

Supplementary Information

Brain Gene Silencing with Cationic Amino-Capped Poly(Ethylene Glycol) Polyplexes

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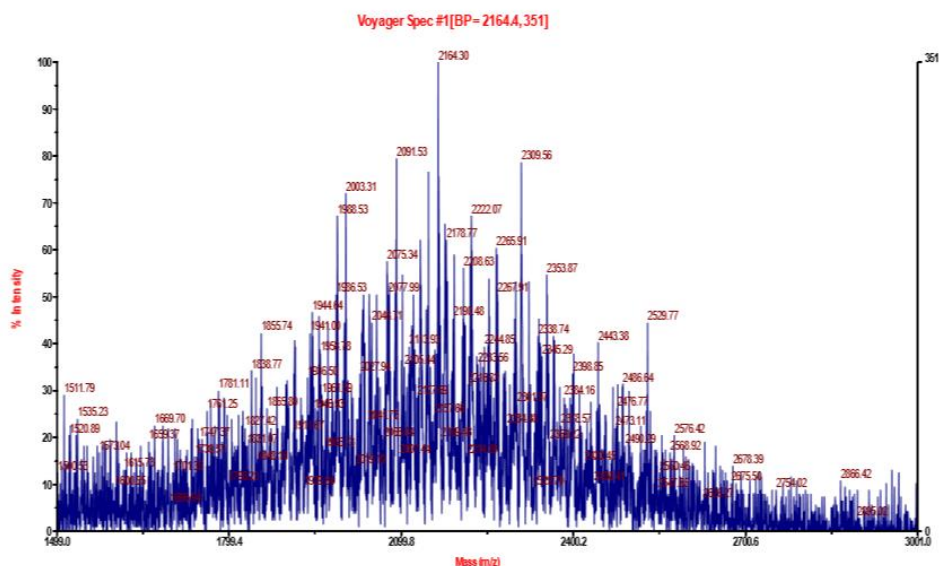


Figure S1. 4APPA MALDI-TOF spectrum

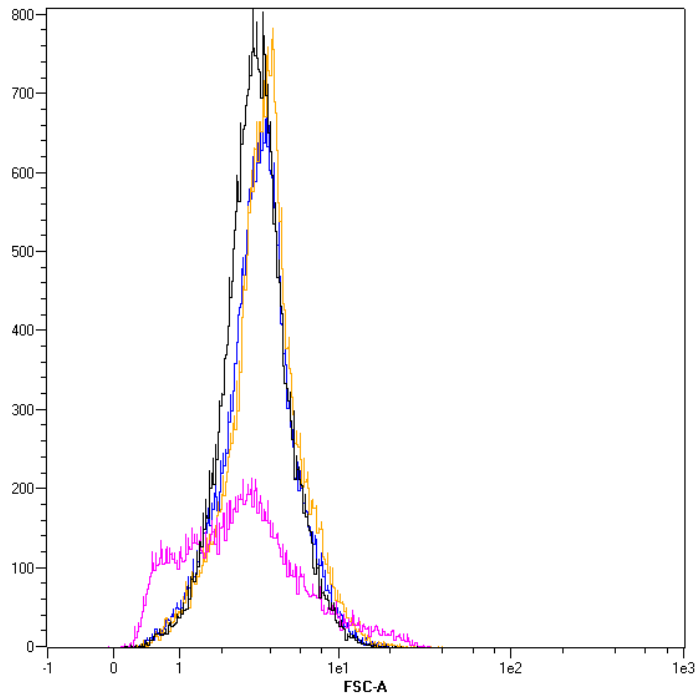


Figure S2: Fluorescent activated cell sorting histograms from the Forward Scatter (FSC) channel following incubation of FAM[™]-siRNA with A431 cells for 6 hours (black = cells alone, yellow = cells plus FAM[™]-siRNA alone, pink = cells plus FAM[™]-siRNA , Lipofectamine, blue = 4APPA – FAM[™]-siRNA)

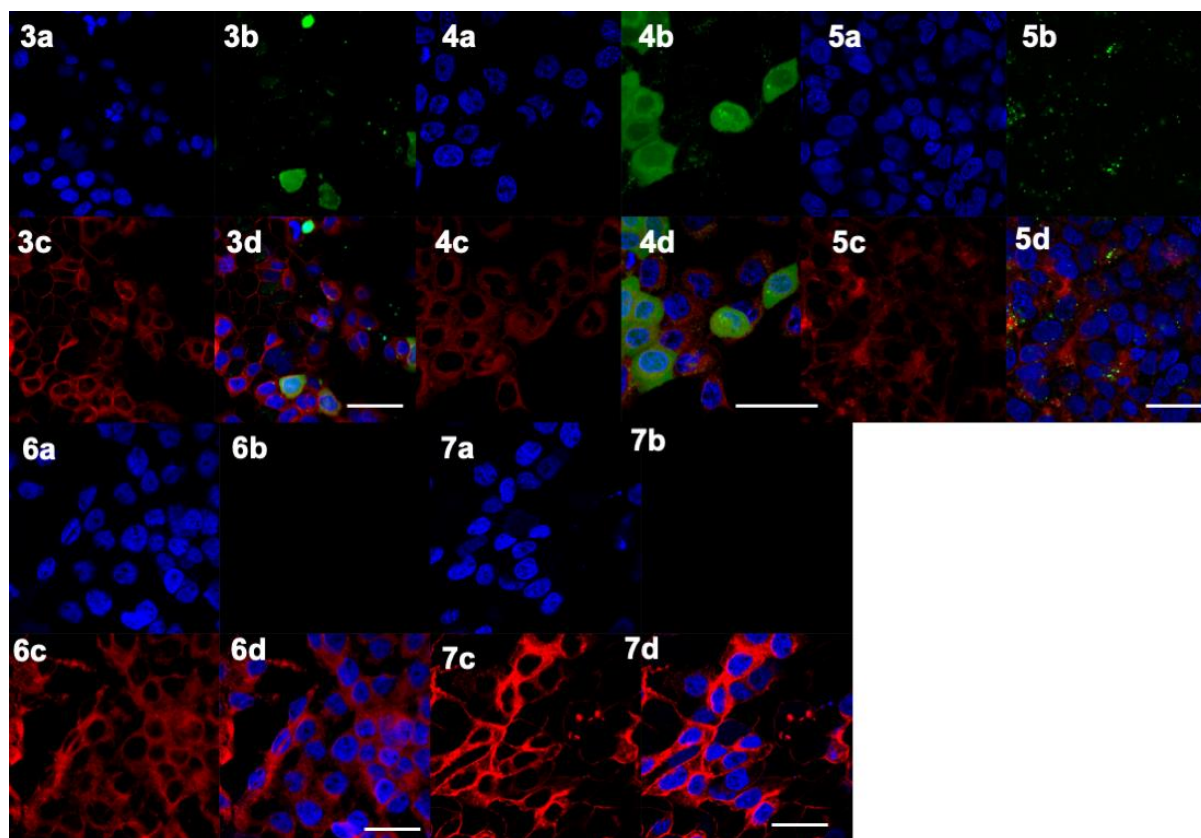


Figure S3: CLSM images of A431 cells (seeded at 150,000 cells per well) with the cell nucleus stained blue (DAPI), cell cytoplasm stained red (CellMask™) and FAM™-siRNA stained green, cells were treated with 4APPA - FAM™-siRNA ($20 \mu\text{g mL}^{-1}$ siRNA, $500 \mu\text{L}$) for 6 hours: a) DAPI channel, b) FAM channel, c) cell mask channel, d) merged image. Bar = $20 \mu\text{m}$.

Figure S4: CLSM images of A431 cells (seeded at 150,000 cells per well) with the cell nucleus stained blue (DAPI), cell cytoplasm stained red (CellMask™) and FAM™-siRNA stained green, cells were treated with 4APPA - FAM™-siRNA ($20 \mu\text{g mL}^{-1}$ siRNA, $500 \mu\text{L}$) for 6 hours: a) DAPI channel, b) FAM channel, c) cell mask channel, d) merged image. Bar = $20 \mu\text{m}$.

Figure S5: CLSM images of A431 cells (seeded at 150,000 cells per well) with the cell nucleus stained blue (DAPI), cell cytoplasm stained red (CellMask™) and FAM™-siRNA stained

green, cells were treated with Lipofectamine - FAMTM-siRNA (100 µg mL⁻¹ siRNA, 30 µL, made according to the manufacturer's instructions) for 6 hours: a) DAPI channel, b) FAM channel, c) cell mask channel, d) merged image. Bar = 20 µm.

Figure S6: CLSM images of A431 cells (seeded at 150,000 cells per well) with the cell nucleus stained blue (DAPI), cell cytoplasm stained red (CellMaskTM) and FAMTM-siRNA stained green, cells were treated with PBS (500 µL) for 6 hours: a) DAPI channel, b) FAM channel, c) cell mask channel, d) merged image. Bar = 20 µm.

Figure S7: CLSM images of A431 cells (seeded at 150,000 cells per well) with the cell nucleus stained blue (DAPI), cell cytoplasm stained red (CellMaskTM) and FAMTM-siRNA stained green, cells were treated with FAMTM-siRNA alone (20 µg mL⁻¹ siRNA, 500 µL) for 6 hours: a) DAPI channel, b) FAM channel, c) cell mask channel, d) merged image. Bar = 20 µm.

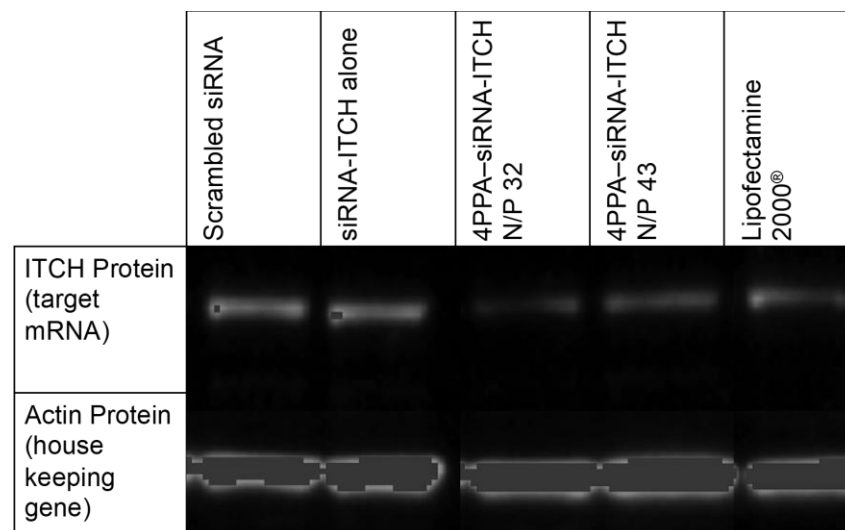


Figure S8: Western blot analysis of ITCH gene silencing when compared to siRNA alone (n = 5 replicates and representative gel of 3 separate experiments) in A431 cells (seeded at 400,000 cells per flask) incubated for 6 hours with scrambled siRNA (20 µg mL⁻¹ siRNA, 500 µL in phosphate buffer - 2 mM), siRNA-ITCH alone (20 µg mL⁻¹ siRNA, 500 µL, in phosphate

buffer - 2 mM), 4APPA-siRNA ($20 \mu\text{g mL}^{-1}$ siRNA, 500 μL , N, P ratio = 32 in phosphate buffer - 2 mM), 4APPA-siRNA ($20 \mu\text{g mL}^{-1}$ siRNA, 500 μL , N, P ratio = 43 in phosphate buffer - 2 mM), Lipofectamine 2000TM-siRNA ($100 \mu\text{g mL}^{-1}$ siRNA, 30 μL) prepared according to the manufacturer's instructions.

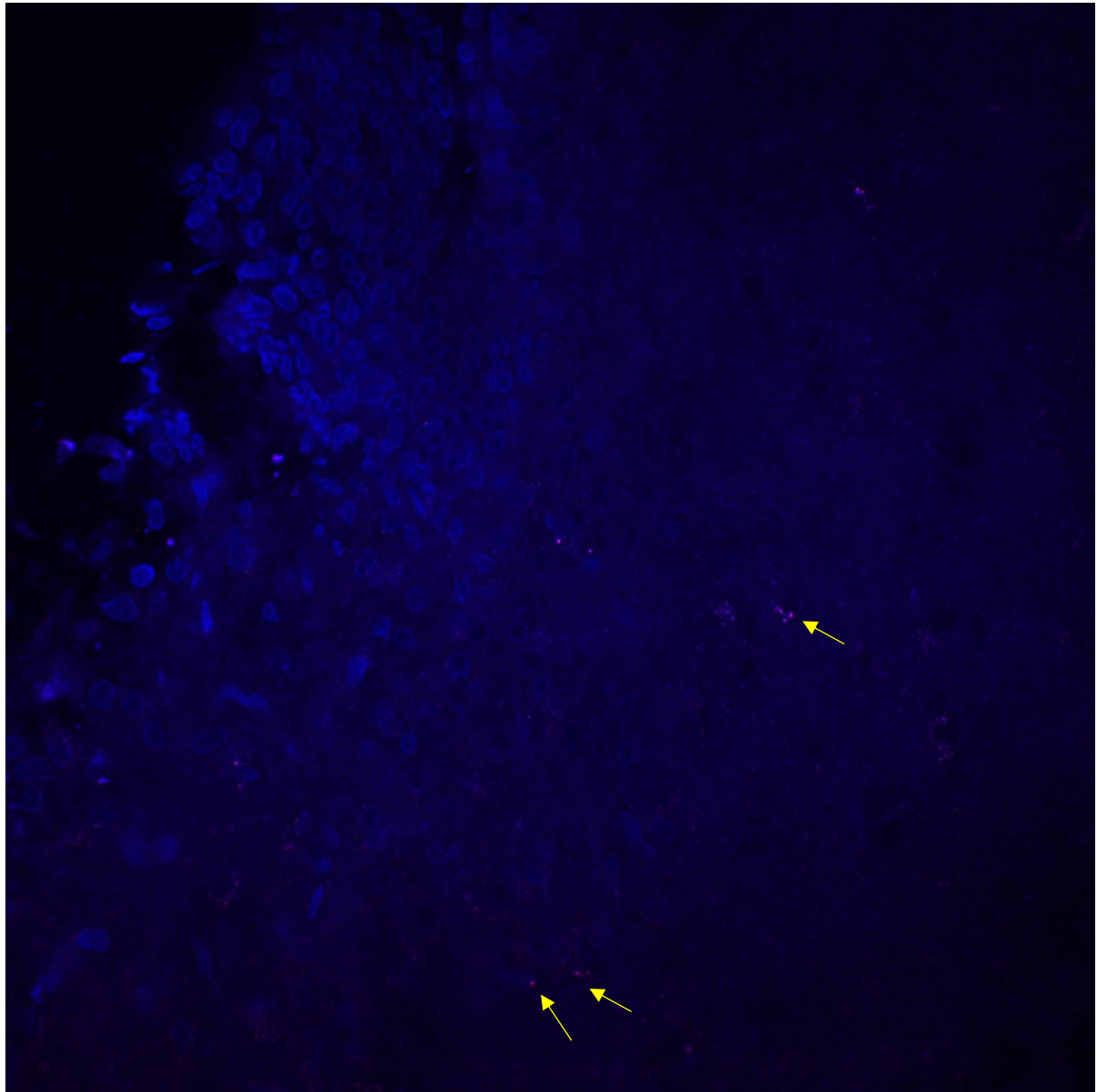


Figure S9: CLSM image (with the nucleus stained blue with DAPI and Cy³-siRNA-GAPDH stained red) of the rat mid brain 1 hour after the intranasal administration of 4APPA- Cy³-

siRNA-GAPDH polyplexes (0.444 mg mL^{-1} siRNA, $45 \text{ }\mu\text{L}$, N, P ratio = 32). Arrows show sparse fluorescent signals originating from the fluorescent siRNA.