Supporting Information

Cationic amino acid-based lipids as effective non-viral gene delivery vectors for primary cultured neurons

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Synthesis

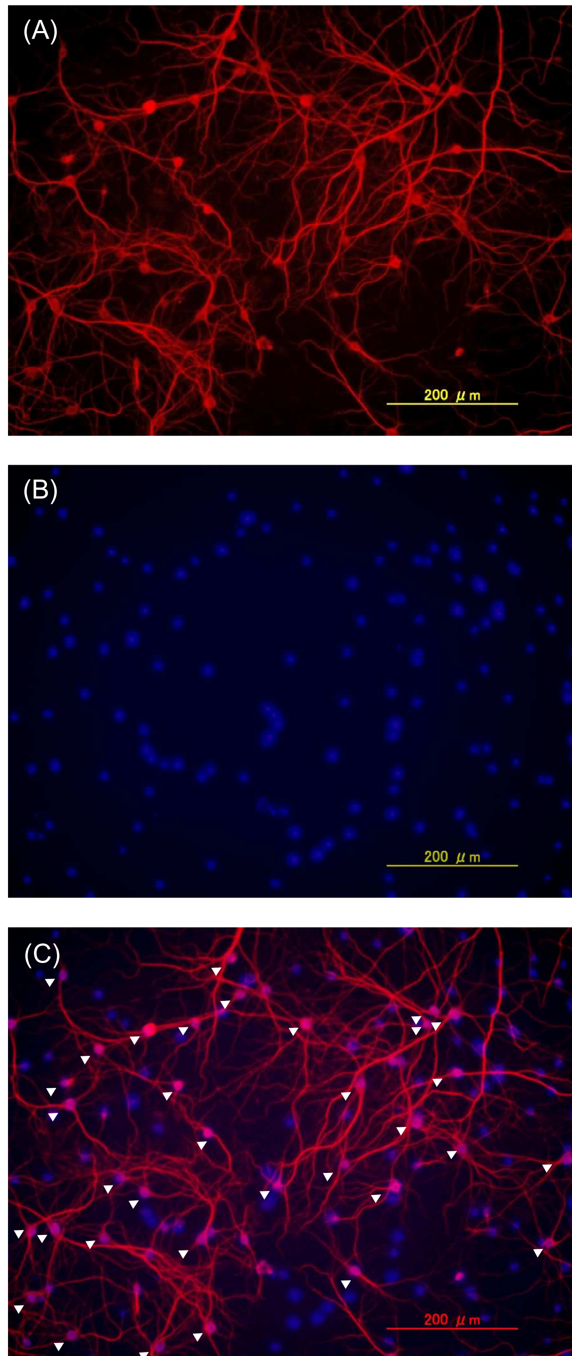
Supporting information Figure S1: Representative fluorescence images of primary cultured cells stained with anti-MAP-2 antibody and 4',6-diamidino-2-phenylindole (DAPI) for purity evaluation.

Supporting information Figure S2: Representative fluorescence images of primary cultured cells stained with calcein-AM and anti-MAP-2 antibody to count live neurons for cytotoxicity assay.

Supporting information Figure S3: All data of the transfection efficiency of the outstanding samples in Fig.4.

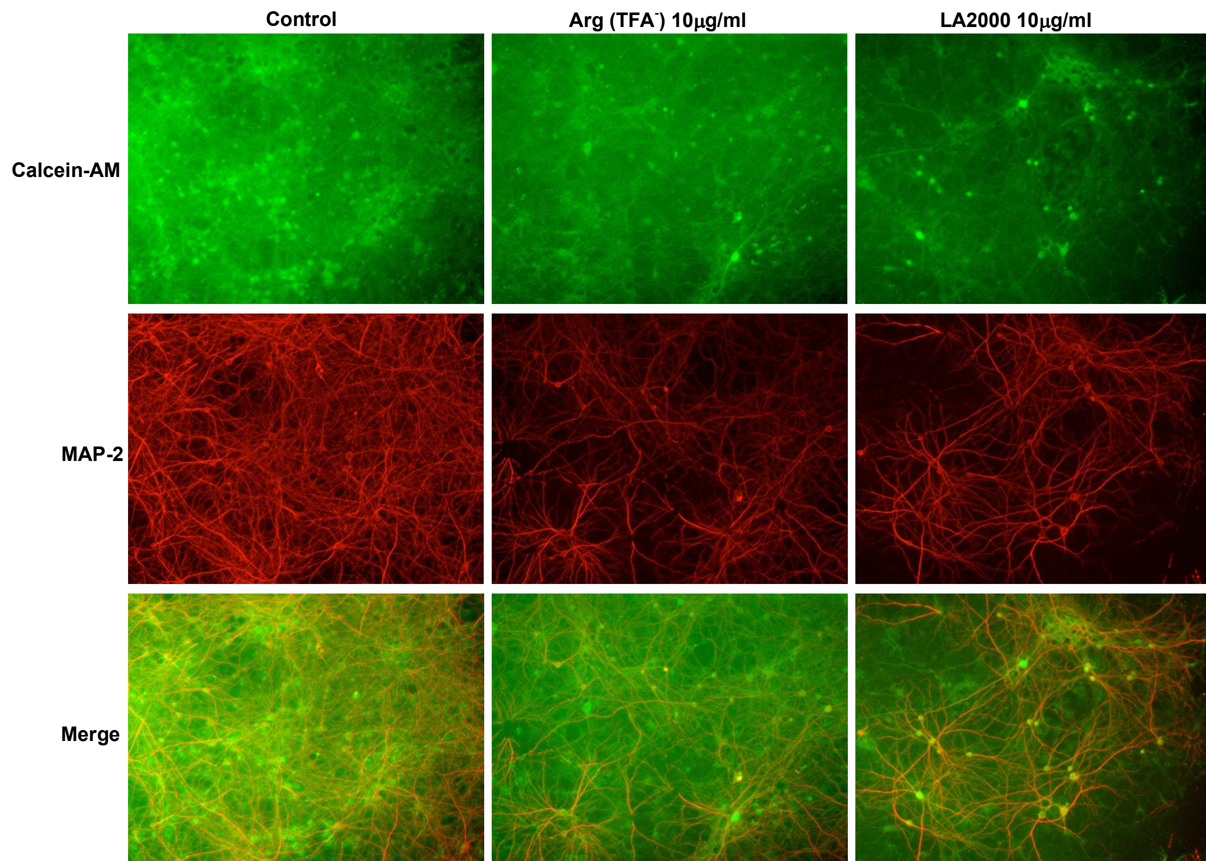
Synthesis

Briefly, 1,5-ditetradecyl-L-glutamate ($Glu2C_{14}$) was synthesized by dehydration synthesis between glutamic acid and n-tetradecyl alcohol. The amino group of 4-amino butyric acid was protected by di-*tert*-butyl dicarbonate (Boc anhydride), and then the protected 4-amino butyric acid was coupled with $Glu2C_{14}$ in the presence of (benzotriazol-1-yloxy)-tris(dimetylamino)phosphonium hexafluorophosphate (BOP) followed by deprotection with trifluoroacetic acid (TFA) to obtain 1, 5-ditetradecyl-*N*-trityl-L-glutamate (NH_2-$(CH_2)_3$-CO-$Glu2C_{14}$). For the synthesis of the arginine-based lipid, dehydration synthesis was performed with the latter compound and Boc-Arg$(Boc)_2$-OH in the presence of BOP to obtain 1,5-ditetradecyl-*N*-butoxycarbonyl-*N*-trityl-*N*-arginyl-L-glutamate (Boc-Arg$(Boc)_2$-$(CH_2)_3$-CO-$Glu2C_{14}$). For the synthesis of the lysine-based lipid, dehydration synthesis was performed with the latter compound and Boc-Lys(Boc)-OSu to obtain 1,5-ditetradecyl-*N*-butoxycarbonyl-*N*-trityl-*N*-lysl-L-glutamate (Boc-Lys(Boc)-$(CH_2)_3$-CO-$Glu2C_{14}$). Then, lipids with the TFA ion as the counter ion, Arg (TFA^-) and Lys (TFA^-), were deprotected with TFA; lipids with the chloride ion as the counter ion, Arg (Cl^-) and Lys (Cl^-), were deprotected with HCl (4 M) in ethyl acetate. Finally, both TFA and HCl were removed under reduced pressure followed by flushing with nitrogen and the lipids were obtained.



Supporting information Figure S1: Representative fluorescence images of primary cultured cells stained with anti-MAP-2 antibody and 4',6-diamidino-2-phenylindole (DAPI) for purity evaluation. Neurons were immunospecifically tagged with anti-MAP-2 antibody (red) and nuclei from all cells are stained with DAPI (blue). (A) Neurons tagged with anti-MAP-2 antibody. (B) Nuclei from all cells stained with DAPI. (C) Merge image of (A) and (B). Arrows indicate the nuclei of neurons.

Percentage of neurons was calculated as follows, Percentage of neurons = (number of nuclei from MAP-2 positive cells) / (total number of nuclei from all cells) × 100



Supporting information Figure S2: Representative fluorescence images of primary cultured cells stained with calcein-AM and anti-MAP-2 antibody to count live neurons for cytotoxicity assay. These images were obtained in control which was not exposed to lipids, for Arg (TFA^-) 10μg/ml which was exposed to Arg (TFA^-) at 10μg/ml for 48 hours, and LA2000 10μg/ml which was exposed to Lipofectamine^{TM} 2000 at 10μg/ml for 48 hours. Live neurons were counted as calcein-AM and MAP-2 positive cells.

Supporting information Figure S3: All data of the transfection efficiency of the outstanding samples in Fig.4. ‘Arg(TFA^{-})’ indicates transfection efficiency of Arg(TFA^{-}) at the lipid-to-pDNA ratio of 1 (W/W), [pDNA]=2.0μg/well, [lipid]=10μg/mL. ‘LA2000(10)’ indicates transfection efficiency of LA2000 at the ratio of 1 (W/W), [pDNA]=2.0μg, [lipid]=10μg/mL. ‘LA2000(30)’ indicates transfection efficiency of LA2000 at the ratio of 1 (W/W), pDNA=2.0μg, [lipid]=30μg/mL. (n=8)