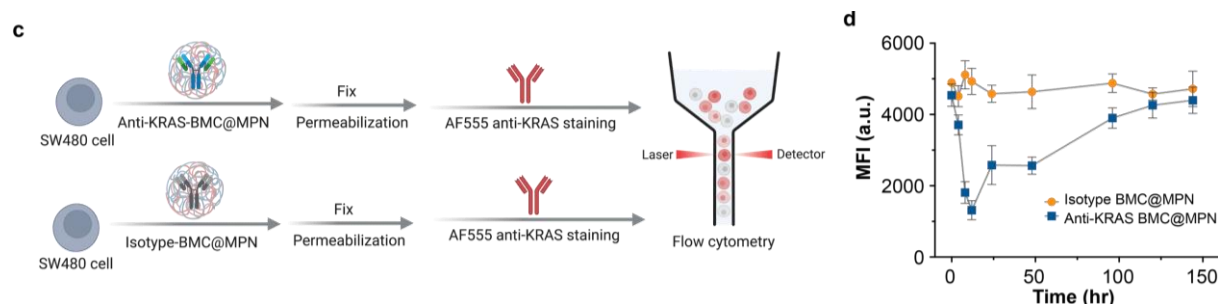
Supplementary Fig. 16 | No degradation was observed in cells treated with isotype-BMC@MPNs, serving as the negative control.

Fig. 3c: shows KRAS levels returning to normal by 6 days. Presumably this occurs due to incomplete degradation of KRAS which could be a limitation of this approach. The authors should discuss this observation.

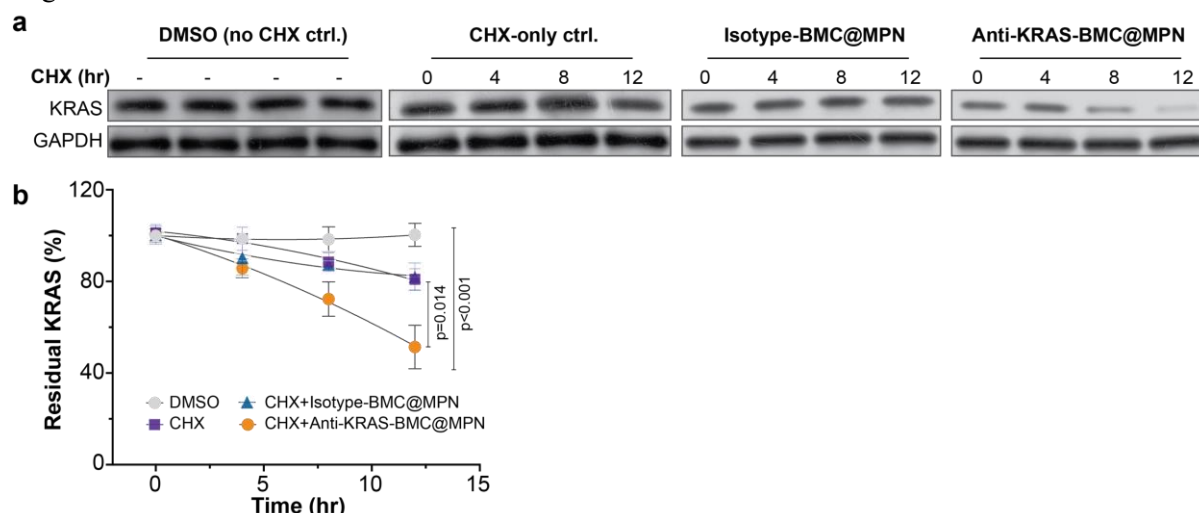
Response: Our results showed that KRAS levels gradually return to baseline over 6 days following a single treatment, **which reflects the dynamic balance between KRAS degradation and ongoing protein synthesis**. In this experiment, **no global protein synthesis inhibitor (such as cycloheximide, CHX) was applied during this period; thus, endogenous KRAS synthesis continues throughout**. Literature reports estimate that the half-life of KRAS proteins ranges from approximately 22 to 24 hours^{29,33}, depending on isoform, cell type, and cellular context. Therefore, following initial depletion, newly synthesized KRAS gradually replenishes the intracellular pool over time.

This recovery reflects the physiological turnover of KRAS. Indeed, the observed maximal reduction (~67% at 48 hours) demonstrates that IgG-BMC@MPNs can effectively engage and degrade a significant portion of mutant KRAS in its native expression context without the need to suppress global translation. While repeated or sustained delivery may further enhance long-term KRAS suppression, our results highlight the platform's ability to induce substantial degradation without halting protein synthesis, thus preserving overall cell function and viability during treatment. We have now added this discussion to the revised manuscript.

In addition, **we performed a CHX-chase assay to directly measure KRAS degradation half-life under conditions where new protein synthesis is blocked**. As shown in the updated Supplementary Fig. 17, CHX treatment revealed a significantly accelerated KRAS degradation rate (half-life ~12 hours) and extent (**57% reduction with anti-KRAS-BMC@MPNs vs. 14% with isotype control**) compared to controls (no CHX, CHX-only, and isotype-BMC@MPNs). **These results confirm that the observed KRAS reduction reflects active degradation rather than decreased synthesis. Notably, anti-KRAS-BMC@MPNs shortened the KRAS half-life from its native turnover (~22–24 hours^{33,34}) to under 12 hours, further demonstrating the efficacy of intracellular antibody-mediated degradation.** The corresponding data and discussion have been incorporated into the revised manuscript (Supplementary Fig. 17).



Supplementary Fig. 16 | KRAS degradation kinetics induced by anti-KRAS-BMC@MPNs in SW480 cells. **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**, 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.



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.

I suggest replacing Fig. 3e with the full WB that includes NC and isotype control data (i.e. Supp Fig. 15) – as the bar chart (Fig. 3f) would make more sense to the reader if the whole WB is placed next to it.

Response: In the revised manuscript, we have replaced the original Fig. 3d with the full western blot, including both negative control (NC) and isotype control data, as shown previously in Supplementary Fig. 15. This adjustment allows the corresponding bar chart (Fig. 3e) to be directly interpreted alongside the complete western blot data, thereby improving clarity and facilitating

comparison across treatment groups. The revised figure and corresponding text have been updated accordingly (Fig. 3d, e).

Are the empty BMCs at all toxic, and if so are they variably toxic to the cell lines used (HT29, H441 and SW480)? I suggest cytotoxicity assays would be helpful and could rule out inherent differences in sensitivity to the BMCs themselves as a cause of some of the observed differences in the cell lines when exposed to KRAS specific variants.

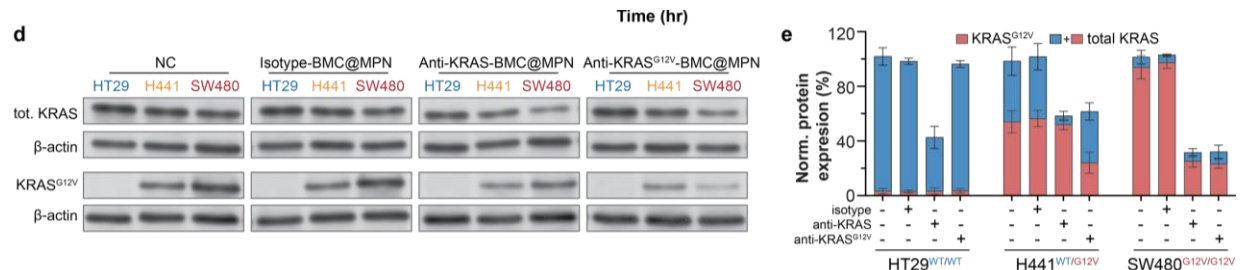
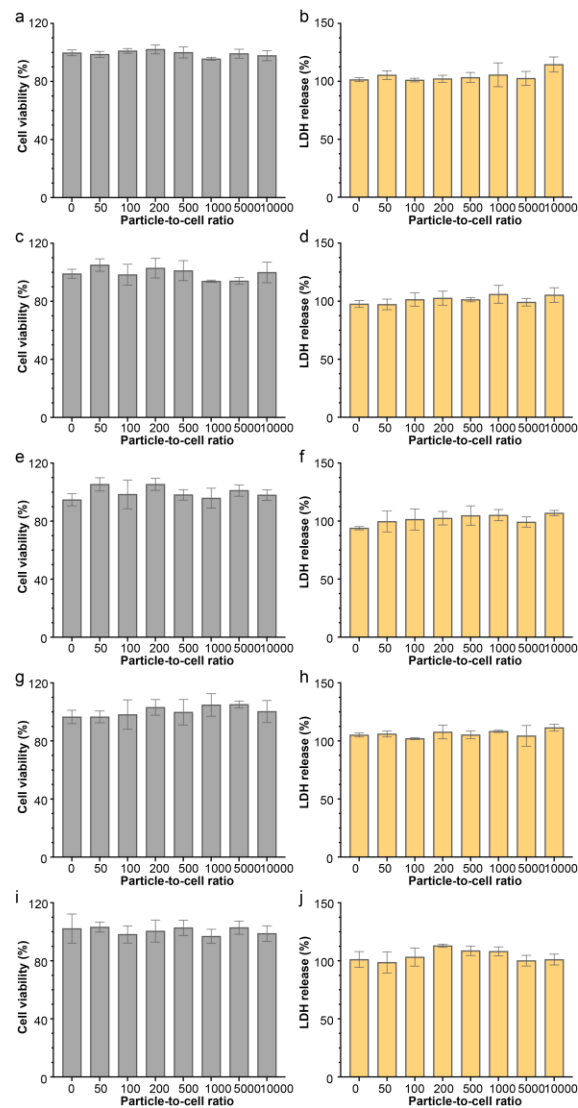


Fig. 3d, e, d, Immunoblots showing variant-selective KRAS degradation. Wild-type, heterozygous, or homozygous mutant KRAS-expressing cells were treated for 2 days with either anti-pan-KRAS-BMC@MPNs or anti-KRAS^{G12V}-BMC@MPNs. **e,** Grayscale quantification of immunoblots. The anti-KRAS^{WT} treatment degraded both KRAS types across all cell lines, whereas the anti-KRAS^{G12V} treatment selectively reduced KRAS^{G12V} levels in heterozygous and homozygous mutant cells. **f,** Schematic of co-culture assay of HT29 and SW480 cells.

Response: In response, we have now performed comprehensive cytotoxicity assessments of empty BMC@MPNs using both CCK-8 and LDH release assays across multiple cell lines, including SW480, HT29, H441, HEK293, and SK-N-SH. All tested cell lines showed no significant cytotoxicity following treatment with empty BMCs, confirming that the observed differences in cellular response to KRAS-specific variants are not attributable to inherent toxicity of the BMCs themselves. These results have been included in the revised manuscript (Supplementary Fig. 32).



Supplementary Fig. 32| Cytotoxicity study of BMC@MPNs in different cell lines. CCK8 and LDH in HT29 (a,b), H441(c, d), SW480 (e,f), HEK293 (g, h), and SK-N-SH cells (i, j). BMC@MPNs have no cytotoxicity at particle-to-cell ratio up to 1:10000.

Fig 4a: Intratumoral injections with BMCs were started 4 days after cancer cells were inoculated. It is surprising that there were detectable tumors at this time point (let alone tumors large enough for accurate intra-tumoral injection) – please comment on this. Intra-tumoral injections are not used in cancer medicine – so a brief discussion of the likely fate of the BMCs if delivered i.v. would be helpful.

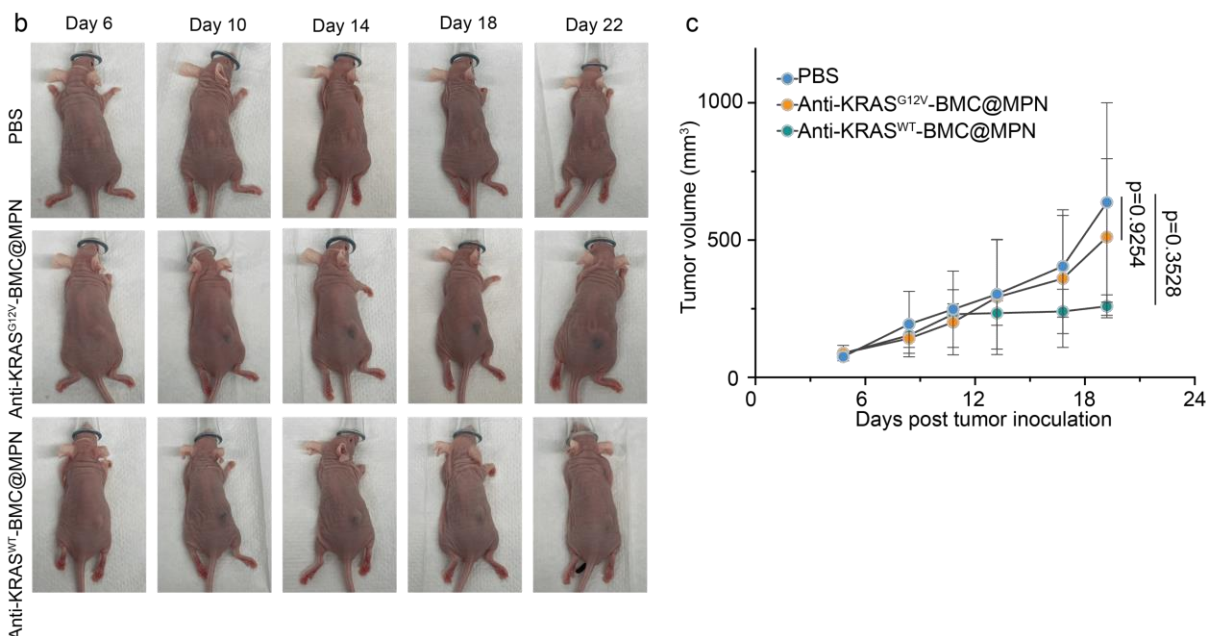
Response: In our study, SW480 tumors were detectable by day 4 post-inoculation, consistent with previous reports using this aggressive xenograft model^{35,36}. While early-stage tumors are indeed small, our protocol allowed for accurate intratumoral injection of **10 μ L volumes**, which was feasible and confirmed by consistent injection efficiency across replicates.

We agree that intratumoral administration is not representative of clinical delivery routes but was chosen here as a controlled method to evaluate local efficacy and establish proof-of-concept for *in vivo* protein degradation. However, given the lack of references for *in vivo* delivery of nano BMCs in the LLPS field (i.e., dosing consideration, injection volume, time duration), we opted for intratumoral injection for maximizing BMC@MPNs' retention in the tumor lesion. Regarding systemic delivery, we agree that the fate of BMCs following intravenous injection is an important consideration and will be a direction of future studies in this field. We have added a brief discussion of these points in the revised manuscript to highlight future directions for systemic delivery.

Ideally the in vivo experiments should have been done with the anti-KRAS^{G12V} variant and to have included a KRAS-WT model as well SW480. Was this attempted and if so what was the result?

Response: KRAS^{G12V} is a well-established **oncogenic driver** and **essential gene** in SW480 cells, and thus its degradation is expected to directly impact tumor growth. Our *in vivo* experiments focused on this mutant to evaluate target-specific degradation and therapeutic efficacy. We agree that including a KRAS^{WT} model helps strengthen the selectivity assessment. We have now added an *in vivo* study in HT29 (KRAS^{WT}) xenografts using the same nude mouse protocol (Supplementary Fig. 23). HT29 tumors did not respond to anti-KRAS^{G12V}-BMC@MPN but did respond to anti-KRAS^{WT}-BMC@MPN, mirroring our *in vitro* results and supporting mutant-selective degradation without perturbing wild-type KRAS. Unlike the SW480 model, where KRAS dependence yields near-complete tumor control, the HT29 model did not show complete inhibition, consistent with its lower KRAS dependence^{28,37}. Nevertheless, anti-KRAS^{WT}-BMC@MPN slowed tumor growth; this effect did not reach statistical significance ($p = 0.3528$).

Other minor points:



Supplementary Fig. 23 (c). 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. (d). Tumor growth curve.

• *I would recommend the use of the term synthesized BMCs (SBMC) as used by other authors (to distinguish these from naturally occurring intracellular BMCs).*

Response: In response, we have revised the introduction to clarify that our system refers to synthetic BMCs, distinguishing them from naturally occurring intracellular condensates. While we acknowledge the utility of the term “SBMCs”, we have opted to retain the abbreviation “BMCs” throughout the manuscript for consistency and simplicity, particularly as this term is used frequently across figures and text. We believe this terminology strikes a balance between clarity and readability.

• *Supp Fig. 1b – the y-axis is mis-labeled*

• *Page 5, Line 2: Should refer to Supp Fig. 2 (not Supp Fig. 5).*

• *Page 6, Line 3: Should refer to Fig. 2i (not 3i)*

Response: We sincerely thank the Reviewer for taking the time to read our manuscript thoroughly. All labeling and indexing issues mentioned above were fixed carefully.

• *P7: It's probably not accurate now to refer to KRAS as “undruggable” (with small molecule inhibitors in clinical trial)*

Response: In response, we have revised the manuscript to refer to KRAS as a “**challenging-to-target**” protein, acknowledging recent advances in small-molecule inhibitors. We also emphasize the advantages of our BMC-based approach in addressing a broader range of KRAS mutants and enabling proteome-level degradation. These changes are reflected in the revised Introduction and Discussion sections.

• *Page 10: In several places Fig.4 is referred to, but it should be Fig. 3.*

Response: We sincerely thank the Reviewer for taking the time to review our manuscript thoroughly. All labeling and indexing issues mentioned above were fixed carefully.

• *There is an inconsistency between the legend for Fig. 4 which indicates 5 intra-tumoral doses were given– but elsewhere in the text (and in the Fig. 4) 6 injections are mentioned.*

Response: We confirm that **six intratumoral doses were administered during the treatment regimen**. We have corrected the figure legend and any inconsistent references in the text to accurately reflect the six-dose schedule. We appreciate the reviewer’s attention to detail.

Reviewer #3 (Comments for the Author):

Overall comments:

This report details a delivery approach applied to IgG antibodies which have been engineered to promote protein degradation of their targets. The authors apply this approach to degrade KRAS and mutant KRAS derivatives. Overall, this work is similar to other studies that use designer particles to attempt to deliver biologics for targeted protein degradation. This remains an extremely attractive concept, but fails in practice due to problems of protein delivery and targeting certain cells for delivery. Major questions need to be addressed to differentiate this work from other work, including what advantages might exist compared to other delivery strategies (direct CPP conjugation, LNPs), what the targeting ability of these BMCs are, the mechanism of degradation, why this would be more advantageous than mRNA delivery, etc. Additionally, the lack of specificity and marginal degradation ability appear to be significant drawbacks from a basic science perspective. The long time needed for degradation compared other other approaches (TRIM away), and the large number of off targets observed in proteomics in particular place it far from standards in the field. From a translational perspective, the practical attributes of this approach remain unclear. As it is peptide-based, immunogenicity remains a concern compared to other LNP based approaches. I am not sure these BMCs offer anything different than lipid nanoparticle formulations (for example, this recent report: Chan et al. Nat. Commun. 15, 5808, 2024. This would need to be explained much more clearly. Finally, there are a number statements in the introduction and main text that are incorrect, poorly referenced, too broadly generalizing, or not demonstrating scholarship of the field of targeted protein degradation. Being precise about what is the advance, the state of the art, and the current advances beyond a different delivery formulation are essential. If the delivery formulation is the advance, much more rigorous characterization would need to be done in terms of bioavailability, clearance, targeting, and immune consequences.

Response: We thank the Reviewer for the feedback. We fully recognize the importance of improving degradation performance compared to the state-of-the-art TPD strategies and appreciate the opportunity to clarify how our approach differs from existing methods.

Our work presents a carrier-based strategy that condenses full-length IgGs into peptidic biomolecular condensates (BMCs) via biomimetic liquid-liquid phase separation (LLPS), enabling efficient cytosolic delivery and proteasome engagement for variant-specific degradation.

Compared to existing approaches, our method requires no (1) chemical conjugation to client IgGs, (2) removal of non-functional heavy chains, or (3) genetic engineering to express specific degradation adaptors. This distinguishes our platform from CPP-conjugated antibodies, which often suffer from short half-lives and immunogenicity^{38,39}, or lipid nanoparticles (LNPs), which typically present antibodies as surface-targeting ligands or exhibit suboptimal lumen encapsulation of macromolecules^{40,41}.

In response to the Reviewer's comments, we have:

1. Expanded the Introduction and Discussion to clearly compare our BMC platform with CPPs and LNPs, citing relevant literature, including the work by Chan et al. (*Nat. Commun.* 2024).

2. Clarified the mechanistic distinction of our system, which enables proteasome-dependent degradation through direct cytosolic trafficking of full-length antibodies, without requiring for genetic modification.
3. Provided additional discussion on the practical and translational attributes of our system, including its modularity, cargo generality, and potential strategies to achieve cell-specific targeting.
4. Protein depletion is inherently constrained by natural turnover rates, which vary across different proteins based on their synthesis and degradation dynamics. To contextualize our degradation timeline and extent, we have included comparisons with existing KRAS G12V degradation studies in both the Main and Discussion sections.
5. Included X-fect as a benchmark CPP-based method in our delivery experiments, highlighting the delivery advantages of BMCs in maintaining intracellular antibody activity, trafficking efficacy, and degradation induction.
6. Lastly, we have carefully revised the manuscript for accuracy, proper referencing, and precision in claims regarding the current state of the TPD field. We believe these revisions better articulate the contribution of our approach and its potential to expand the intracellular delivery toolkit for functional biologics.

Comments on the abstract:

The statement "However, the application of antibody-based TPD is limited to extracellular targets owing to the difficulty of delivering these agents inside cells." is incorrect. The TRIM-Away method uses antibodies to degrade intracellular targets, as does the follow up work using TRIM21 tags. Additionally, antibody fragments have been linked to cell penetrating peptides and used for intracellular protein delivery.

Response: We have revised the section to emphasize that our degradation strategy **directly engages the proteasome without requiring co-expression of adaptor proteins** (e.g., TRIM21) **or target labeling** (e.g., TRIM21 tags). We believe our design streamlined the need for genetic modification, which distinguishes from TRIM21-based methods.

While our aim is to develop a carrier-mediated strategy for intracellular antibody delivery that offers protection against proteolytic degradation and reduces immunogenicity, we agree that

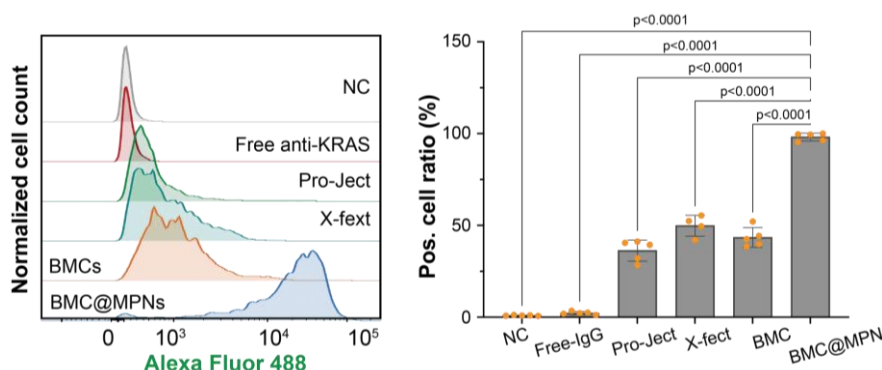


Fig. 2h | Flow cytometry raw data comparing cell internalization efficacy among free-IgG, commercial transfection reagents (Pro-Ject and X-fect), BMCs, and BMC@MPN. BMC@MPNs have 99% transfection efficacy (quantified by positive cell ratio), outperforming BMCs (42%), X-fect (51%), Pro-Ject (39%), and free-IgGs (2%).

comparison with CPP-based systems is important. In response, we have expanded the Introduction to discuss the limitations of CPP–antibody complexes regarding their immunogenic risks and their inability to preserve IgG functionality before reaching intracellular targets^{25,26}. Additionally, we included X-fect, a representative CPP-based transfection reagent as a benchmark in our experimental evaluation of delivery efficiency to highlight the advantages of our design (Fig. 2h)

Comments on Introduction:

The statement "Molecules like proteolysis-targeting chimeras (PROTACs)1-3, lysosome-targeting chimeras (LyTACs)4-6, and molecular glues7-9 facilitate the binding between target proteins and the degradation system, initiating the breakdown of targets." is fairly imprecise and broad-sweeping. For example, PROTACs and some molecular glues induce interactions with E3 substrate adaptors and target proteins, while LYTACs induce interactions with internalizing receptors that result in lysosomal translocation and degradation.

The statement "The selectivity of TPD depends on precise binding to targets, making antibodies ideal for achieving precision due to their exceptional affinity and specificity for antigen recognition12,13" is also not correct. Several PROTACs have been developed which use promiscuous or weaker binders to achieve protein degradation.

As stated above, the statement "Despite the success with receptor proteins, antibody degraders are limited to extracellular targets because of their large sizes and other properties that render them impermeable to cell membranes17" is not correct. TRIM-away and the follow up work, as well as bioPROTACs, all use antibody or antibody-like binders to achieve degradation. Additionally, nanobodies have been used for degradation by delivering with cell penetrating peptides. The authors should be much more specific about the point they are trying to make and differentiate from the current state of the literature.

Evidence of this statement "or conjugation of naked antibodies with cell-penetrating peptides, which can provoke an unwanted immune response." should be provided or referenced.

Response: We thank the reviewer for the feedback and have revised the Introduction to better clarify the scope of our work, which focuses on phenotype-selective targeted protein degradation (TPD), specifically the selective degradation of pathogenic KRAS variants while sparing wild-type KRAS.

We have clarified the distinctions between existing TPD platforms: Comparing to PROTACs and molecular glues, our system recruit E3 ligases to drive proteasomal degradation without requiring prior gene modification, enabling their application to diverse intracellular targets in unmodified cytosolic environments.

We have also distinguished our approach from intracellular antibody degradation strategies such as TRIM-Away, bioPROTACs, and nanobody-CPP conjugates, which often rely on genetic manipulation, elevated TRIM21 expression, or face challenges with intracellular stability, folding, and endosomal escape. In contrast, our BMC@MPN platform protects full-length antibodies during delivery, enabling functional engagement with cytosolic targets without genetic engineering or chemical conjugation.

In addition, BMC@MPNs are fundamentally distinct from conventional delivery systems. BMCs use phase-separated condensates to scaffold and concentrate antibodies while preserving functionality, which offers advantages of (1) rapid condensing and sequestering guest molecules

within seconds⁴², (2) an aqueous fabrication process that preserves functionality of guest molecules, and¹ (3) viscoelastic properties that offer unique bio-nano interactions⁷.

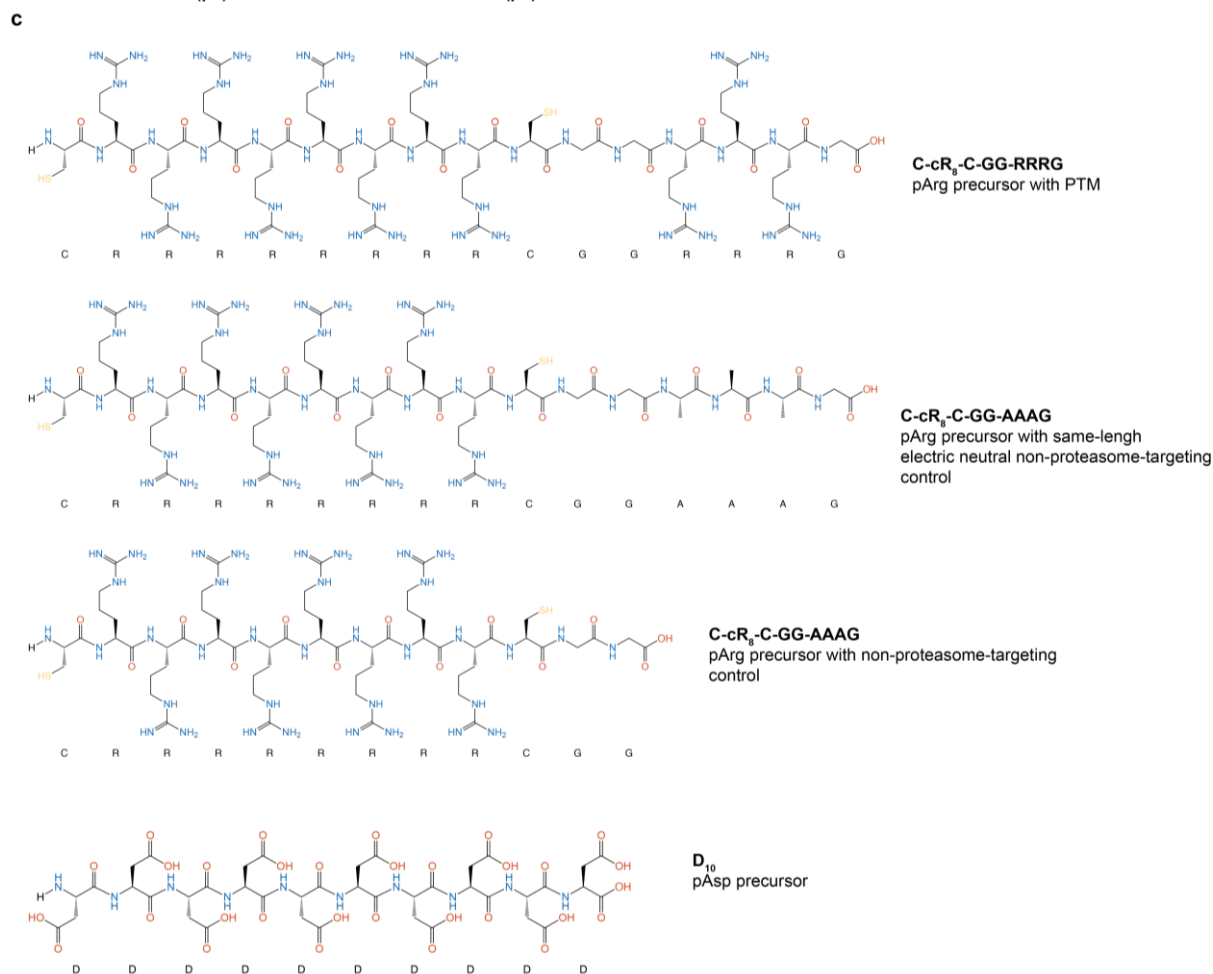
Finally, we have now included proper supporting references to the Introduction section.

Comments on Figure 1:

The structure of the BMCs is not clear in a, for example, what do the plus signs indicate? A less stylistic and more clear description of the exact chemical nature of the BMCs would be extremely helpful. Are these just three pictures of the same thing?

Response: The plus signs in Fig. 1a indicate positively charged moieties, specifically guanidinium groups, while negatively charged groups such as carboxylates are also labeled. To improve clarity, we have revised the figure legend accordingly and included the full chemical structures of the BMC components in Supplementary Fig. 2.

The illustrations in Fig. 1 are intended as conceptual representations of molecularly crowded, condensed phases (i.e., biomolecular condensates), rather than depictions of atomic-level structure or precise chemical composition. The three panels in Fig. 1a illustrate: (1) the full-length sequences used (e.g., C-cR₈-C-GG-RRRG and D10, left); (2) the key functional groups responsible for inducing LLPS (i.e., guanidinium and carboxylate groups, top right); and (3) a simplified schematic using tildes (left), consistent with the visual style used in Fig. 1b and Fig. 2a.



Supplementary Fig. 2 | Chemical structure of precursors used in this study.

KRAS is not a transmembrane protein, I am not sure why it is being shown that way.

Response: We have moved KRAS to the inner surface of the cell membrane in our illustration.

Comments on Figure 2:

For mechanistic studies of endocytosis, usage of acidification inhibitors such as BafA and Chloroquine are the standard. Inclusion of biochemical inhibitors is also important for demonstrating mechanism.

Response: We agree that the use of acidification inhibitors such as Bafilomycin A1 (BafA) is important for studying endocytosis and degradation mechanisms.

For the endocytosis study section (Fig 2 and Supplementary Fig 12, 14), we have now included experiments using BafA in the Supplementary Information (Supplementary Fig. 14) to assess the role of endosomal acidification in cytosolic delivery of BMC@MPNs. These data support the conclusion that endosomal escape is a critical step in the intracellular trafficking of our system, attributed to MPN semipermeable coating, in alignment with previous studies^{14,15}.

For the degradation mechanism study, (Fig 5 and Supplementary Fig. 26) we have expanded and clarified our mechanistic studies in the revised manuscript. Specifically:

1. We moved the Bafilomycin A1 (BafA1) experiment from the original Supplementary Fig. 17 to the main manuscript (now Fig. 5a–b) to highlight its relevance: **treatment with BafA1 did not rescue KRAS degradation, suggesting that lysosomal acidification is not involved in the degradation process.**
2. We have also included new data using chloroquine, another lysosomal inhibitor, **which similarly failed to prevent KRAS degradation** (Supplementary Fig. 26).
3. In contrast, MG132, a proteasome inhibitor, successfully blocked degradation, **indicating that the process is proteasome-dependent but lysosome-independent** (now Fig. 5a–b).
4. **This observation was consistent with using bortezomib**, an additional proteasome inhibitor (Supplementary Fig. 26).
5. Furthermore, we added experiments with TAK-243, a ubiquitin-activating enzyme inhibitor (Supplementary Fig. 26). **TAK-243 did not rescue KRAS levels, suggesting that degradation is also independent of ubiquitination.**

Collectively, these results indicates that degradation induced by IgG-BMC@MPNs is a **lysosome-independent**, **ubiquitin-independent**, and **proteasome-dependent degradation** process. We have added these findings in our revised manuscript to strengthen our mechanistic understanding of intracellular degradation via IgG-BMC@MPNs and are now fully detailed in the revised main text and figures.

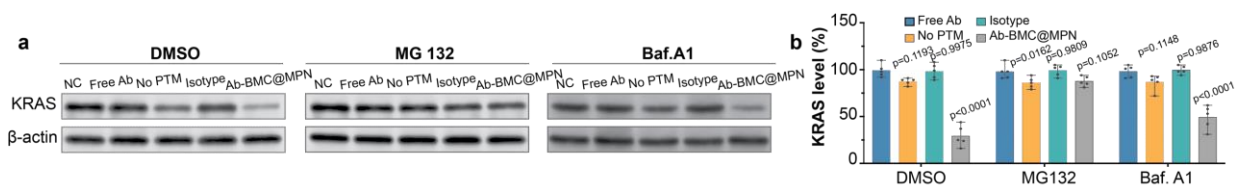
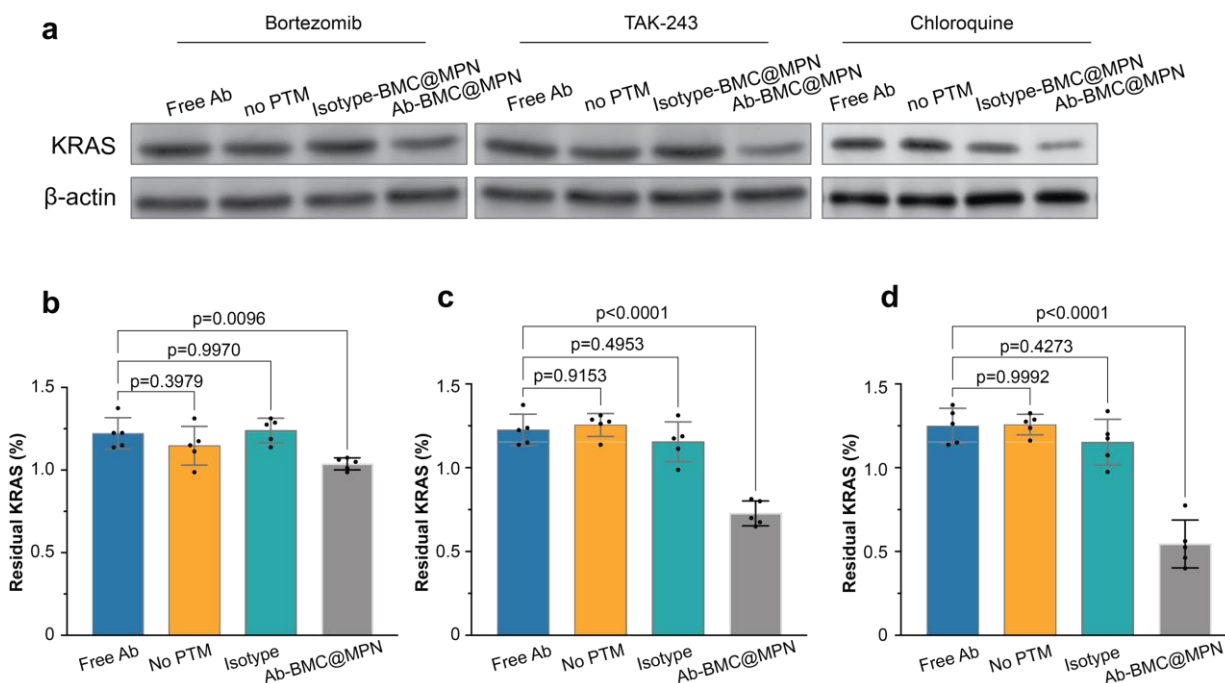


Fig. 5 | Proteasome-mediated degradation enables the disruption of intracellular targets. a, Representative WB analysis of KRAS degradation in SW480 cells pretreated with MG132 or Bafilomycin A1 (Baf. A1) followed by anti-KRAS-BMC@MPNs treatment. KRAS levels were reduced to 27% in untreated cells, rescued by 91% with MG132 but not by Baf. A1. **b,** grayscale quantification histogram.



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 Chloroquine, and an additional autophagy-lysosome pathway inhibitor. **b,** Grayscale quantification of bortezomib (**b**), TAK-243 (**c**), and chloroquine (**c**) blots.

In Figure 2h, comments are made comparing different protein transfection reagents regarding delivery efficiency. It is well known these reagents are primarily just getting endocytosed and not undergoing endosomal escape. I don't think it is accurate to conflate cytoplasmic delivery to overall cell uptake, which is an issue throughout this section.

Response: In this study, the comparison in Fig. 2h was intended to benchmark overall cellular internalization efficiency under equivalent dosing conditions. **These transfection reagents were used as benchmark references for cytosolic delivery of functional molecules**, such as cationic lipid nanoparticle (LNP)-based transfection reagent (Pro-Ject) and CPP-based transfection reagent (Xfect)¹.

Both Pro-ject and Xfect are designed for cytosolic delivery of macromolecules of functionality. Pro-ject is capable of inducing endosomal escape, as described in their manufacturers' manuals. Xfect is designed for delivering proteins and antibodies of biological activity, which indicates that cytosolic delivery occurs after entering the cells. These two products are popular protein transfection reagents and have been widely used for transfecting functional proteins, and were cited by many studies. Additionally, the experiment comparison to evaluate the endocytosis efficacy of particles has been used by a previous study in Nat Chem¹. Therefore, we think it is proper to include them as controls for our cell entry experiment in this section.

Product Overview
Ask our AI about this Product
Figures
Recommendations
Documents

Thermo Scientific Pierce Protein Transfection Reagent (formerly Pro-Ject) is a cationic lipid mixture for complexation with proteins, peptides, antibodies and other biologically active molecules to allow their direct intracellular delivery.

Features of Protein Transfection Reagent:

- No need to isolate, clone and transfect gene sequences—Saves days of work
- Optimum delivery in three to four hours after incubation—Fast protein delivery
- Delivery complex is non-covalent—Proteins and peptides retain biological activity after delivery
- Works with proteins, peptides and fluorescently labeled antibodies—Versatility

This is a unique cationic lipid mixture (previously called Pro-Ject Reagent) which, when complexed with proteins or peptides, allows direct intracellular delivery of proteins, peptides, antibodies and other biologically active molecules. Reagent protein complexes attach to negatively charged cell surfaces and either can directly fuse with the membrane and deliver the captured protein into the cell or be endocytosed by the cell and then fuse with the endosome, releasing the captured protein into the cytoplasm. Because the complex formed is noncovalent it does not interfere with the protein's biological activity.

Molecules that have been successfully delivered with the transfection reagent include β -galactosidase, fluorescent-antibody, green fluorescent protein (GFP), high and low molecular weight dextran sulfate, phycoerythrin-BSA, caspase 3, caspase 8 and granzyme B.

Specifications

Product Type	Protein Transfection Reagent
Product Line	Pierce™
Quantity	250 μ L
Unit Size	Each

Contents & Storage

Upon receipt store at -20°C. Product is shipped on dry ice.

Note: For optimal results, do not repeatedly freeze-thaw the β -galactosidase and FITC-antibody controls. Store the control proteins in aliquots suitable for one experiment.

Xfect™ Protein Transfection Reagent

Catalog Nos.

631323

631324

Amount

30 rxns

100 rxns

Description

The Xfect Protein Transfection Reagent allows the delivery of proteins and antibodies into cell lines in less than two hours. Delivery efficiency is high (60–99%), and proteins retain their biological activity. This kit contains Xfect Protein Transfection Reagent, Xfect Protein Buffer, deionized water, and a Beta-Galactosidase Control.

To avoid potential confusion, we have revised the text to explicitly distinguish between total cellular uptake (Fig 2h-k) and functional cytosolic delivery, reflected by (1) target protein engagement and (2) target protein degradation (Fig 3a-b, Supplementary Fig 15), emphasizing that high uptake observed in BMC@MPNs are attributed to direct cytosolic release following internalization, endosomal escape and condensate dissolution, rather than being trapped in endosome and losing their biofunctions. These distinctions have been clarified in the revised manuscript.

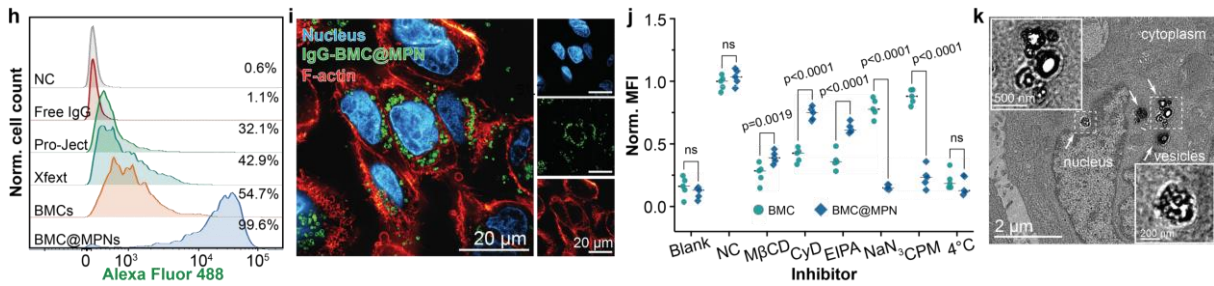
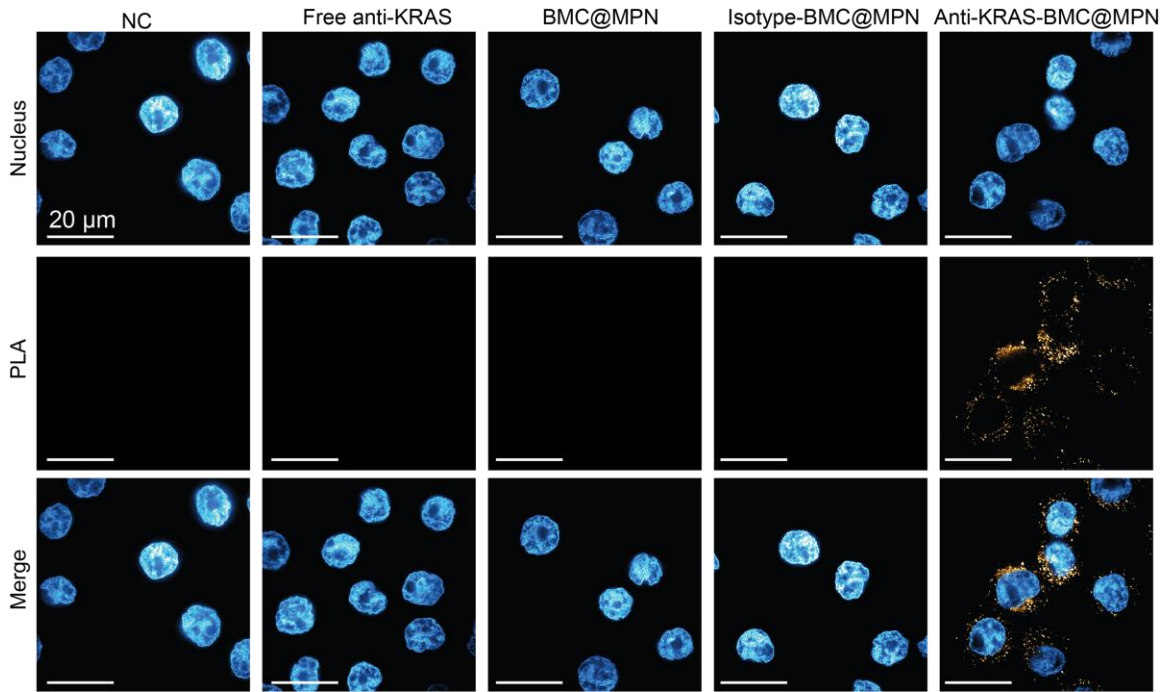


Fig. 2 | Engineering of IgG-BMC@MPNs with enhanced cell uptake. **h**, FACS analysis of SW480 cells transfected with AF488-anti-KRAS existing in its free form, Pro-Ject, Xfect, BMCs, and BMC@MPN. NC, non-treated control. **i**, Fluorescence micrographs of SW480 cells incubated with AF488-IgG-BMC@MPNs. F-actin (red) was stained to outline the cell membrane and indicate the relative intracellular localization of BMCs (green). **j**, FACS analysis of SW480 cells pretreated with cell uptake inhibitors before incubation with AF488-IgG-BMC@MPNs or BMCs. Data normalized to no-inhibitor-treated group. MFI, mean fluorescence intensity **k**, TEM images of BMC@MPNs after being internalized by SW480 cells. BMC@MPNs were enclosed within intracellular vesicles. The electron-sparse inner core corresponds to the BMC, while the electron-dense outer shell, rich in iron, represents the MPN coating.



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.

In the image in 2i, fluorescence is primarily in small, circular structures. What is the proposed reason for these structures and staining pattern? Do these co-stain with proteins in the endocytic pathway or lysosomal markers? It would be helpful to have a sense of what these represent, since they primarily appear to vesicular structures.

Response: These structures are our IgG-BMC@MPNs. There are no co-staining biomarkers F-actin (red) was stained to outline the cell membrane and indicate the relative intracellular localization of BMCs (green). We have used a larger font in the figure legend in the panel and added a description as well.

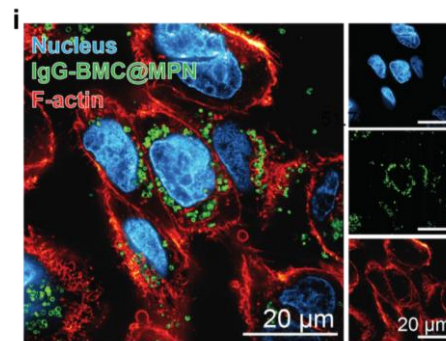
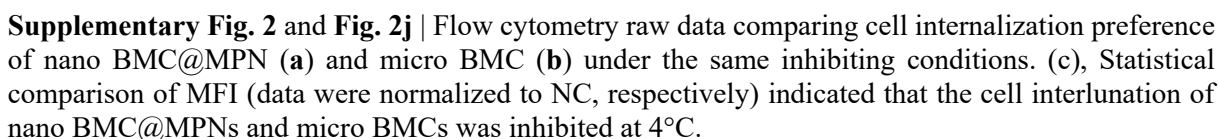


Fig. 2 | Engineering of IgG-BMC@MPNs with enhanced cell uptake. i, Fluorescence micrographs of SW480 cells incubated with AF488-IgG-BMC@MPNs. F-actin (red) was stained to outline the cell membrane and indicate the relative intracellular localization of BMCs (green).

This statement: "Uptake occurred at low temperatures (4 °C), implying direct penetration through cell membranes did not require endocytosis, ascribed to the function of the CPP domain (Fig. 2j)37" Is not supported by the data. Uptake and penetration are different properties, and surface adherence has not been ruled out for this condition.

Response: The residual fluorescence signal in the no-BMC control group may result from strong membrane adsorption of BMC@MPNs rather than true cellular uptake. To address this, we optimized the washing protocol by using 0.1% Tween 20 in PBS instead of PBS alone to remove uninternalized material. The revised data (Fig. 2j; Supplementary Fig. 12, relevant figures are shown below) show **no statistically significant difference** in normalized median fluorescence intensity (MFI) between the **4 °C group** and the **Blank group** ($p = 0.2912$, MFI was normalized to the non-inhibitor-treated group, denoted as NC), supporting that no uptake occurred under conditions where receptor activity and membrane fluidity are theoretically inhibited.



P-values are missing for all histogram data in this figure.

This statement "a 21 kDa cytoplasmic protein devoid of druggable binding sites, as the target. " Is not correct. Multiple KRAS mutants have been drugged now, including mutant and pan-KRAS.

Response: We have revised the statement to clarify our specific focus. While significant progress has been made in developing covalent inhibitors for certain KRAS mutants such as G12C (e.g., AMG 510, adagrasib^{33,43,44}) and emerging pan-KRAS inhibitors (e.g., RMC-6236^{33,34}), selective degradation of specific non-G12C variants, including endogenous KRAS G12V, remains a major challenge due to the absence of accessible pockets for direct binding^{29,33,45}. We have now clarified our points in the revised introduction:

“Although KRAS G12C inhibitors have shown strong target engagement and clinical benefit, the cysteine-specific targeting approach is not applicable to other KRAS mutants^{33,43}. In particular, the G12V variant, prevalent across multiple cancers and linked to poorer overall survival, remains a therapeutic challenge due to the lack of selective targeted therapies⁴⁵. This highlights the urgent need for strategies that can address a broader range of KRAS mutations”.

For the IP experiments in Fig. 3a, performing a cytoplasmic extraction step to separate from membrane enclosed compartments seems like an essential experiment. During cell lysis, contents could easily be mixed enabling pulldown when in the live cell context, this is not true.

Response: KRAS localizes to the inner leaflet of the plasma membrane via C-terminal lipid modifications, while remaining exposed to the cytoplasm to engage downstream effectors and

regulate signaling pathways⁴⁶. Therefore, using whole-cell lysates for IP experiments is appropriate to capture both membrane-associated and cytoplasmic KRAS. **Many studies have used whole-cell lysates for KRAS immunoprecipitation**, as membrane-bound and cytosolic fractions remain accessible following lysis^{30,47,48}. While compartment mixing can occur during lysis, whole-cell IP remains a widely accepted approach for detecting KRAS–antibody interactions.

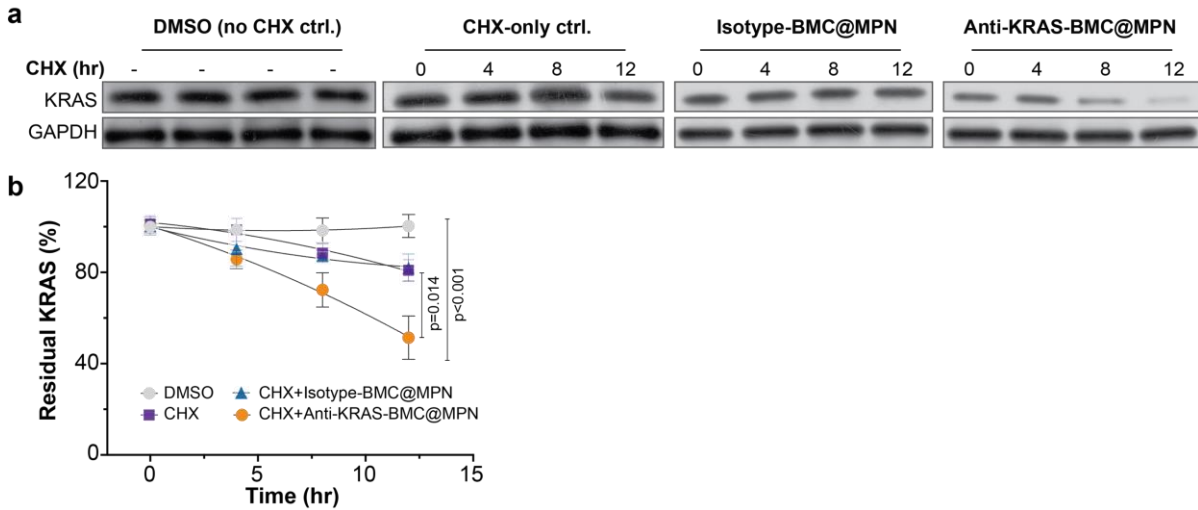
This statement, "Efficient degradation of the mutated protein is observed, with WB results indicating an average degradation level of 67% within 48 hrs (Fig. 3b)." Is not accurate. This is relatively slow degradation (inefficient) compared to other degrader technologies.

Response: We agree that the degradation kinetics observed here are slower than some of the degraders, especially TRIM21-based systems, which can achieve degradation within minutes^{49,50}. **However, our aim is to engineer TPD tools for use in native, unmodified cytosolic environments** and does not rely on co-expression of degradation adaptors such as TRIM21, representing **a mechanistically distinct strategy**.

Importantly, **degradation efficiency is governed by the balance between endogenous protein synthesis and clearance, which varies across targets**⁵¹. While some proteins with short half-lives can be rapidly degraded, mutant KRAS has a basal turnover of 22–24 hours^{34,43}, making swift degradation inherently more difficult.

Moreover, **degradation kinetics are also influenced by the size and structure of the target protein**—larger proteins like **Tau (70 kDa)** or **EGFR (180 kDa)** are often more amenable to rapid clearance, while smaller proteins such as **KRAS (21 kDa)** and **α -synuclein (17 kDa)** tend to have slower turnover rates⁵².

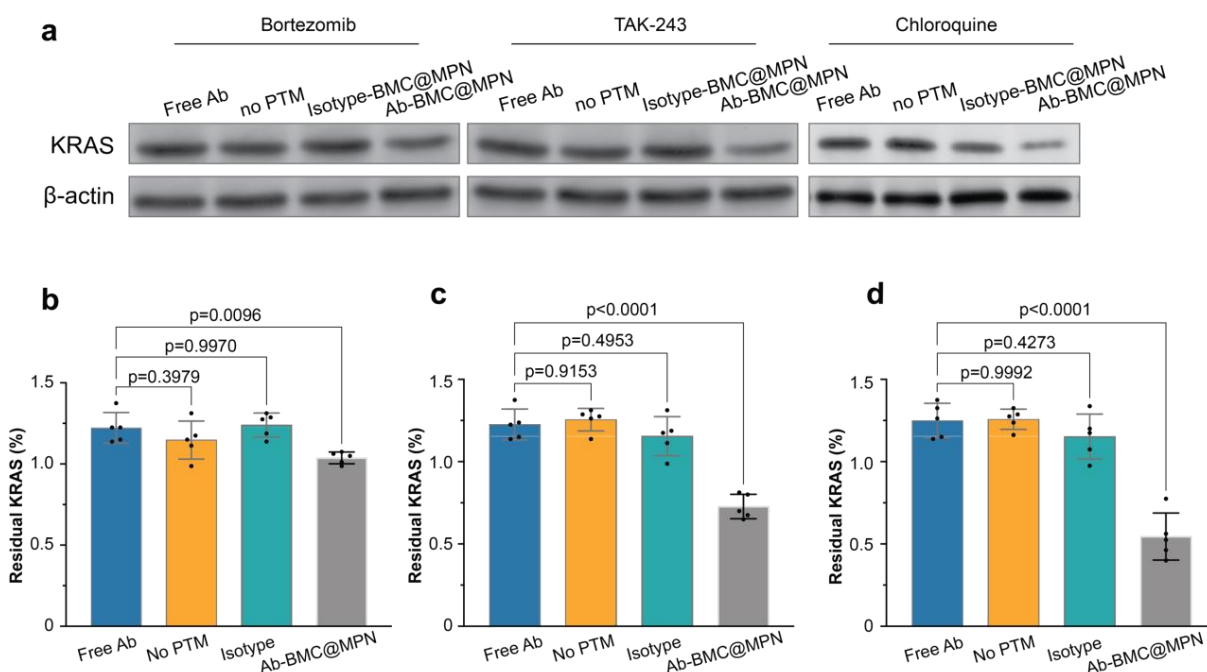
To benchmark our results, we compared them with peer studies employing intracellular antibodies for degradation without genetic modification. These typically report degradation half-lives of 12–16 hours^{47,48}. In contrast, our cycloheximide (CHX)-chase assay revealed a KRAS^{G12V} degradation half-life of 8–12 hours, demonstrating accelerated clearance relative to the endogenous baseline and outperforming comparable systems. We have attached our CHX-chase assay result below.



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, 100 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.

For the degradation experiments in Fig. 3, some biochemical characterization would be helpful and is the standard in the field. How does treatment with various inhibitors of the proteasome, neddylation, endocytosis, lysosomal proteases, E1 activity, etc. impact degradation?

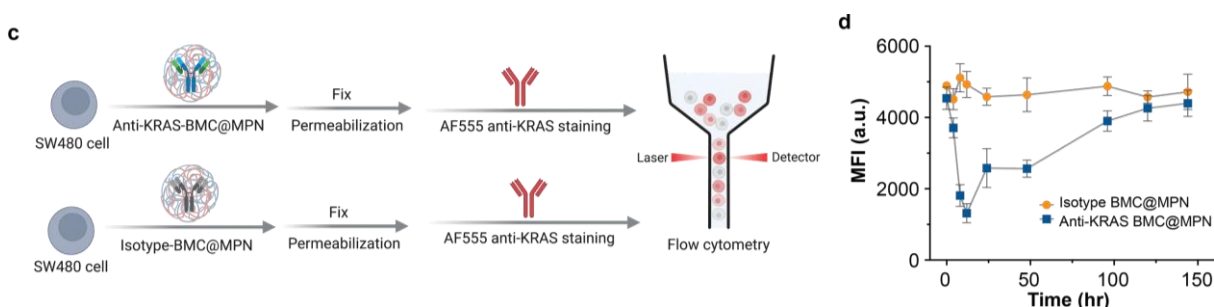
Response: We have now added additional inhibitors in our updated Supplementary Fig.17, including (1) Bortezomib (proteasome inhibitor), (2) TAK-243 (E1 inhibitor), and (3) Chloroquine for the degradation effect rescue study. Our results showed that Bortezomib inhibited degradation, while TAK-243 and chloroquine failed to inhibit degradation, supporting our conclusion that the degradation induced by IgG-BMC@MPNs is a lysosome-independent, ubiquitin-independent, and proteasome-dependent process.



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 Chloroquine, and an additional autophagy-lysosome pathway inhibitor. **b**, Grayscale quantification of bortezomib (**b**), TAK-243 (**c**), and chloroquine (**d**) blots.

How was the experiment in Fig. 3c performed? Is this flow cytometry data after a fix and permeabilization step?

Response: Yes, the experiment in Fig. 3c was performed using flow cytometry following fixation and permeabilization. Cells were first treated with the respective BMC@MPNs, then fixed with 4% paraformaldehyde and permeabilized using 0.1% Triton X-100. Intracellular KRAS levels were subsequently stained with a fluorophore-conjugated anti-KRAS antibody and analyzed by



Supplementary Fig. 16| KRAS degradation kinetics induced by anti-KRAS-BMC@MPNs in SW480 cells. **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**, 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.

flow cytometry to quantify protein abundance. We have now included the schematic of this experiment in our revised Supplementary Fig 16.

This statement "Oncogenic KRAS is characterized by a single amino acid mutation that current degraders are unable to selectively target under their native expression conditions^{40,42}." seems misleading. Most state-of-the art therapies targeting KRAS are not degraders for several reasons, but covalent and non-covalent inhibitors are already able to suppress KRAS activity (for example, the recent compounds from Revolution Medicines). I think comparing to the gold standard in loss of function activity is more important than comparing to other inefficient degraders (some of which are actually mutant selective, i.e. KRASG12C).

Response: We thank the Reviewer for the comment. We appreciate the opportunity to clarify our statement:

Although recent advances, such as covalent and non-covalent inhibitors developed by Revolution Medicines and others, have shown promising activity against KRAS^{G12C}, there are currently no reported degraders or inhibitors specifically targeting KRAS^{G12V} in clinical trials^{29,30,32,43,44}.

We have revised our introduction and other related parts to clarify that our demonstration is for the KRAS^{G12V} mutant:

"Although KRAS G12C inhibitors have shown strong target engagement and clinical benefit, the cysteine-specific targeting approach is not applicable to other KRAS mutants^{33,43}. In particular, the G12V variant, prevalent across multiple cancers and linked to poorer overall survival, remains a therapeutic challenge due to the lack of selective targeted therapies⁴⁵. This highlights the urgent need for strategies that can address a broader range of KRAS mutations".

For the experiments in Fig. 3h-m, I think a better control would be an isotype or other antibody that targets a non-essential protein equally in both cells. Comparing to untreated doesn't reflect cell-specific differences in response to the BMCs.

Response: We agree that an isotype control targeting a non-essential protein equally in both cell types would be a valuable comparator. In this set of experiments, we used the untreated condition as the negative control **to account for the intrinsic difference in baseline growth rates between the two cell lines. This is to make sure that any observed growth suppression could be attributed to the treatment rather than natural proliferation differences.**

Comments on Figure 4:

In this xenograft study, does the anti-KRAS have cross-reactivity with mouse KRAS?

Response: Yes. Our anti-KRAS^{G12V} was ordered from Cell Signaling Technology (K-Ras (E2M9G) Rabbit mAb #71835), which has reactivity with human, mouse, rat, and monkey.

The data in Fig. 4h should be quantified, and scaled appropriately with an internal standard. Raw images here are not informative given the resolution.

h

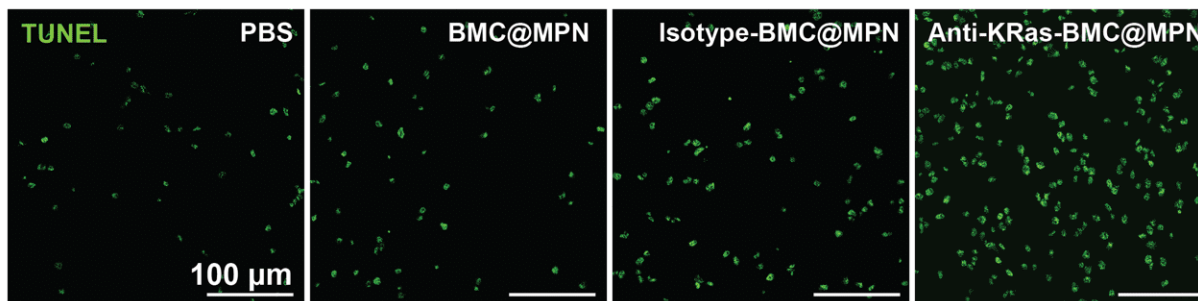


Fig. 4 | Targeted degradation of KRAS^{G12V} inhibit tumor growth *in vivo*. **h**, TUNEL assay showed elevated apoptosis level in the anti-KRAS^{G12V}-BMC@MPNs treatment group (stained in AF488).

Response: We have enlarged the panel to better visualize the TUNEL signal (green) and revised the figure legend for improved clarity. Cell membrane and nucleus staining were used as internal references to calculate TUNEL-positive cell ratios per view.

This statement: "These findings indicate that IgG-BMC@MPNs is safe and effective TPD modalities for in vivo anti-tumor therapy." seems premature. For example, what is the bio distribution of BMCs end up body wide? What happens when you target something in a syngeneic model? CPPs have failed to translate over ~30 years of development because of off target problems among other things. This seem like similar concerns for these particles.

Response: We agree that, while our results demonstrate promising *in vivo* efficacy and tolerability in the current intratumoral xenograft model, additional studies are needed to comprehensively evaluate biodistribution, off-target effects, and immunological responses in more clinically relevant settings.

To that end, we agree the importance of characterizing the systemic fate of BMCs, including organ-level biodistribution and clearance. Although this study focused on proof-of-concept efficacy through local delivery, future work will incorporate quantitative biodistribution studies and syngeneic or immunocompetent models to assess immune compatibility and off-target effects under systemic administration.

We also recognize the long-standing translational challenges faced by CPPs, particularly regarding nonspecific interactions. Unlike traditional CPPs, however, our BMC@MPNs are stabilized phase-separated condensates with tunable physical properties and modular targeting potential because of the semipermeable coating, which would help reduce off-target uptake. Furthermore, the transition from micro- to nano-scale BMCs enables active endocytosis rather than relying solely on passive membrane fusion, potentially improving both delivery specificity and biodistribution by activating receptor-mediated endocytosis.

We appreciate the reviewer's concerns and have revised the manuscript to discuss the limitations in our conclusion section:

"While this study demonstrates the feasibility of using intratumoral injection for IgG-BMC@MPNs, future work should focus on incorporating cell- or organ-specific targeting features to enable systemic (e.g., intravenous) administration of BMCs. The improved cellular uptake

observed with nanoBMCs likely results from their reduced size, which shifts uptake from passive membrane fusion—common in microBMCs—to active endocytic pathways. However, how this transition affects in vivo biodistribution remains unclear and warrants further investigation. While our observation supports a proteasome-dependent, ubiquitination-independent degradation process, the exact molecular mechanism requires deeper mechanistic studies. Finally, we demonstrated the degradation of α -synuclein as a proof of concept, and future research may want to expand this strategy to a wider range of intracellular targets with clinical relevance".

Comments on Figure 5:

The experiments in Fig. 5a would fit better with figure 3, and should be quantified in triplicate western blot in accordance with the highest rigor of the field.

Response: We included the experiment in Fig. 5a as part of our mechanistic study on the degradation process. Fig. 3 was designed to demonstrate the selectivity of the degradation effect rather than its underlying process. Additionally, we actually have **five replicates** and quantified in Fig. 5a, b.

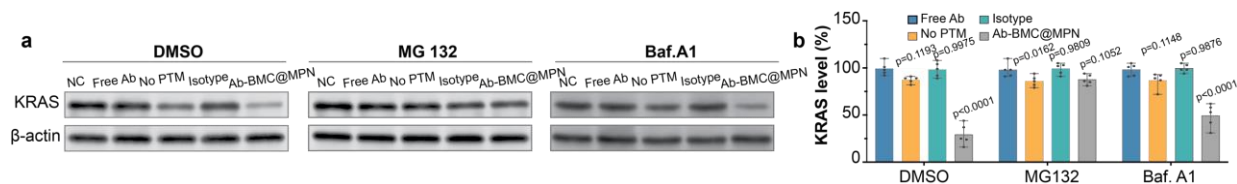


Fig. 5 | Proteasome-mediated degradation enables the disruption of intracellular targets. a, Representative WB analysis of KRAS degradation in SW480 cells pretreated with MG132 or Bafilomycin A1 (Baf. A1) followed by anti-KRAS-BMC@MPNs treatment. KRAS levels were reduced to 27% in untreated cells, rescued by 91% with MG132 but not by Baf. A1. **b,** grayscale quantification histogram.

Have the authors demonstrated that the "PTM domain" is essential for degradation? For example, IgGs are well-known to engage with TRIM21 to clear proteins through the proteasome. Additionally, the statement "The absence of ubiquitination bands validated the hypothesis that anti-KRAS-BMC@MPNs-induced proteolysis is initiated by direct proteasome recruitment via the PTM domain." seem premature. Proteasome inhibition, followed by IP of KRAS and Ub blot would be the better experiment, and further, the data with a positive control are not shown so this is purely speculative.

Response: We agree that additional mechanistic studies would further strengthen the claim regarding the role of the PTM domain in mediating degradation. In our current study, the functional importance of the PTM domain was supported by the significant reduction in degradation observed when using BMCs lacking the PTM motif (no PTM-BMC@MPNs), as shown in Fig. 5d,e. This suggests that the PTM domain plays a critical role in facilitating proteasomal degradation.

Meanwhile, we used experimental validation using RRRG or AAAG as PTMs to show that only the RRRG fusion induced degradation, as shown by immunoblot (Fig. 5d, e). These results demonstrate that electrostatic interaction with PSMD9 is essential for proteasome recruitment and highlight the importance of rational PTM design in enabling ubiquitin-independent degradation through direct proteasome targeting.

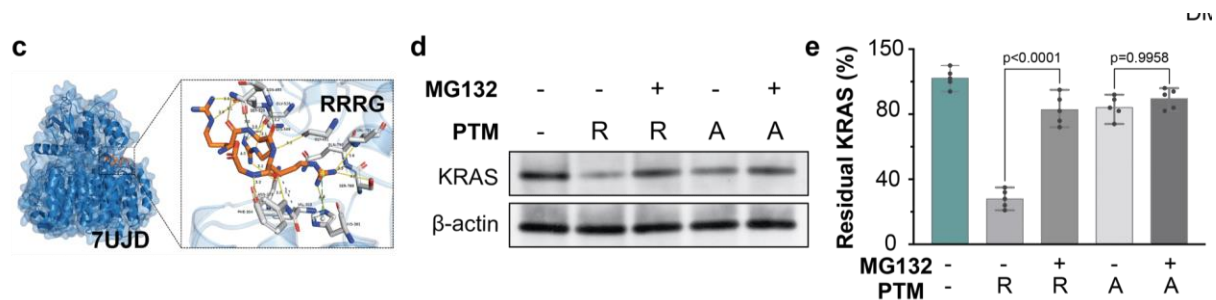


Fig. 5 | Proteasome-mediated degradation enables the disruption of intracellular targets. **c**, Representative pose of the interaction between PTM and the 7UJD proteasome regulatory subunits, shown in a space-filling representation. **d**, WB analysis of KRAS degradation using RRRG (R) or AAAG (A) negative control. Only the RRRG motif induced KRAS degradation, demonstrating that electrostatic interaction with PSMD9 is essential for proteasome recruitment. **e**, grayscale quantification histogram. **f**, 48-hr quantitative α -syn degradation analysis using SDS-PAGE and greyscale analysis.

Docking experiments would be fine for validating experimental data. It seems straightforward for the authors to just use anti-KRAS without a "PTM" domain as a way to validate that proteasomal direct targeting is occurring.

Response: We use AAAG as a negative control because we want to keep the identical length of the PTM peptide and the control. This is because a shorter peptide that simply removes functional domain changes size, flexibility, electrostatics, degree of freedom, and genuine flanking-residue contacts, which will affect docking scores. Therefore, we use length-matched neutral charge peptides here as the negative control instead⁵³. However, we now use a non-PTM peptide as a control in our WB validation (Fig. 5d,e). The results show that the non-PTM peptide fails to induce KRAS degradation, confirming the specificity of the observed effect.

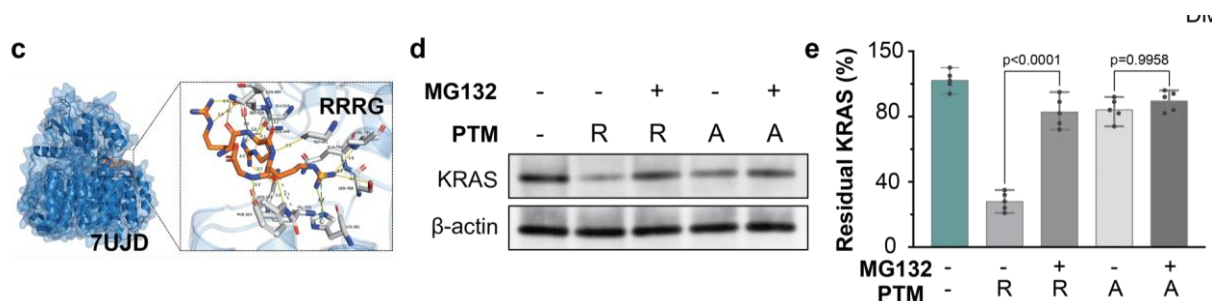


Fig. 5 | Proteasome-mediated degradation enables the disruption of intracellular targets. **c**, Representative pose of the interaction between PTM and the 7UJD proteasome regulatory subunits, shown in a space-filling representation. **d**, WB analysis of KRAS degradation using RRRG (R) or AAAG (A) negative control. Only the RRRG motif induced KRAS degradation, demonstrating that electrostatic interaction with PSMD9 is essential for proteasome recruitment. **e**, grayscale quantification histogram. **f**, 48-hr quantitative α -syn degradation analysis using SDS-PAGE and greyscale analysis.

For the data in Fig. 5d, p values are essential. I do not think these data would reflect degradation when quantified.

Response: We have updated p-values in our new Fig. 5f. The degradation efficacy for α -syn was not as significant as KRAS, possibly due to different subcellular location, as a certain amount of BMC@MPNs will disassemble before reaching the nucleus. However, we did quantify the degradation level of α -syn and have significance after 12-hr treatment in our updated Fig 5f.

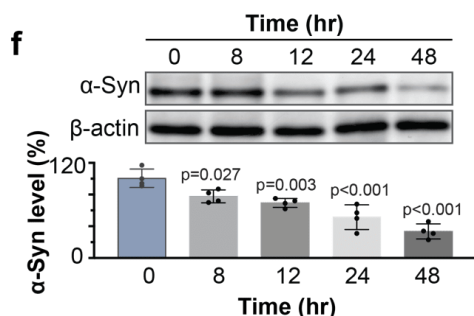
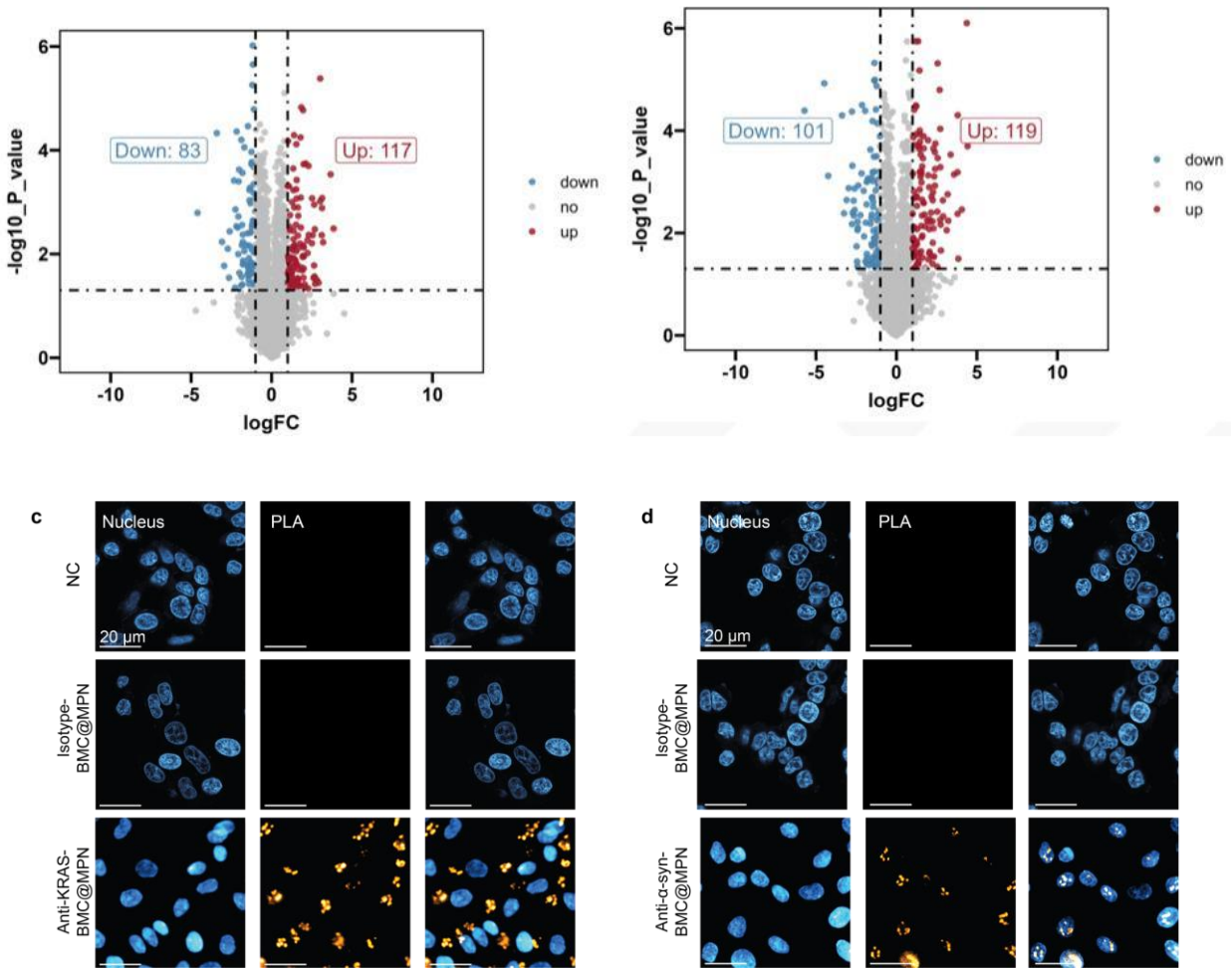


Fig. 5 | Proteasome-mediated degradation enables the disruption of intracellular targets. f, 48-hr quantitative α -syn degradation analysis using SDS-PAGE and greyscale analysis.

The substantial number of off-target degradation events in Fig. 5i,j is troubling for an approach that should be highly surgical in its ability to downregulate a specific target. I think this essential piece of data shows that there are some issues with the approach, with the targets chosen, or with the nature of the degradation which takes a long time and thus makes quantitative MS difficult.

Response: We would like to clarify that many of the proteins identified in our original Fig. 5i, j are not off-targets but instead functionally **related effectors that naturally interact with the target protein in active signaling complexes, rather than direct degradation by our construct**⁵⁴⁻⁵⁶. Importantly, the number of off-target hits we observed (83 downregulated and 117 upregulated out of >3500 signals for KRAS; 101 downregulated and 119 upregulated out of >3500 signals for α -syn) is comparable to or even lower than those reported in recent studies using intracellularly delivered antibodies or degrader constructs without genetic modification^{57,58}.

To further validate the hits (i.e., proteasome subunits and target proteins, KRAS or alpha-synuclein), we now included a proximity ligation assay (PLA) in our Supplementary Fig. 31 to evaluate the interaction between PSMD9—a 19S proteasomal subunit—and the target protein. The PLA signal supports a direct recruitment mechanism involving the proteasome, strengthening the specificity of our degradation strategy. We agree that long degradation windows can complicate MS quantification, and we are actively optimizing experimental timing to minimize such effects in future studies.



Supplementary Fig. 31 | Proteasome-mediated degradation enables the disruption of intracellular targets. g-h, PLA detecting PSMD9–target interactions during KRAS (g) and α -syn (h) degradation. Distinct punctate PLA signals were observed in the cytosol of SW480 cells and the nucleus of SK-N-SH cells following treatment with anti-KRASG12V-BMC@MPNs and anti- α -syn-BMC@MPNs, respectively, indicating spatially resolved PSMD9 engagement and target degradation within their native subcellular compartments.

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We sincerely thank the Reviewers and Editors for their feedback and suggestions. In response, we have expanded our discussion to address the points raised. We have revised the manuscript and provided a point-by-point response below. For clarity, Reviewer comments are shown in blue italics, followed by our responses in black, with key points highlighted in bold. We hope our revised manuscript will be further considered for publication at *Nature Communications*.

Reviewer #1

1. This might be matter of semantics, but what do the authors mean by “passive entry” for macro-size BMCs (page 7, line 16; page 16, line 19 and 23, and Supp. Fig. 12a) ? Filopodia formation during BMCs engulfment involves active cytoskeleton remodeling, see for instance the review by Gleeson and co-workers¹ or the study by Kress et al². Thus, naming this process “passive” seems a bit misleading. Both entry mechanisms shown in Supp. Fig. 12 are active, although they involved different entities and length scales? I’d like the author to provide a more accurate differentiation here.

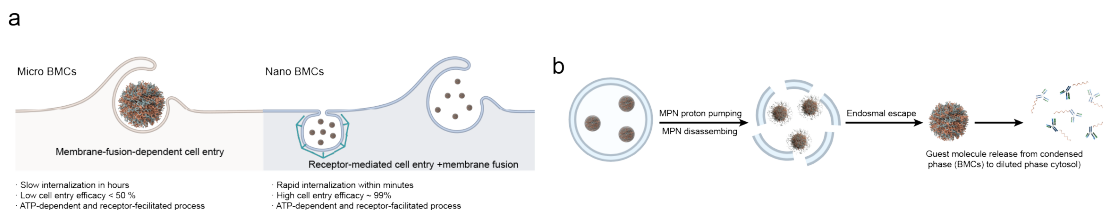
Response:

We have replaced the term “passive membrane fusion” throughout the manuscript with “membrane fusion”.

We originally used the term “passive membrane fusion” to describe the entry process of micro-BMCs, which primarily relies on plasma membrane remodeling rather than receptor-mediated uptake (i.e., clathrin-dependent endocytosis), in contrast to nano-BMCs. We hope this term more accurately supports our discussion that nano-BMCs achieve higher internalization efficiency because they can engage additional receptor-mediated uptake pathways, which are not accessible to micron-scale BMCs due to their size constraints.

In response, we have revised the following contents in our manuscript:

1. Page 7, line 16: “... indicating that nano BMC@MPNs are less dependent on **passive membrane fusion** and activate alternative entry pathways due to size reduction.”
2. Page 16, line 19: “... but their reliance on **passive membrane fusion** for cell entry and propensity for droplet fusion limit their *in vivo* applications^{28, 56}.”
3. Page 16, line 23: “... and shifter the entry process from **passive membrane fusion** to active, energy-dependent endocytosis.”
4. Supplementary Fig.12a: We have revised the text in the panel accordingly (shown below).



Supplementary Fig. 12 Cell internalization mechanism study. **a,b** Schematic of cell entry and endosomal escape process of BMCs.

2. While in their system, nanoscale BMCs indeed appear to perform better than micro-scale BMC, the authors cannot make generalized statements that nano-BMCs are superior to micro-BMCs in terms of transfection efficiency and uptake kinetics. There are many recent studies showing that micro-BMCs can safely achieve above 90% efficiency and cargo release within minutes. For example, in Sun et al³ some peptide condensates achieved > 95% transfection rate with cargo release observed within a few minutes. In other words, while size can be a factor, they are many other variables that play an equally or even more critical role in mediating cell entry, including the viscoelastic properties of the condensates, their surface charge, specific peptide motifs, etc...The authors should make it clear that the superiority of the nano-scale BMCs over micro BMCs is valid for their system, but this is not generalized for all BMCs. Supp. Fig. 12 should be corrected accordingly.

Response: We have revised the manuscript to clarify that our comparison is based on nano- and micro-condensates on the specified chemical composition.

In our system, nano-BMCs and micro-BMCs are derived from identical peptide precursors without alterations to the amino-acid residues, such that particle size represents the primary variable. Under these conditions, nanoBMCs consistently exhibit higher internalization efficiency and faster uptake kinetics than micro-BMCs.

In addition, nanoBMC uptake produced a more uniform intracellular fluorescence distribution in the monolayer cell uptake assay (Supplementary Fig. 11-12), reflected by a narrower full width at half maximum (flow cytometry) relative to microBMCs under non-inhibitor-treated (denoted as NC in our panel) conditions, and particle number internalized per cell (confocal microscopy). These results suggest that nano-BMCs enable more homogeneous cellular uptake, which may be advantageous in contexts where precise dosing control is important (i.e., quantifying BMC uptake per cell relative to the number of KRAS copies degraded).

In response, we have revised the following contents in the manuscript:

1. Page 7, line 21: In addition, BMC@MPNs produced a more uniform intracellular fluorescence distribution, suggesting that nano-BMCs enable a homogenous cellular uptake, which is important for precise dosing control (Supplementary Fig. 11-12).

3. In their reply, the authors state that the intracellular delivery of intact biomolecules (IgGs) is largely an unmet challenge that remains unexplored in the field of LLPS.

I believe that in this context, they are specifically referring to IgG antibody delivery, which they indeed demonstrate retain efficient intracellular binding activity? (although IgG delivery has been demonstrated for micro-BMCs by Bao et al JACS, 2025⁴. However, in the broader context of biomacromolecules, there have been numerous recent examples that coacervates can very efficiently deliver large macromolecules cargos in cells, with unaffected biological activity, including large enzymes (e.g. 430 kDa beta-galactosidas); cytotoxic proteins (saporin), mRNA, pDNA, or CRISP-Cas modalities (see for instance Forenzo and Larsen, ACS Mol. Pharma. 2023⁵, Sun et al, ACS Nano 2023⁶). The authors may want to clarify this in the introduction or discussion.

Response: We have revised the *Introduction* and *Discussion* sections to clarify the distinction between general biomacromolecule delivery by condensates and the advances presented here.

Beyond IgG delivery, our work positions BMCs as a platform that enables IgG-guided targeted protein degradation without the need of engineering ubiquitin systems or introducing exogenous degradation machinery. In our early experiments, we tested BMCs assembled from different peptide precursors (Arg/Asp, Lys/Glu, and Lys/Asp). While all these LLPS precursors enabled efficient cytosolic delivery of IgG, they induced markedly different levels of KRAS degradation. We therefore reasoned that the LLPS precursors themselves facilitate proteasome recruitment, specifically, arginine-rich peptides in our system. In our final formulation, we strengthen the mechanism by incorporating RRRG, a motif reported to represent the minimal residues capable of recruiting the proteasome^{7,8}, into the BMC design.

In response, these points are reflected in the following sections in the manuscript:

1. Page 2, line 19: Moreover, intracellular antibody binders rely on genetic overexpression of degradation adaptors to induce degradation restricting their use in unmodified cytosolic contexts¹⁷⁻¹⁹.
2. Page 17, line 17: This strategy integrates rapid IgG condensation, efficient cytosolic delivery via active endocytosis, mutation-selective target recognition, and direct engagement of the endogenous proteasome machinery, offering a genetically unmodified, ubiquitination-independent route to intracellular protein degradation.

On a different note, I have also read the comments of the other Reviewers, and in my view the criticism of Reviewer #3 “I am not sure these BMCs offer anything different than lipid nanoparticle formulations” is unwarranted. Peptide-based BMCs are entirely different from LNPs from many different aspects: they are made from completely different chemical building blocks, their materials (visco-elastic) properties are very distinct from LNPs, their fabrication process is fundamentally different and much simpler and does not use of organic solvents, etc...In short, BMCs are indeed an entirely different class of delivery vehicles, with their own merits and advantages over other intracellular drug carriers and should be recognized as such.

Response: We thank the Reviewer for this supportive comment and for clarifying that BMCs represent a fundamentally different class of delivery vehicles from LNPs. As noted, their unique material basis and functional properties set BMCs apart from conventional lipid-based carriers, and we appreciate the Reviewer highlighting this important difference.

Reviewer #2

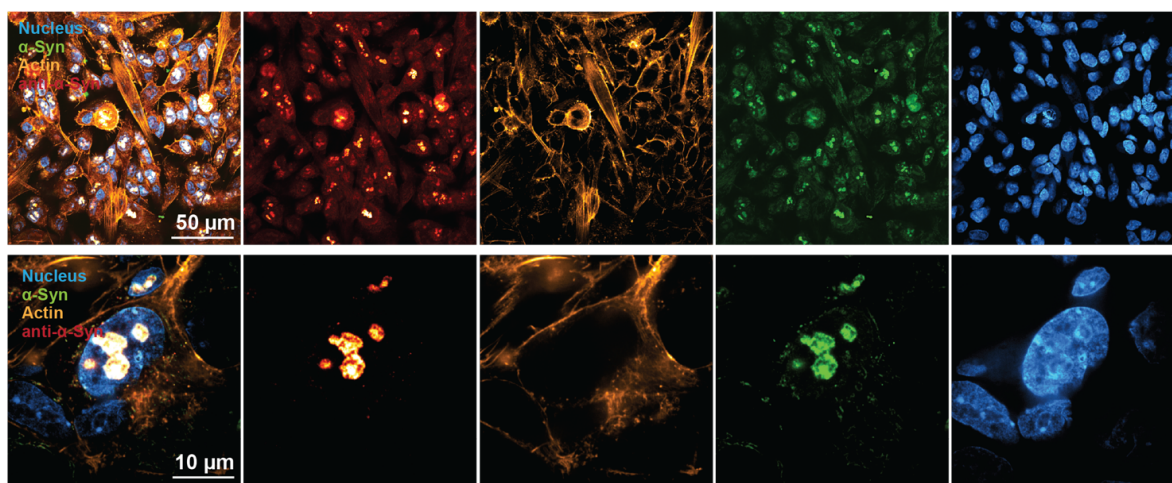
The authors have provided a detailed response to the previous reviews, with the addition of substantial new data.

An earlier concern was the lack of information about the mode of cellular uptake of the BMCs and their subsequent release into the cytoplasm. The inclusion of additional cell uptake inhibitors has allowed the authors to define the mechanism of uptake as clathrin-mediated endocytosis (Fig. 2j). They have now provided some evidence that the BCM@MPNs trigger endosomal release (Supp Fig. 14).

1. The addition of PLA to demonstrate direct interaction between the internalised antibody and its target (KRas) is a helpful addition (Supp Fig. 15). PLAs have now also been done to demonstrate interaction of the BMCs with PSMD9 (Fig. 31): in the case of the anti- α -syn-BMC@MPNs (Supp Fig 31d), the authors may want to speculate on the likely mechanism by which the antibodies reach the nucleus – given their large size relative to nuclear pore size?

Response: While the nuclear pore complex typically restricts diffusion of large macromolecules, α -synuclein dynamically shuttles between the cytoplasm and nucleus, which may facilitate its accessibility to cytosolic degraders^{8,9}.

Additionally, we observed IgG signals within the nucleus (Supplementary Fig. 28, shown below, red signals denotes the IgG cargo location), which suggested that the IgG binders reached their targets. The precise mechanism of nuclear entry of BMCs remains unexplored, but it could be related to their deformability or vescoelastic properties. In our early experiments, we incorporated nuclear localization signal (NLS) peptides into the LLPS precursors and observed enhanced nuclear accumulation. However, because NLS motifs introduce longer peptide sequences that compromise LLPS efficiency and BMC formation, we ultimately used precursors without NLS. Even in the absence of NLS, detectable IgG signals were still observed in the nucleus.



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.

2. Additional controls have been added as suggested in the previous reviews. One example is the new *in vivo* experiment shown in Supp. Fig. 23 – demonstrating the relative lack of efficacy of the anti-KRASG12V-BMC@MPN in a HT29 (KRASWT) xenograft model.

A minor point is that the authors were asked to justify their claim that CPPs have in the past been shown to be immunogenic. However, the papers they have cited concern ADCs rather than CPPs.

Response: We have updated the citation to include an additional reference discussing the potential immunogenic risks and endosomal escape challenges of CPPs:

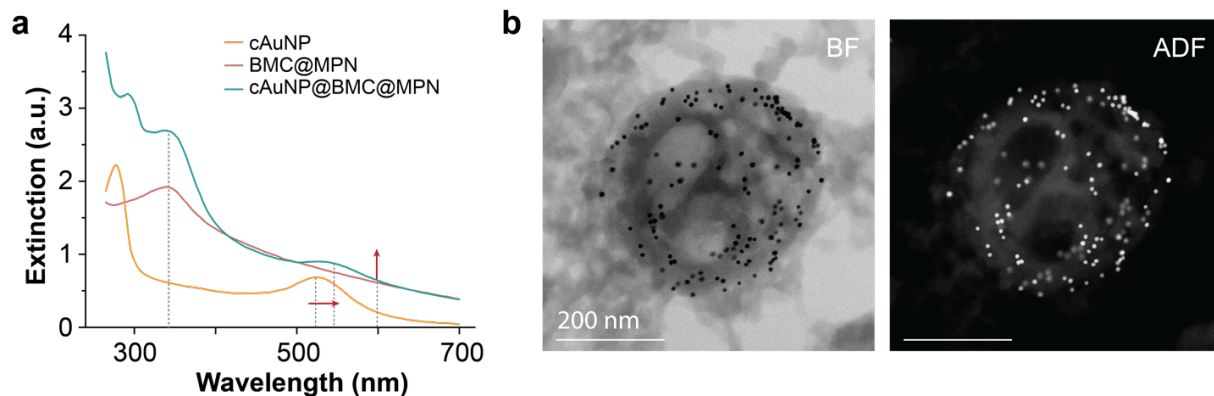
Page 2, line 17: “Current delivery methods, like cell-penetrating peptide conjugates, may pose immunogenic risks and fail to preserve IgG functionality long enough for intracellular targets to be reached¹⁵⁻¹⁷.

17. Xie, J. et al. Cell-Penetrating Peptides in Diagnosis and Treatment of Human Diseases: From Preclinical Research to Clinical Application. *Frontiers in Pharmacology* Volume 11 - 2020, (2020).”

3. In the rebuttal the authors provide the calculation of the total IgG accumulation per cell: this is interesting and could be added to the Supp Materials.

Overall, the authors have more precisely stated the novelty of their delivery system, and their conclusions are now better supported by the addition of the new data.

Response: Thank you for the suggestion. We have updated our Supplementary Fig. 7 to include this discussion, shown below.



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.

Reviewer #3

The authors have addressed some of the reviewer concerns from the prior round of review, but some gaps remain in the work that need to be clarified. In addition to the points below, further clarification of exactly how peptides remain bound to the antibodies after dissolution of the BMC in the cytoplasm and how this results in proteasome recruitment, and why free peptides do not compete with binding to the proteasome is needed.

Response to responses of initial comments:

Reviewer Comment: In the image in 2i, fluorescence is primarily in small, circular structures. What is the proposed reason for these structures and staining pattern? Do these co-stain with proteins in the endocytic pathway or lysosomal markers? It would be helpful to have a sense of what these represent, since they primarily appear to vesicular structures.

Response: These structures are our IgG-BMC@MPNs. There are no co-staining biomarkers F-actin (red) was stained to outline the cell membrane and indicate the relative intracellular localization of BMCs (green). We have used a larger font in the figure legend in the panel and added a description as well.

I do not see evidence that intracellular markers do not co-stain, was this experiment performed? If so, please include the data, if not, it seems like an important experiment to define where these complexes go inside cells.

Response: The aim of Fig. 2i is to demonstrate the cytosolic accessibility of BMCs rather than to characterize their intracellular trafficking routes. In this experiment, we incubated a cell monolayer with Alexa Fluor 488–labeled BMCs, followed by staining of actin and nuclei to delineate cell boundaries and intracellular structures. There is no co-stain of intracellular markers other than actin and nuclei. The green channel denotes BMCs, the blue channel marks the nucleus, and the red channel outlines the cell boundaries. As shown in Fig. 2i, BMCs are clearly internalized and distributed within the cytosolic region of the cells.

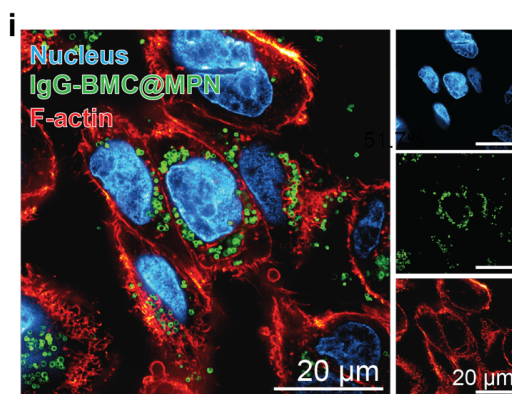
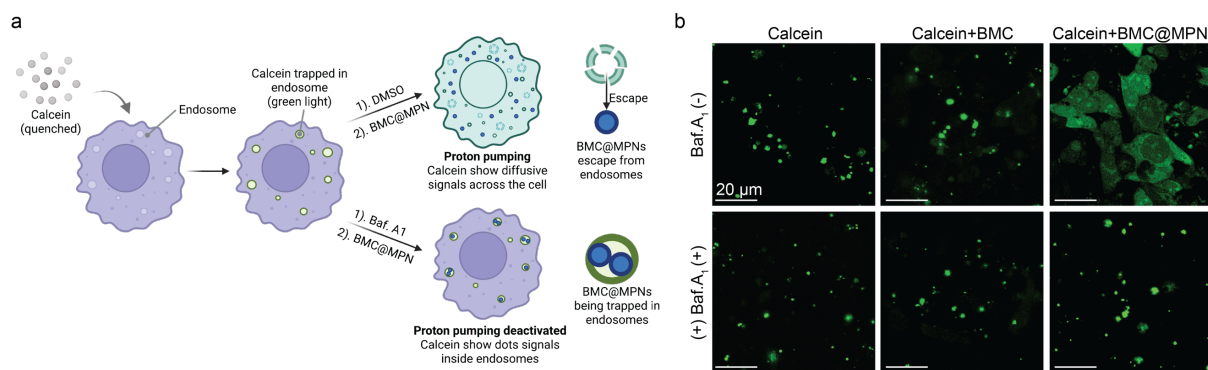


Fig. 2 | Engineering of IgG-BMC@MPNs with enhanced cell uptake. i, Fluorescence micrographs of SW480 cells incubated with AF488-IgG-BMC@MPNs. Blue, nucleus; green, IgG-BMC@MPNs; red, F-actin.

In addition, we included an endosomal escape assay (Supplementary Fig. 14, shown below). Calcein leakage indicates that BMCs can escape from lysosomes, likely enabled by the proton sponge effect of the MPN coating. We hope these experiments support our conclusion that BMCs gain access to the cytosol, where they can reach intracellular protein targets.



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.

Reviewer Comment: The substantial number of off-target degradation events in Fig. 5i,j is troubling for an approach that should be highly surgical in its ability to downregulate a specific target. I think this essential piece of data shows that there are some issues with the approach, with the targets chosen, or with the nature of the degradation which takes a long time and thus makes quantitative MS difficult.

Response: We would like to clarify that many of the proteins identified in our original Fig. 5i, j are not off-targets but instead functionally related effectors that naturally interact with the target protein in active signaling complexes, rather than direct degradation by our construct54-56. Importantly, the number of off-target hits we observed (83 downregulated and 117 upregulated out of >3500 signals for KRAS; 101 downregulated and 119 upregulated out of >3500 signals for α -syn) is comparable to or even lower than those reported in recent studies using intracellularly delivered antibodies or degrader constructs without genetic modification.

To further validate the hits (i.e., proteasome subunits and target proteins, KRAS or alpha-synuclein), we now included a proximity ligation assay (PLA) in our Supplementary Fig. 31 to evaluate the interaction between PSMD9—a 19S proteasomal subunit—and the target protein. The PLA signal supports a direct recruitment mechanism involving the proteasome, strengthening the specificity of our degradation strategy. We agree that long degradation windows can complicate MS quantification, and we are actively optimizing experimental timing to minimize such effects in future studies.

Whole cell-proteomics should be utilized to demonstrate selectivity of degradation.

Response: The aim of Fig. 5i–j is to demonstrate that the target protein is degraded through engagement with the proteasome subunit. Therefore, we believe that pull-down proteomics and the proximity ligation assay (PLA) performed represent more appropriate experimental approaches for probing such interactions.

In contrast, whole-cell proteomics will capture global upstream and downstream protein changes besides targets, which makes the fold change of the target less interpretable. Moreover, changes in overall proteasome subunit abundance may not be detectable, as the system is designed to degrade a specific target protein rather than alter the global levels of proteasome components.

References

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We sincerely thank the Reviewers and Editors for their feedback and suggestions. In response, we have expanded our discussion to address the points raised. We have revised the manuscript and provided a point-by-point response below. For clarity, Reviewer comments are shown in blue italics, followed by our responses in black, with key points highlighted in bold. We hope our revised manuscript will be further considered for publication at *Nature Communications*.

Reviewer #1

The authors have addressed all Reviewers' comments. I recommend acceptance of the manuscript.

Reviewer #2

The authors have satisfactorily responded to my questions/points in the last round of review.

Reviewer #3

The authors have chosen not to perform the requested revision experiments, but to clarify their aims. While this is their prerogative, the original questions raised by the requested experiments remain unanswered.

Author response from last round: The aim of Fig. 2i is to demonstrate the cytosolic accessibility of BMCs rather than to characterize their intracellular trafficking routes. In this experiment, we incubated a cell monolayer with Alexa Fluor 488–labeled BMCs, followed by staining of actin and nuclei to delineate cell boundaries and intracellular structures. There is no co-stain of intracellular markers other than actin and nuclei. The green channel denotes BMCs, the blue channel marks the nucleus, and the red channel outlines the cell boundaries. As shown in Fig. 2i, BMCs are clearly internalized and distributed within the cytosolic region of the cells.

1. Reviewer's comment: Without some intracellular co-stain, this experiment could just be showing endoscopes which is not particularly informative or useful. The original comment understood the aim of the figure, but request further information to provide additional value to the experiment.

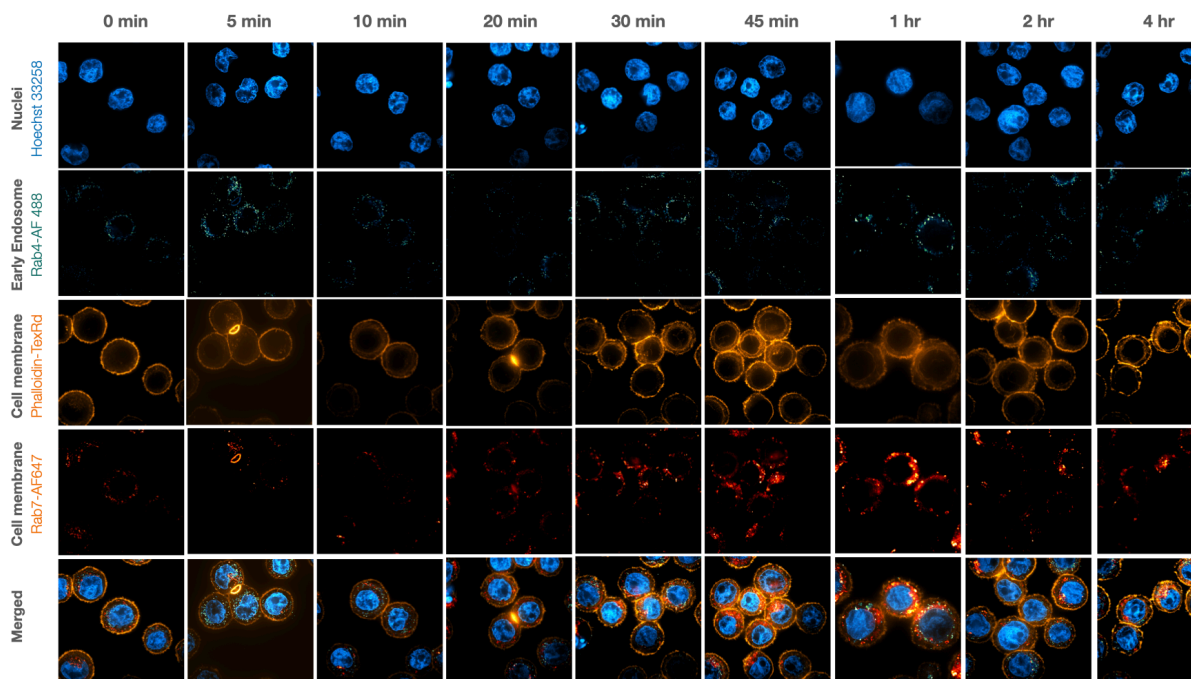
Response: We assumed the “endoscope” mentioned here means endosome, and we assumed the requested “additional value” mentioned here means the information of intracellular distribution pattern of BMCs.

Firstly, we clarify that the original experiment (Fig. 2i) neither depicts nor discusses endosomal compartments. Both the morphology and fluorescent signals are attributed exclusively to internalized biomolecular condensates (BMC@MPNs), rather than other intracellular structures. The purpose of Fig. 2i is to demonstrate cytosolic accessibility of BMCs. A complete time-resolved uptake series in cell monolayers is provided in Supplementary Fig. 11.

Additionally, intracellular distribution of BMCs is best resolved by electron microscopy, as BMC@MPNs can dynamically quench noncovalently labeled fluorescent signals^{1,2}, making fluorescence-based readouts less robust. In transmission electron microscopy (TEM), BMCs appear as electron-lucent (bright) structures, whereas intracellular vesicles, including endosomes, are electron-dense (dark) due to osmium and uranyl acetate staining. Thus, intracellular

distribution can be distinguished based on morphology and electron contrast. Representative TEM images of BMC@MPNs are provided in Fig. 2k and Supplementary Fig. 13.

In response to the reviewer's comments, we here provide a **time-resolved endosome activity assay** (shown below). Co-staining of Rab4 and Rab7 was used to track early and late endosomal dynamics, respectively, as established previously³. Early endosome activity increased within the first 10 min following BMC@MPN entry, declined thereafter, and returned to baseline by 30 min, whereas late endosome activity increased progressively over time.



Time-dependent endosome activity assay. Early endosome activity increased within the first 10 min following BMC@MPN entry, transiently declined, and returned to baseline by 30 min. In contrast, late endosome activity increased steadily over time.

However, we note that the transmembrane entry and intracellular routing of BMCs remain largely unexplored in the liquid–liquid phase separation (LLPS)-based materials field. BMCs engage cells through a distinct mechanism governed by wetting-driven interactions and viscoelastic behavior^{4,5}. Accordingly, fluorescence imaging alone cannot conclusively resolve their intracellular fate. Our ongoing work is using genome-wide omics and correlative light–electron microscopy (CLEM) to establish a global landscape for BMC trafficking.

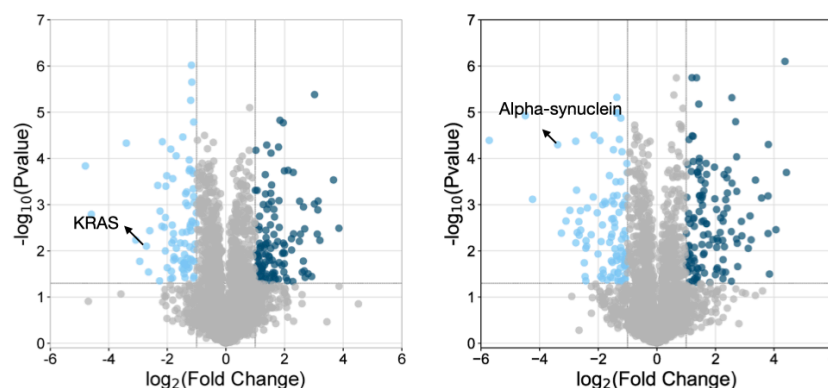
In this study, our primary objective is to establish cytosolic accessibility and functional delivery of BMCs, which is directly supported by time-resolved uptake imaging (Fig. 2i) and the proton pump assay (Supplementary Fig. 11).

Author response from last round: The aim of Fig. 5i–j is to demonstrate that the target protein is degraded through engagement with the proteasome subunit. Therefore, we believe that pull-down proteomics and the proximity ligation assay (PLA) performed represents more appropriate experimental approaches for probing such interactions. In contrast, whole-cell proteomics will capture global upstream and downstream protein changes beyond the targets, making the target's fold change less interpretable. Moreover, changes in overall proteasome subunit abundance may not be detectable, as the system is designed to degrade a specific target protein rather than alter the global levels of proteasome components.

2. Reviewer's comment: While the aim of the figure is as stated, it shows that there a substantial number of off targets and interactions that would complicate selective degradation. The aim of the requested experiment is to clarify how selective this approach can be, as without selectivity there is very little reason to use a complicated delivery vehicle for an antibody.

Response: We summarized the the key requests of this comment are to (1) provide whole-cell proteomics data, (2) demonstrate the selectivity of our BMC design, and (3) explain the possible origin of non-target hits in pull-down proteomics data.

(1). In response to the request of whole cell proteomics data: We have included 24-hour post treatment whole-cell proteomics data requested (shwon below), showing a 2.7-fold of KRAS and 3.8-fold of α -synuclein reduction relative to the untreated control. The presence of non-target proteins and interactions has been addressed in our previous response, as both KRAS and α -synuclein are in complex networks involving multiple isoforms and upstream and downstream effectors with relatively high dissociation constants ^{6,7}. Furthermore, whole-cell proteomics is inherently dependent on the sampling time point and reflects global cellular states, including changes in cell cycle progression and metabolism following target protein loss-of-function. Accordingly, these secondary effects are reflected in the proteomic profile alongside the targeted protein signals.



24-hour whole-cell proteomics analysis of KRAS and α -syn degradation in SW480 cells (left) and SK-N-SH cells (right).

(2). In the response to the discussion of selectivity:

We agree that the proteomics approach may not be the best way to demonstrate degradation selectivity, as whole-cell LFQ largely depends on the sampling time point and cell state, and pull-down LFQ requires a mild detergent to preserve protein-protein interactions, which also preserves upstream and downstream binding proteins.

In our work, we have systematically discussed selectivity at the molecular and cellular levels in our Fig 3. Key findings are summarized below.

(i). **Molecular selectivity** is supported a 50-50 degradation of KRAS wildtype vs G12V variant in H441^{G12V/WT} heterozygous cells when treated by anti-G12V BMC@MPNs (Fig. 3c, shown below), reflecting allele-specific targeting.

(i). **Biological selectivity** is demonstrated in a co-culture of HT29^{WT/WT} and SW480^{G12V/G12V} homozygous cells, where BMCs selectively suppress SW480 proliferation without affecting HT29. (Fig. 3f, shown below).

(iii). Besides demonstrated selectivity, we **highlight the benefit of BMC as a protein degradation tool for its ubiquitin-independent proteasomal degradation property, which does not require genomic elevation of degradation adaptor (e.g., TRIM 21). We claim this as an improvement over current protein degradation platforms.**

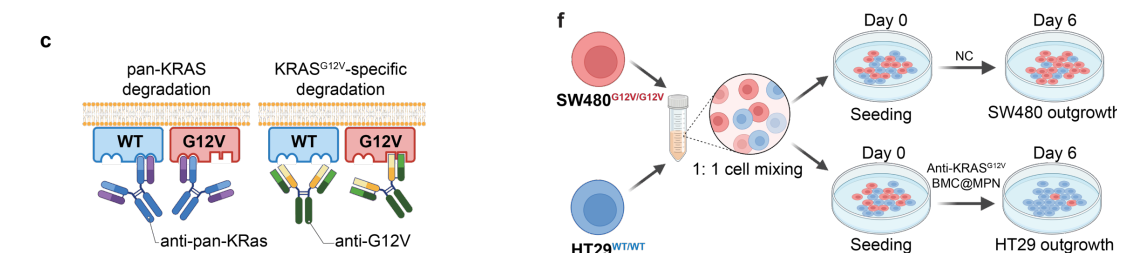


Fig. 3 | Antibody-guided targeting enabled KRAS^{G12V}-selective degradation. c, Schematic of orthogonal-antibody blotting. Cells were treated with anti-pan-KRAS or anti-KRAS^{G12V}-BMC@MPNs. The residual total KRAS or KRAS^{G12V} was resolved using detection antibodies that bind to distinct epitopes. f, Schematic of co-culture assay of HT29 and SW480 cells.

(3). In response to the concern regarding non-target signals in the pull-down label-free quantification (LFQ) analysis: As noted in our previous response, the observed changes and interacting proteins are consistent with the KRAS signaling network, supported by gene set enrichment analysis (GSEA; Supplementary Fig. 31). Additionally, to preserve protein-protein interactions (PPIs) involved in the degradation process, we used mild detergent conditions to maintain interactions between KRAS and proteasome subunits. As a result, proteins associated with KRAS may also be detected in the pull-down.

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We sincerely thank the Reviewers and Editors for their feedback and suggestions. In response, we have provided a point-by-point response below. For clarity, Reviewer comments are shown in blue italics, followed by our responses in black, with key points highlighted in bold. We hope our revised manuscript will be further considered for publication at *Nature Communications*.

Reviewer #3

Thanks to the authors for attempting to address the requests. The only additional request prior to publication would be that the proteomics data from the last revision be included in the manuscript, as I do not see it referenced in the main text or supplementary information, and discussed appropriately, with all targets more substantially degraded labeled. After that is done, there are no additional concerns. In general, experiments for revisions should be included in manuscripts.

Response: We thank the Reviewer for their evaluation of our revisions. We agree that additional datasets can enrich the manuscript. However, given the defined scope of this study that focuss on establishing the mechanistic framework and functional validation of BMC-mediated targeted protein degradation, and considering experimental consistency and reproducibility across biological replicates as discussed previously, we believe that including the endosomal activity assays and full proteomics dataset would extend beyond the central focus and may dilute the clarity of the main conclusions. Nevertheless, the full discussion and revision history will be provided in the Peer Review File.

We hope this course of action preserves the conceptual clarity of the manuscript while ensuring transparency and accessibility of all revision details.