

Reporting Summary

Nature Portfolio wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Portfolio policies, see our [Editorial Policies](#) and the [Editorial Policy Checklist](#).

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a Confirmed

- ☐ ☒ The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
- ☐ ☒ A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
- ☐ ☒ The statistical test(s) used AND whether they are one- or two-sided
Only common tests should be described solely by name; describe more complex techniques in the Methods section.
- ☒ ☐ A description of all covariates tested
- ☐ ☒ A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
- ☐ ☒ A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
- ☐ ☒ For null hypothesis testing, the test statistic (e.g. F , t , r) with confidence intervals, effect sizes, degrees of freedom and P value noted
Give P values as exact values whenever suitable.
- ☒ ☐ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
- ☒ ☐ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
- ☐ ☒ Estimates of effect sizes (e.g. Cohen's d , Pearson's r), indicating how they were calculated

Our web collection on [statistics for biologists](#) contains articles on many of the points above.

Software and code

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

Data collection

FEI G20 Lab6 200 kV TEM (FEI, Hillsboro, OR) was used to collect Cryo-TEM data. All ITC experiments were performed using a Malvern MicroCal PEAQ-ITC instrument. AFM images were obtained using a Dimension FastScan AFM (Bruker) with FastScan-C probes, operating in ScanAsyst mode. Bioluminescence imaging was performed using the IVIS Lumina system (Perkin Elmer). Nanoflow analysis was performed using the Nanoflow (NanoFCM Inc., China).

Data analysis

Statistical testing was performed using GraphPad Prism (v.8.0). Analysis of flow cytometry data was performed with FlowJo v10.7. Whole body and organ bioluminescence was analyzed using Living Image Software. Image of the tissue sections were analyzed using ImageJ(v2.9.0). The custom code for the molecular dynamics simulations on the tension test, endocytosis, and translocation of the lipid nanoparticle is available via GitHub at <https://github.com/youliangzhu/pygamd-v1>. The input files for the MD simulations and the custom code for property analysis is available via GitHub at <https://github.com/youliangzhu/lnp.git>.

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

Data

Policy information about [availability of data](#)

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

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

The authors declare that all data supporting the findings of this research are available within the article and Supplementary Information. Source data are provided with this paper.

Research involving human participants, their data, or biological material

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

Reporting on sex and gender	NA
Reporting on race, ethnicity, or other socially relevant groupings	NA
Population characteristics	NA
Recruitment	NA
Ethics oversight	NA

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

Field-specific reporting

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

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

For a reference copy of the document with all sections, see [nature.com/documents/nr-reporting-summary-flat.pdf](https://www.nature.com/documents/nr-reporting-summary-flat.pdf)

Life sciences study design

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

Sample size	Sample size was calculated based on primary hypothesis and determined by referencing similar previous studies in the field, which provided estimates of effect size and variability.
Data exclusions	No data values were excluded in the analysis.
Replication	All samples were processed in technical duplicates, with averaged values of technical duplicates being reported for each biological replicate. All attempts at replication were successful.
Randomization	Cells from the same passage were randomly used for experiment groups or control groups. All animals were randomly allocated into different experimental groups.
Blinding	True binding was not applied in this proof-of-concept study. Data collection and analysis for cell and animal experiments were performed by separate individuals.

Reporting for specific materials, systems and methods

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

Materials & experimental systems

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

Methods

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

Antibodies

Antibodies used	Anti-mouse puromycin antibody (Developmental studies hybridoma bank, PMY-2A4), anti-mouse CK 18 antibody (Abcam, ab315096), anti-mouse F/480 antibody (Servicebio, GB113373), anti-mouse CD31 antibody (Servicebio, GB12063), anti-mouse PCSK9 antibody (Abcam, ab185194), anti-mouse HSP90 (Santa Cruz, sc-101494)
Validation	1. Anti-mouse puromycin antibody has been validated to be used for western blots analysis. The suggested concentration range of this reagent is 0.2-0.5 µg/ml from the manufacturer's website and it also mentioned species reactivity with all species (https://dshb.biology.uiowa.edu/PMY-2A4). We finally use 1 µg/ml for analysis. 2. Anti-mouse CK 18 antibody has been validated to be used for immunofluorescence. The manufacturer's website mentioned species reactivity with mouse (https://www.abcam.com/en-us/products/primary-antibodies/cytokeratin-18-antibody-rm1051-ab315096?srsltid=AfmBOorci3TwHLbyA3KtBsamiLUQV8A3J6GneLM6EkxGDPQOqGt3eEV). We finally use 1/2000 dilution for analysis. 3. Anti-mouse F/480 antibody has been validated to be used for immunofluorescence. The recommended dilution range of this reagent is 1/500-1/1000 from the manufacturer's website, and it also mentioned species reactivity with mouse (https://www.servicebio.com/goodsdetail?id=144932). We finally use 1/200 dilution for analysis. 4. Anti-mouse CD31 antibody has been validated to be used for immunofluorescence. The recommended dilution range of this reagent is 1/200-1/1000 from the manufacturer's website, and it also mentioned species reactivity with mouse (https://www.servicebio.com/goodsdetail?id=141899). We finally use 1/200 dilution for analysis. 5. Anti-mouse PCSK9 antibody has been validated to be used for western blot. The recommended dilution of this reagent is 1/1000 from the manufacturer's website, and it also mentioned species reactivity with mouse (https://www.abcam.com/en-us/products/primary-antibodies/pcsk9-antibody-epr17827-117-ab185194?srsltid=AfmBOopTmHPMeTHHgxDF3nSDIUanrsoS63WtdNWlr9pEckOo2EyMDjN). We finally use 1/1000 dilution for analysis. 6. Anti-mouse HSP-90 antibody has been validated to be used for western blot. The manufacturer's website mentioned species reactivity with mouse (https://www.scbt.com/p/hsp-90-antibody-ac-16?srsltid=ARcRdnp05Yz_6dyEgS140QuuhDs8KAvXe8m1xpoxLSAZjWwOxRmjHfzE). We finally use 1/5000 dilution for analysis.

Eukaryotic cell lines

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

Cell line source(s)	HEK-293T, DC2.4, THP-1 and Hepa1-6 were originally obtained from ATCC. HEK-293T-eGFP cells were obtained from Dr. Yu Haojie's lab from NUS Biochemistry.
Authentication	None of the cell line used were authenticated after receiving from ATCC and Dr. Yu Haojie's lab.
Mycoplasma contamination	HEK-293T-eGFP cells were tested negative of mycoplasma contamination. The other cell lines were not tested for mycoplasma contamination after receiving from ATCC.
Commonly misidentified lines (See ICLAC register)	The cell line used in this article are not in the list of misidentified lines.

Animals and other research organisms

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

Laboratory animals	BALB/c and C57BL/6 mice (6-8 weeks old, female) were purchased from Invivos Pte. Ltd. All mice were housed under specific pathogen-free conditions and used in accordance with the animal experimental guidelines.
Wild animals	No wild animals were used in this study.

Reporting on sex	Female
Field-collected samples	No field-collected samples were applied in this study.
Ethics oversight	All the animal protocols were approved by the National University of Singapore (NUS) Institutional Animal Care and Use Committee (IACUC).

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

Plants

Seed stocks	NA
Novel plant genotypes	NA
Authentication	NA

Flow Cytometry

Plots

Confirm that:

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

Methodology

Sample preparation	Biological resources of samples for flow cytometry in this study were based on in vitro cell lines. Sample preparation process were detailed in Methods section: Cells were seeded in 96-well plates (1×10^4 cells per well) and cultured overnight. Cells were incubated with LNP or MARF LNP for different time points. After treatment, the cells were washed three times with PBS before analysis by flow cytometry after trypsinization.
Instrument	CytoFlex LX (Beckman Coulter)
Software	FlowJo (v10.7)
Cell population abundance	Flow cytometry was used to evaluate mRNA transfection efficiency and cellular uptake via detecting mean fluorescence intensity and ~5000 cells were collected for each sample.
Gating strategy	Gates were drawn based on PBS treated groups. Gating strategy for each experiment were provided in Supplementary information.

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