Science Advances
AAAS

advances.sciencemag.org/cgi/content/full/6/30/eabb4429/DC1

Supplementary Materials for

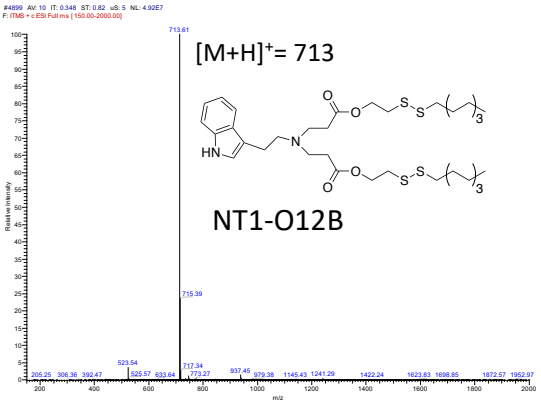
Neurotransmitter-derived lipidoids (NT-lipidoids) for enhanced brain delivery through intravenous injection

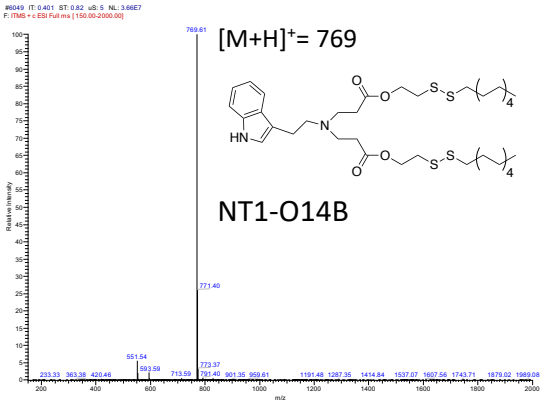
Feihe Ma, Liu Yang, Zhuorui Sun, Jinjin Chen, Xuehui Rui, Zachary Glass, Qiaobing Xu*

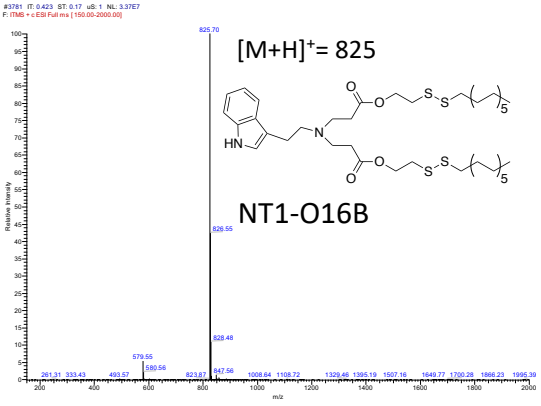
*Corresponding author. Email: Qiaobing.Xu@tufts.edu

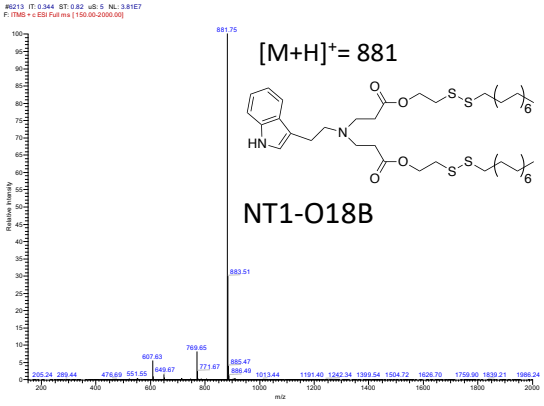
Published 24 July 2020, *Sci. Adv.* **6**, eabb4429 (2020)
DOI: 10.1126/sciadv.abb4429

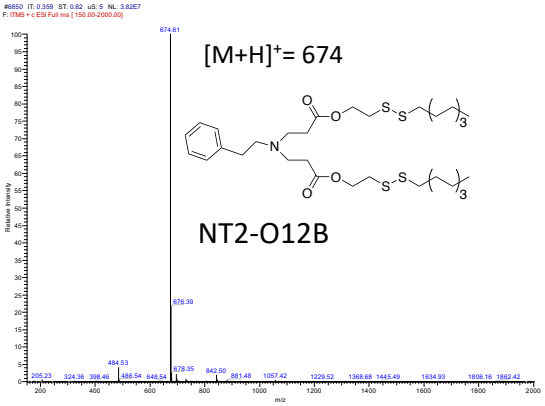
This PDF file includes:

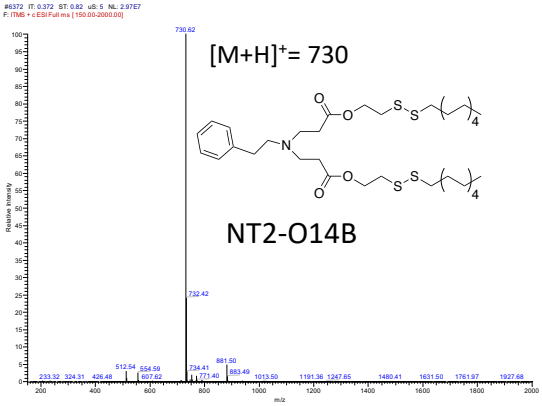

[M+H]+= 713
NT1-O12B

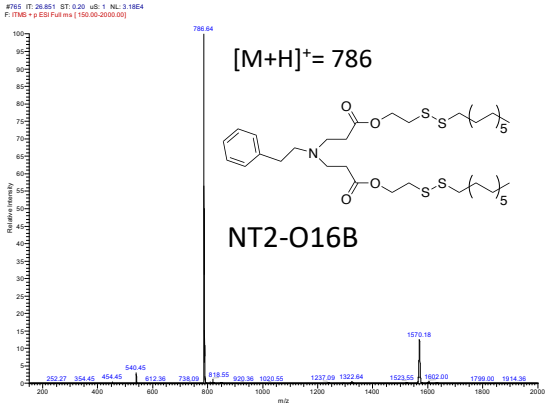

[M+H]+= 769
NT1-O14B

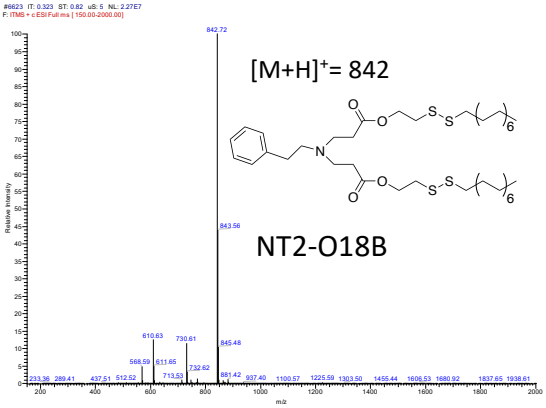

[M+H]+= 825
NT1-O16B

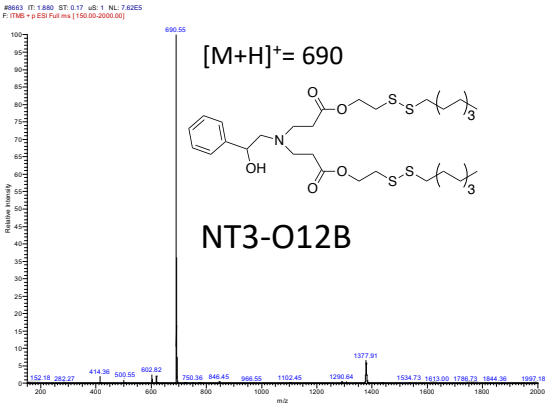

[M+H]+= 881
NT1-O18B

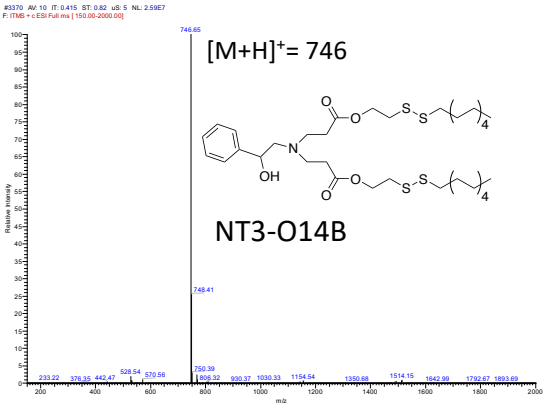

[M+H]+= 674
NT2-O12B

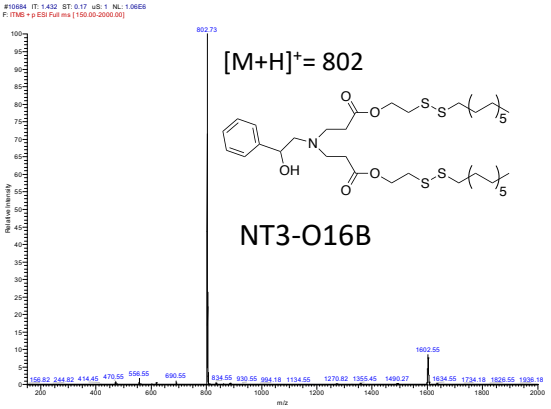

[M+H]+= 730
NT2-O14B

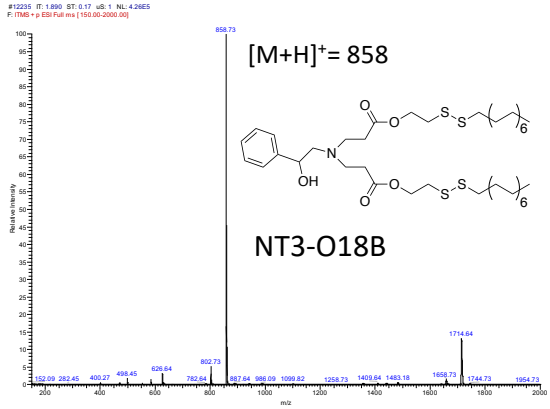

[M+H]+= 786
NT2-O16B

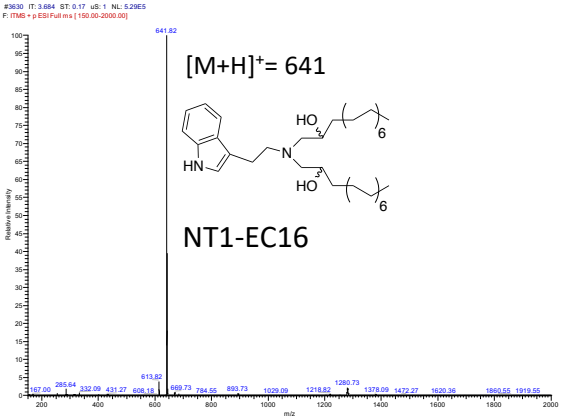

[M+H]+= 842
NT2-O18B

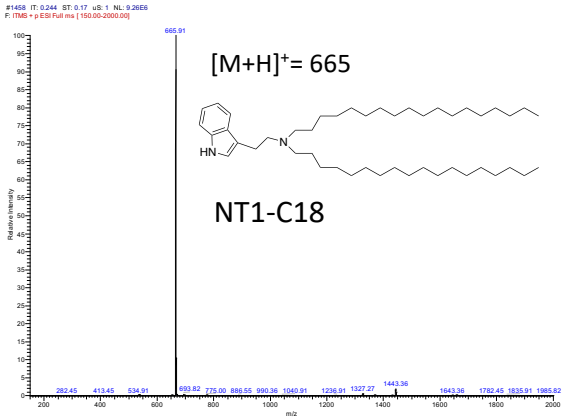

[M+H]+= 690
NT3-O12B

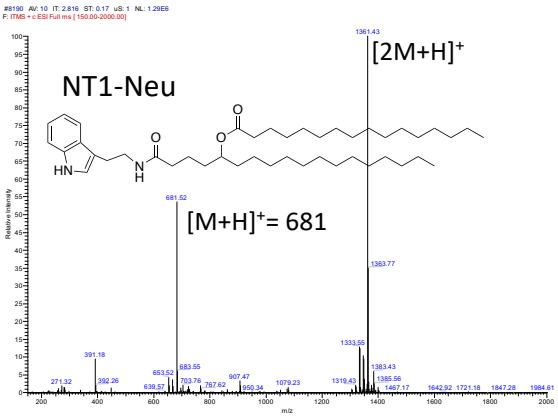

[M+H]+= 746
NT3-O14B

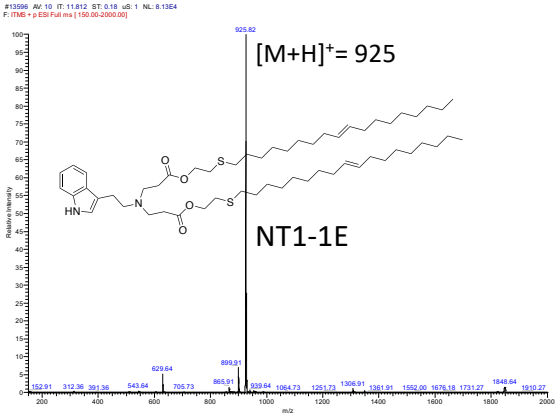

[M+H]+= 802
NT3-O16B


[M+H]+= 858
NT3-O18B


[M+H]+= 641
NT1-EC16


[M+H]+= 665
NT1-C18


[2M+H]+
NT1-Neu
[M+H]+= 681


[M+H]+= 925
NT1-1E

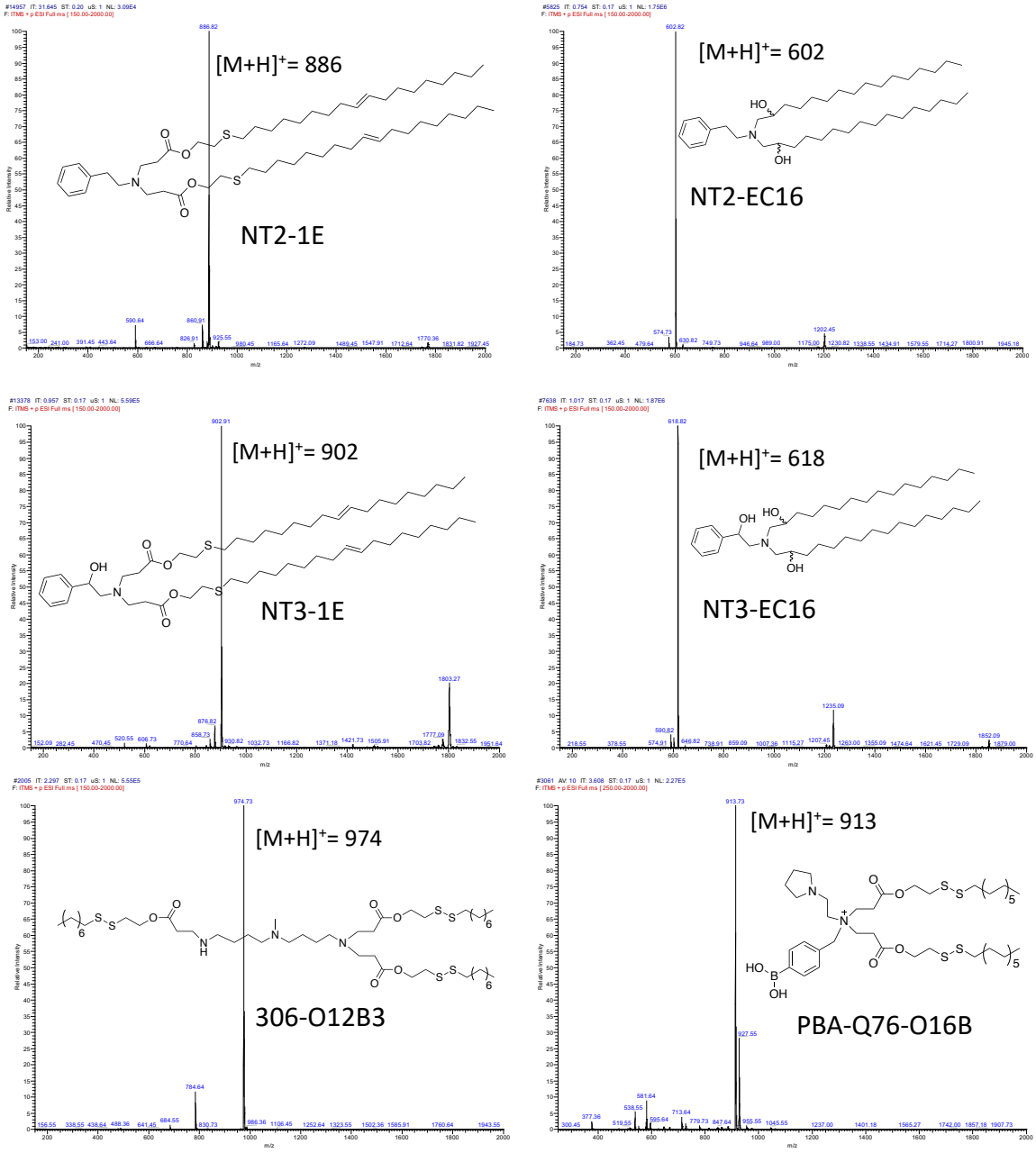


Fig. S1. Chemical structure and ESI-MS Characterization of NT-lipidoids and doping lipidoids.

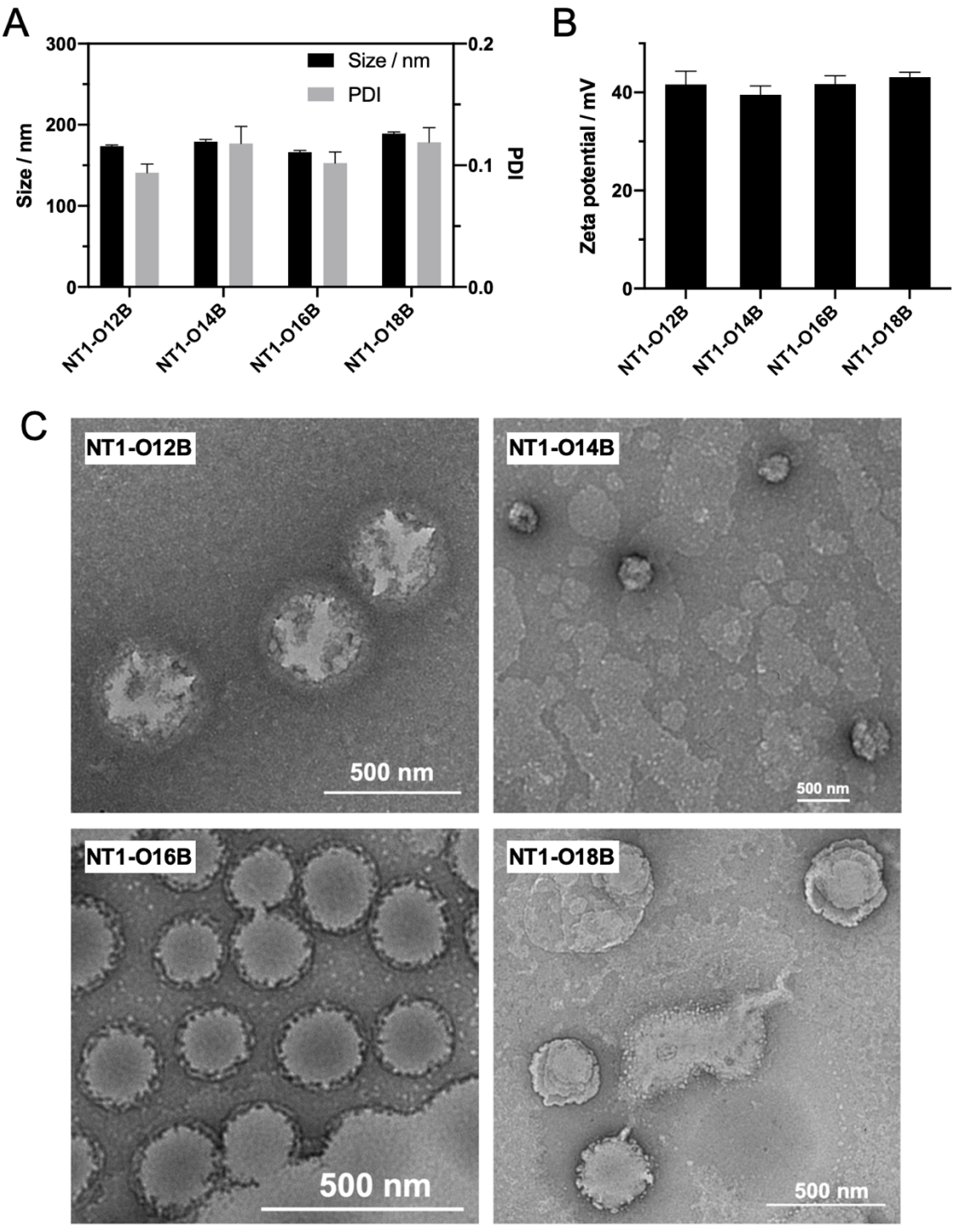


Fig. S2. **Characterization of NT1-LNPs.** (**A**) Hydrodynamic diameters and polydispersity indexes of NT1-LNPs determined by DLS measurements. (**B**) Zeta potential of NT1-LNPs. (**C**) TEM of of NT1-LNPs.

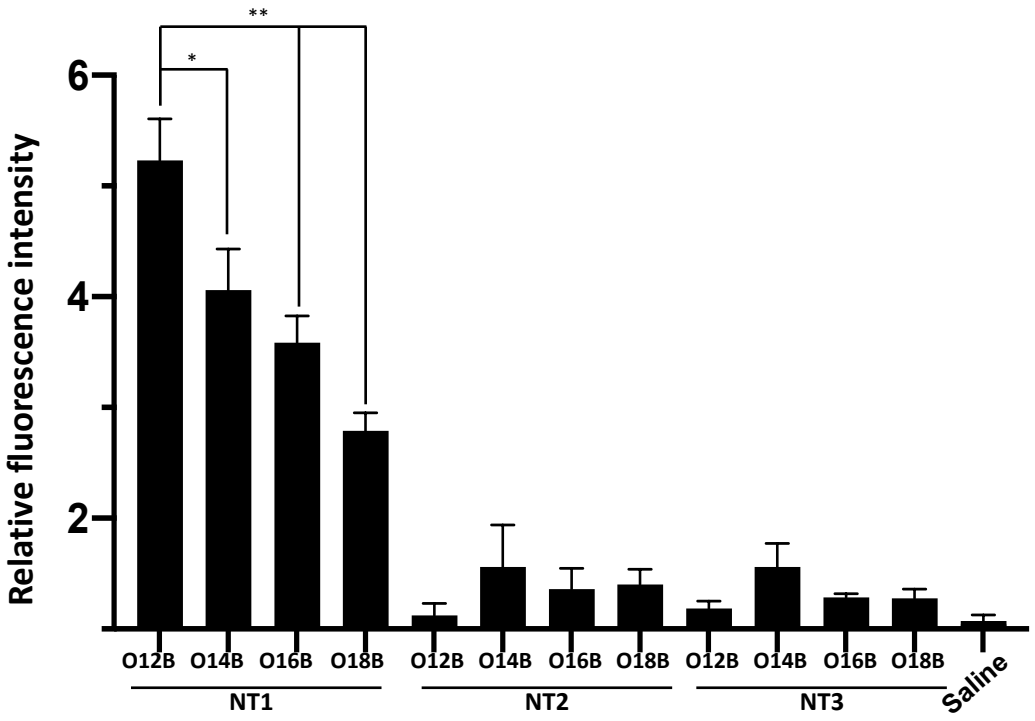
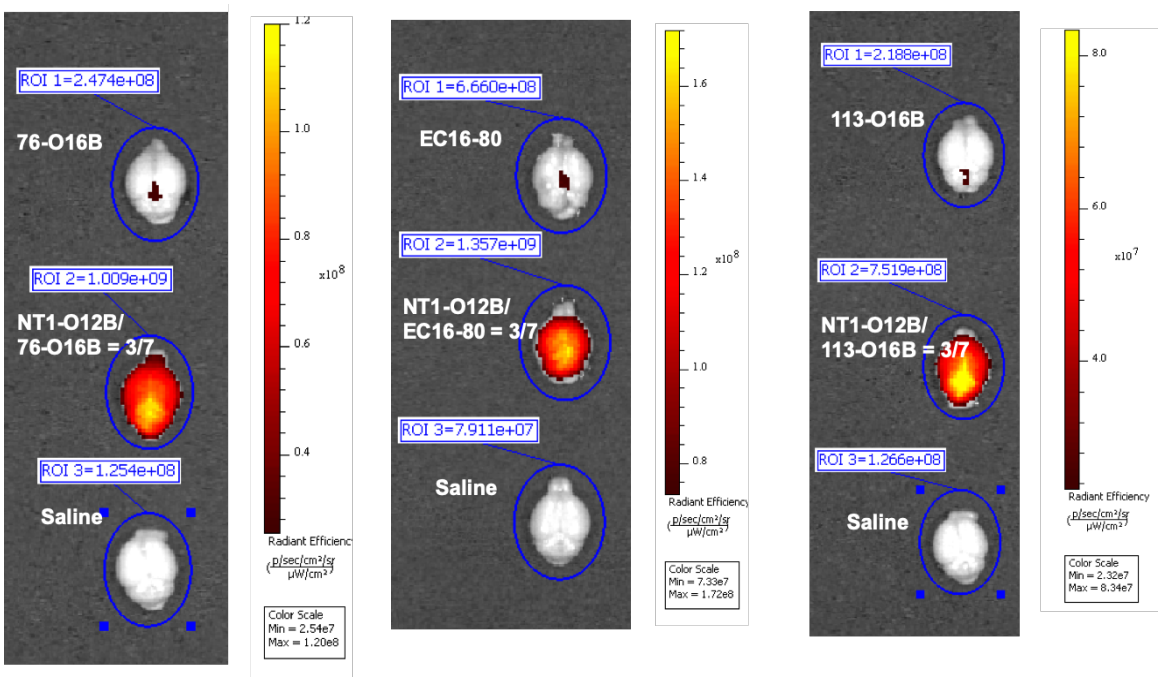


Fig. S3. **Summarized relative fluorescence intensity of the dissected brain tissue after intravenous injection of DiR-labeled NT-LNPs.** The brain tissues were collected 1 h after one-time intravenous injection of 1 mg kg^{-1} DiR-labeled NT-LNPs. DiR was doped into the NT-LNPs with a 10% weight ratio. The mice were perfused with saline before dissection. One-way ANOVA, Sidak post hoc analysis, *$p<0.05$ or **$p<0.01$.



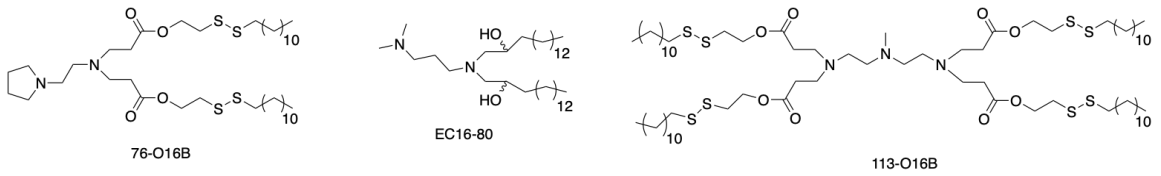


Fig. S4. **BBB-permeability of BBB-impermeable lipid formulations after doping with NT1-O12B lipidoids.** The representative *ex vivo* fluorescence images of the dissected brain 1 h after one-time intravenous injection of 1 mg kg^{-1} DiR-labeled LNPs or NT1-O12B doped NT-LNPs (ratio 3:7, w/w), and the chemical structure of 76-O16B, EC16-80, and 113-O16B. DiR was doped into the NT-LNPs with a 10% weight ratio. The mice were perfused with saline before dissection.

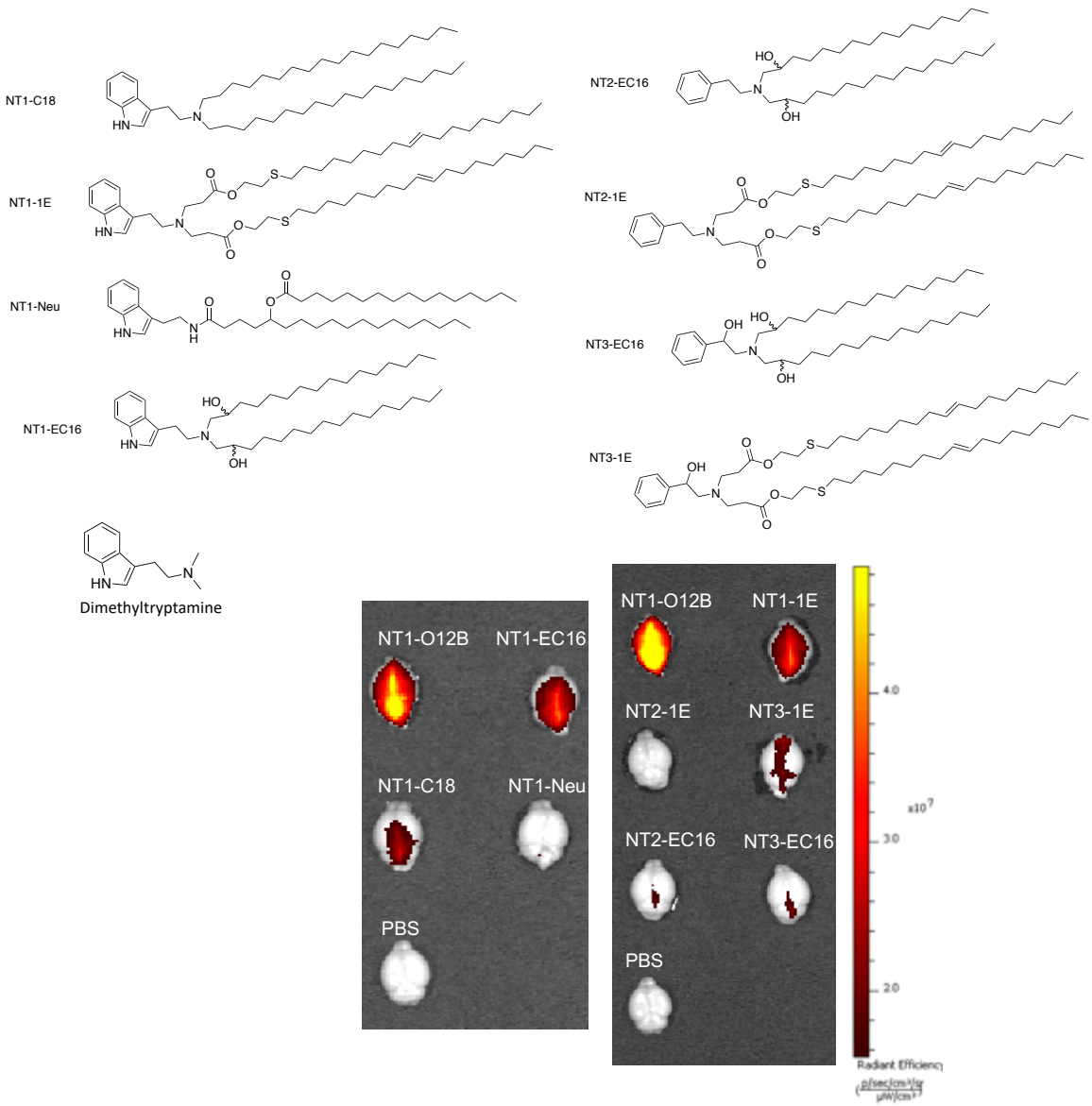


Fig. S5. **BBB-permeability of NT-lipidoids with different chemical structures**. Chemical structure of NT-lipidoids and dimethyltryptamine, and the representative *ex vivo* fluorescence images of the dissected brain 1 h after one-time intravenous injection of 1 mg kg^{-1} DiR-labeled NT-LNPs. DiR was doped into the NT-LNPs with a 10% weight ratio. The mice were perfused with saline before dissection.

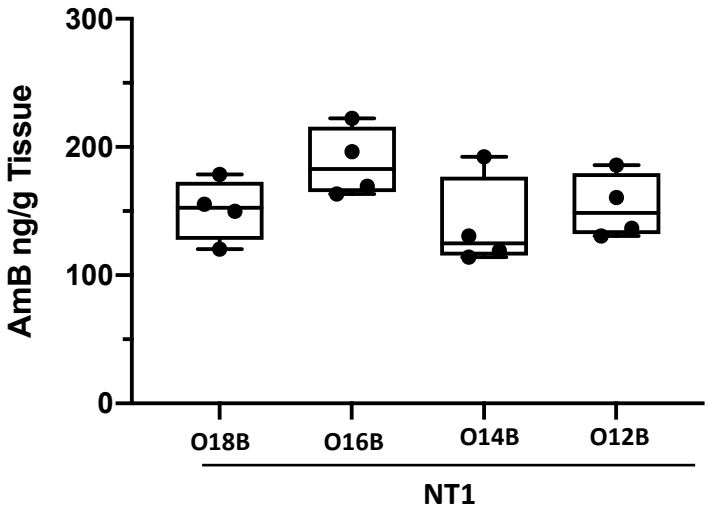


Fig. S6. **Delivery of AmB to mouse brain using pure NT1-lipidoids formulations**. AmB concentration in brain tissues 24hr after intravenous injection of 5 mg/kg AmB in various of NT1 derivatives measured using HPLC. The mice were perfused with saline before dissection.

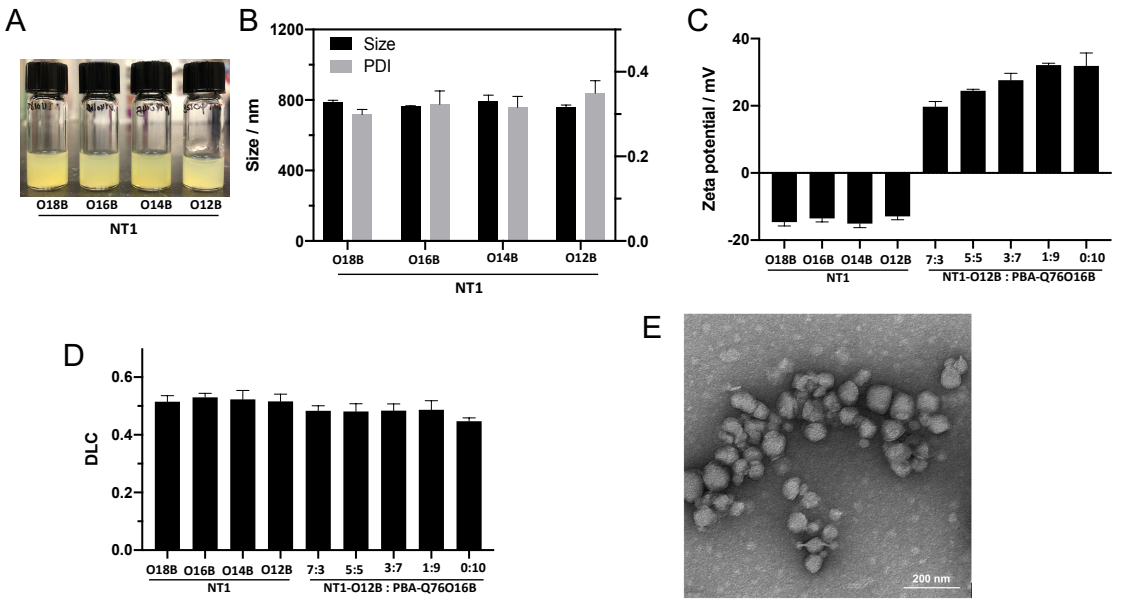


Fig. S7. Characterization of different NT-LNP /AmB formulations. (**A**) Photographs of AmB formulations in NT1-lipidoids with different tail lengths (O18B, O16B, O14B, O12B). All four NT1/AmB encapsulates showed opaque suspension. Photo Credit: Feihe Ma, Tufts University. (**B**) Hydrodynamic diameters and polydispersity indexes of NT-LNP /AmB formulations determined by DLS measurements. (**C**) Zeta potential of NT-LNP /AmB formulations. (**D**) DLC of NT-LNP /AmB formulations. (**E**) TEM of NT1-O12B/PBA-Q76O16B-3/7-AmB complex.

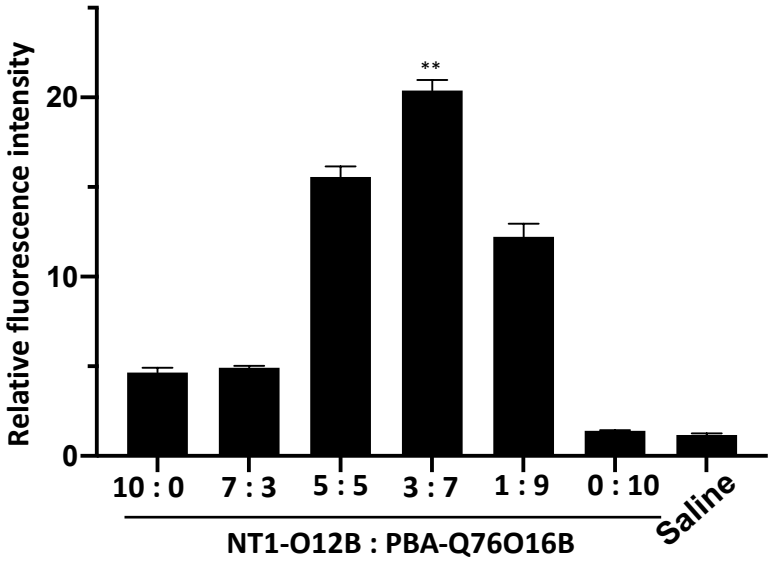


Fig. S8. **Summarized relative fluorescence intensity of the dissected brain tissue after intravenous injection of DiR-loaded NT1-O12B/PBA-Q76-O16B LNPs.** The dissected brain tissues were collected 1 h after one-time intravenous injection 1 mg kg $^{-1}$ DiR-loaded NT1-O12B/PBA-Q76-O16B LNPs. The weight ratio of DiR in LNPs is 10%. **p<0.001. One-way ANOVA, Sidak post hoc analysis.

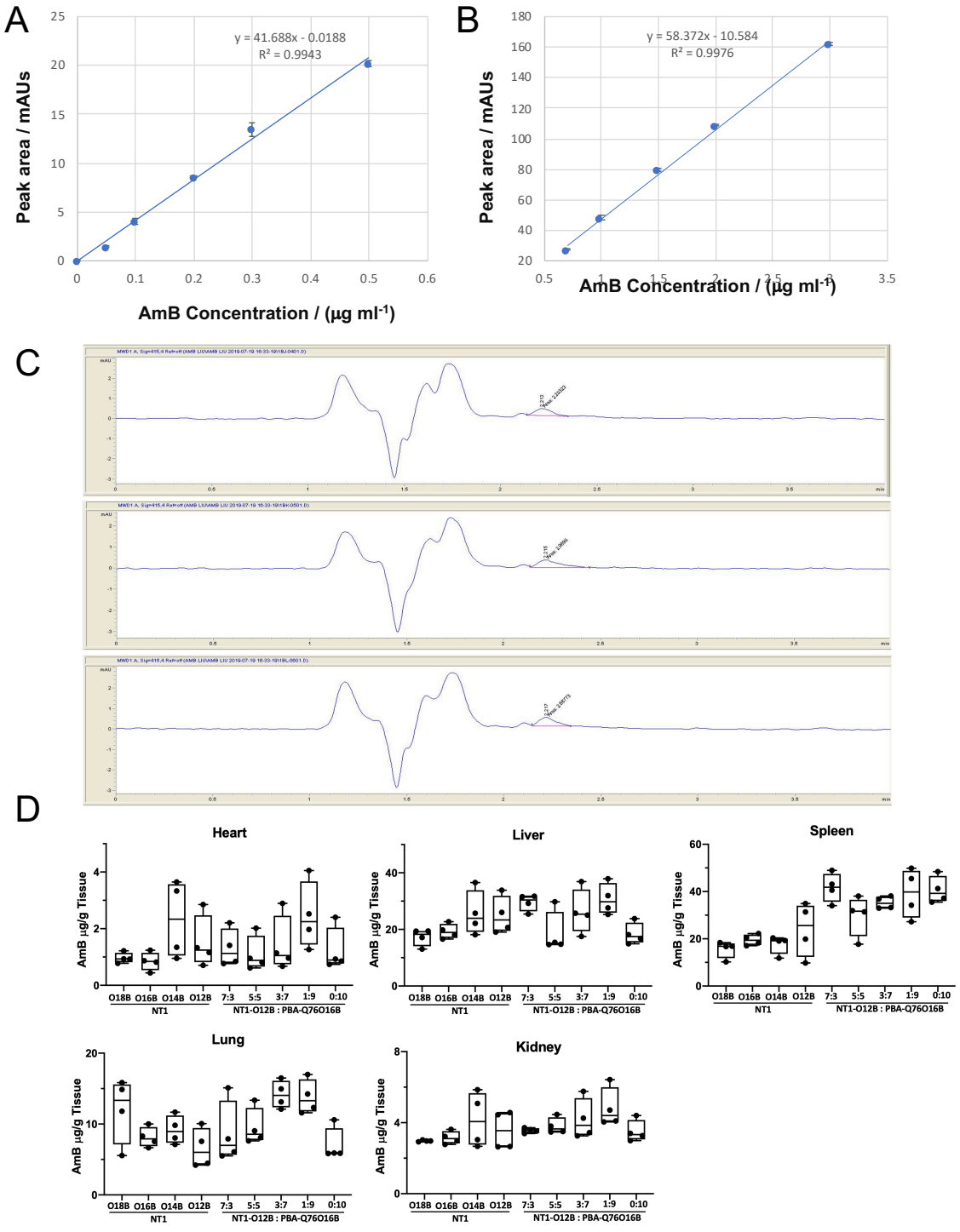


Fig. S9. **Calibration curve, the mAU-time graphs and tissue biodistribution of AmB concentration determined by HPLC.** (**A, B**) The calibration curve of AmB concentration dissolved in methanol ranging from 0.005 to 0.5 μg/mL (**A**) or 0.6 to 3.0

μg/mL (**B**) at 415 nm by HPLC. (**C**) The mAU-time graphs of AmB concentrations in brain tissues 24hr after intravenous treatment with NT1-O12B/PBA-Q76O16-LNPs (ratio: 3/7)-AmB complex at a single dose of 5 mg AmB/kg by HPLC. **(D)** The AmB concentrations in other organs 24hr after intravenous injection of 5 mg/kg AmB measured by HPLC. The mice were perfused with saline before dissection.

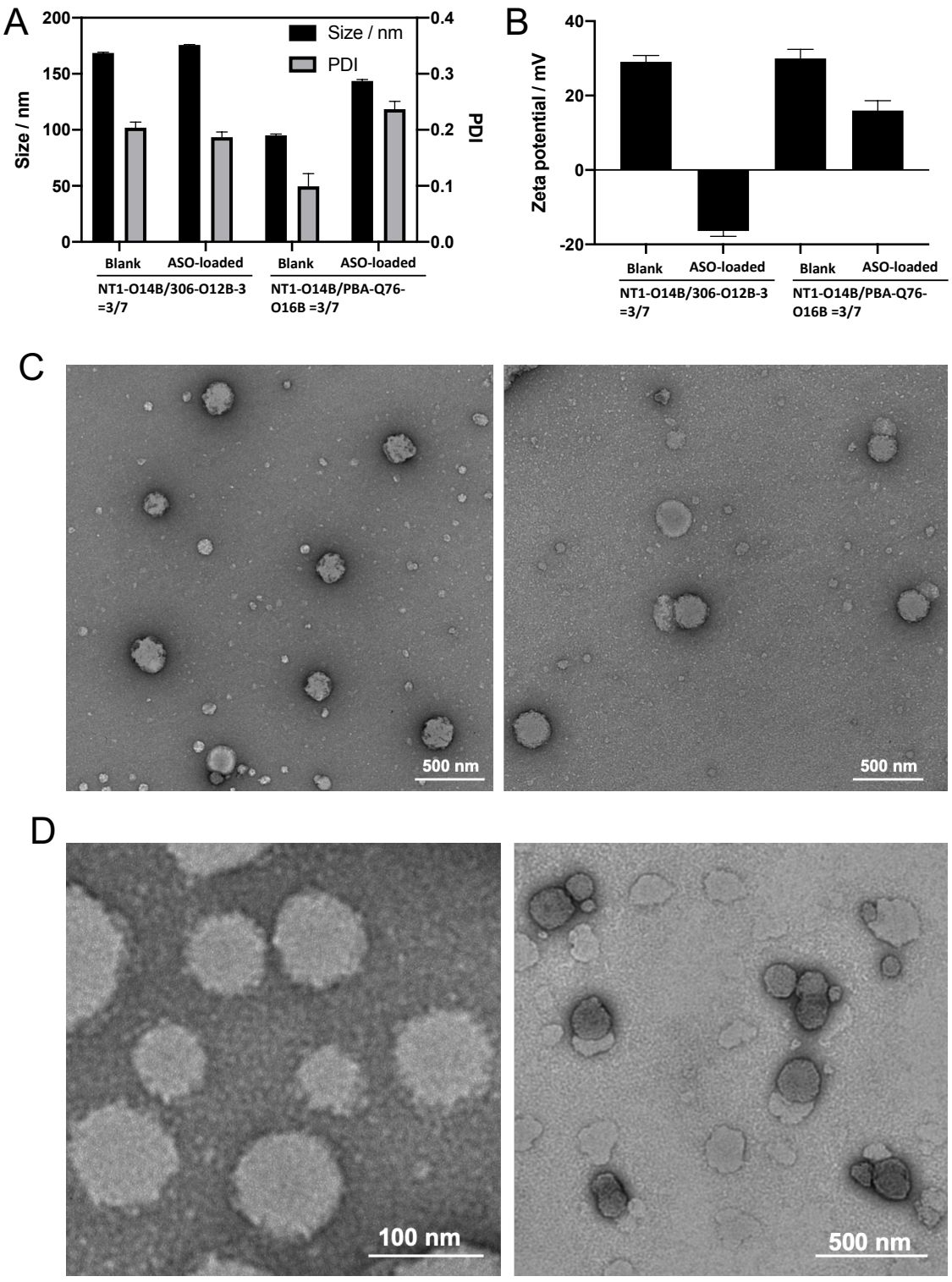


Fig. S10. **Characterization of ASO/NT1-lipidoid and GFP-Cre/NT1-lipidoid nanoparticles formulations.** (**A**) Hydrodynamic diameters and polydispersity indexes of

blank or cargo loaded ASO/NT1-lipidoid and GFP-Cre/NT1-lipidoid nanoparticles formulations. (**B**) Zeta potential of blank or cargo loaded ASO/NT1-lipidoid and GFP-Cre/NT1-lipidoid nanoparticles formulations. (**C**) TEM of blank (left) and ASO loaded (right) NT1-O14B/306-O12B-3 (ratio: 3/7) nanoparticles. (**D**) TEM of blank (left) and (-27)GFP-Cre loaded (right) NT1-O14B/PBA-Q76O16B (ratio: 3/7) nanoparticles.