

## Supplementary Information

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

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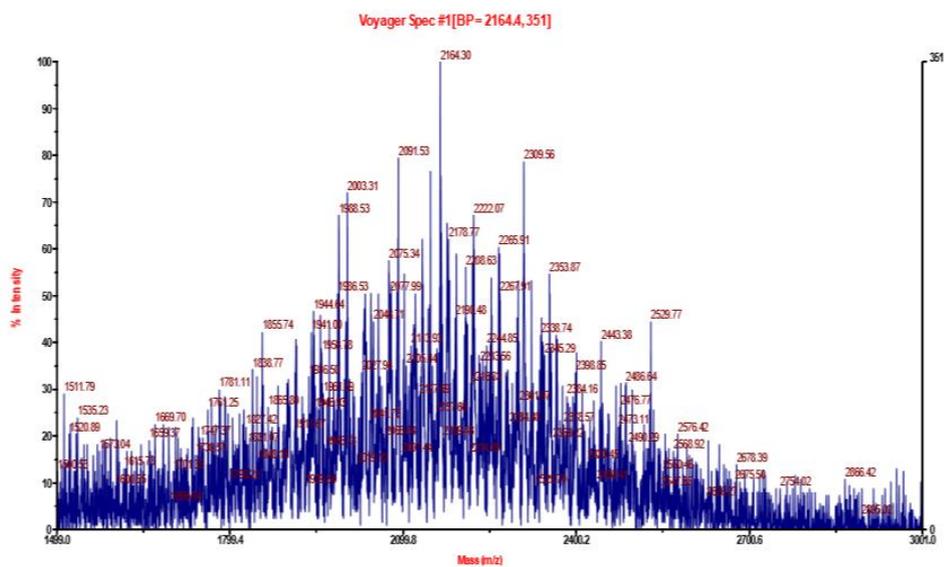


Figure S1. 4APPA MALDI-TOF spectrum

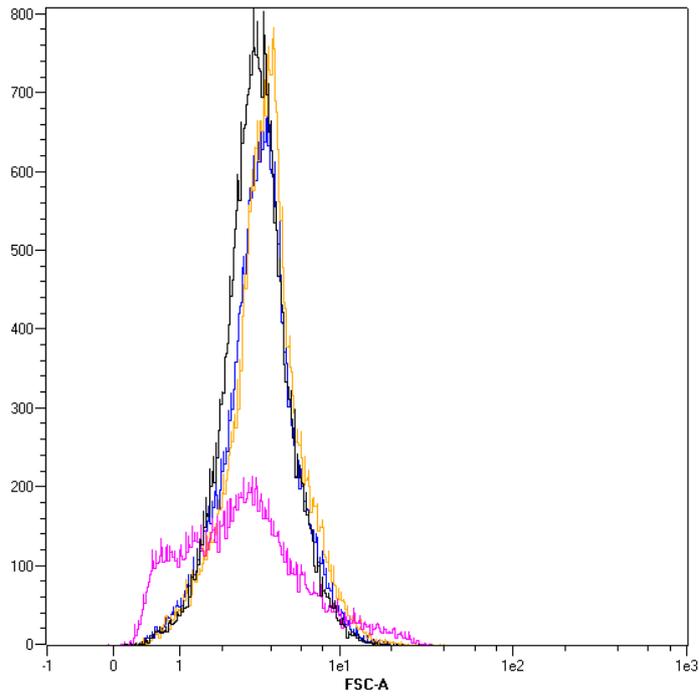


Figure S2: Fluorescent activated cell sorting histograms from the Forward Scatter (FSC) channel following incubation of FAM<sup>TM</sup>-siRNA with A431 cells for 6 hours (black = cells alone, yellow = cells plus FAM<sup>TM</sup>-siRNA alone, pink = cells plus FAM<sup>TM</sup>-siRNA , Lipofectamine,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

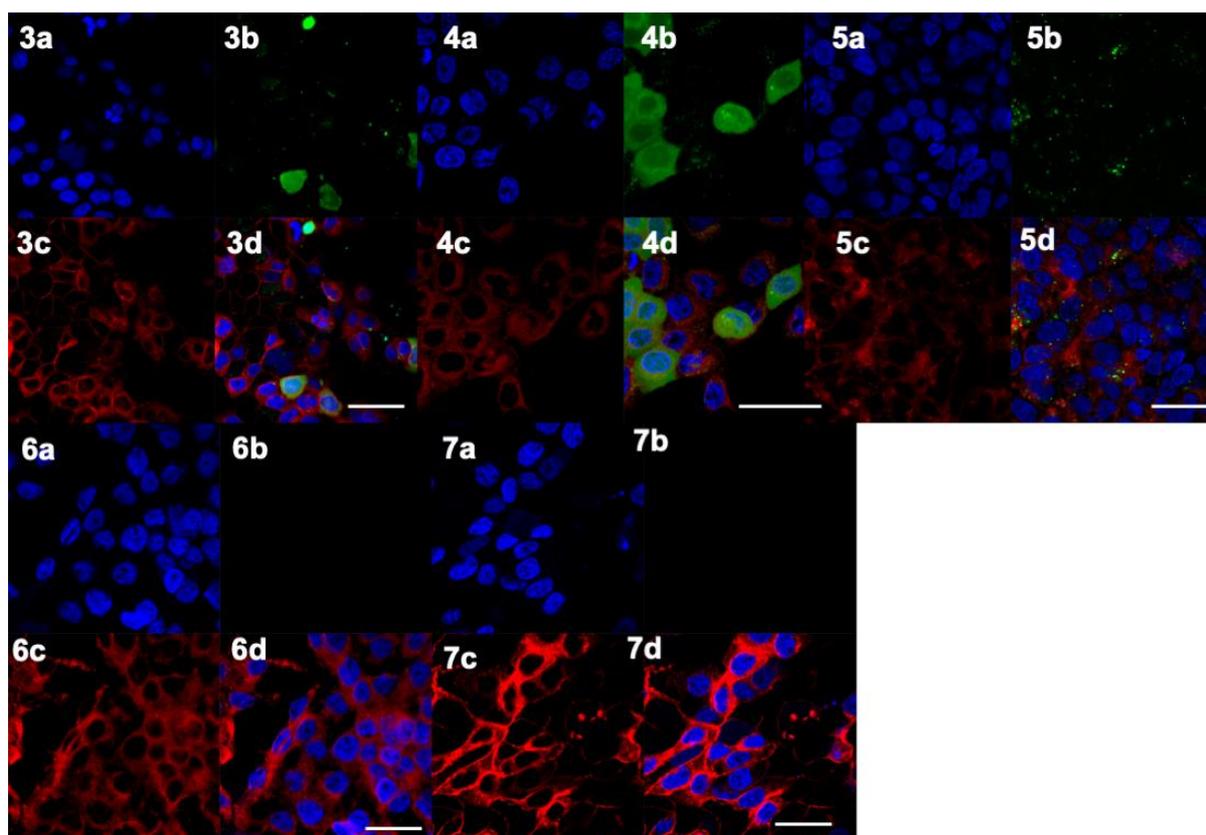


Figure S3: CLSM images of A431 cells (seeded at 150,000 cells per well) with the cell nucleus stained blue (DAPI), cell cytoplasm stained red (CellMask™) and FAM™-siRNA stained green, cells were treated with 4APPA - FAM™-siRNA ( $20 \mu\text{g mL}^{-1}$  siRNA,  $500 \mu\text{L}$ ) for 6 hours: a) DAPI 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 - FAM<sup>TM</sup>-siRNA (100 µg mL<sup>-1</sup> 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 (CellMask<sup>TM</sup>) and FAM<sup>TM</sup>-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 (CellMask<sup>TM</sup>) and FAM<sup>TM</sup>-siRNA stained green, cells were treated with FAM<sup>TM</sup>-siRNA alone (20 µg mL<sup>-1</sup> siRNA, 500 µL) for 6 hours: a) DAPI channel, b) FAM channel, c) cell mask channel, d) merged image. Bar = 20 µm.

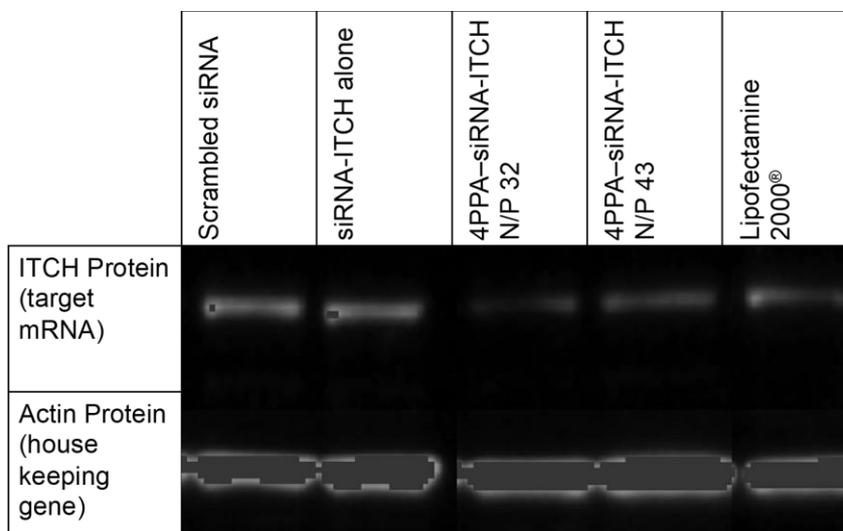


Figure S8: Western blot analysis of ITCH gene silencing when compared to siRNA alone (n = 5 replicates and representative gel of 3 separate experiments) in A431 cells (seeded at 400,000 cells per flask) incubated for 6 hours with scrambled siRNA (20 µg mL<sup>-1</sup> siRNA, 500 µL in phosphate buffer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

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

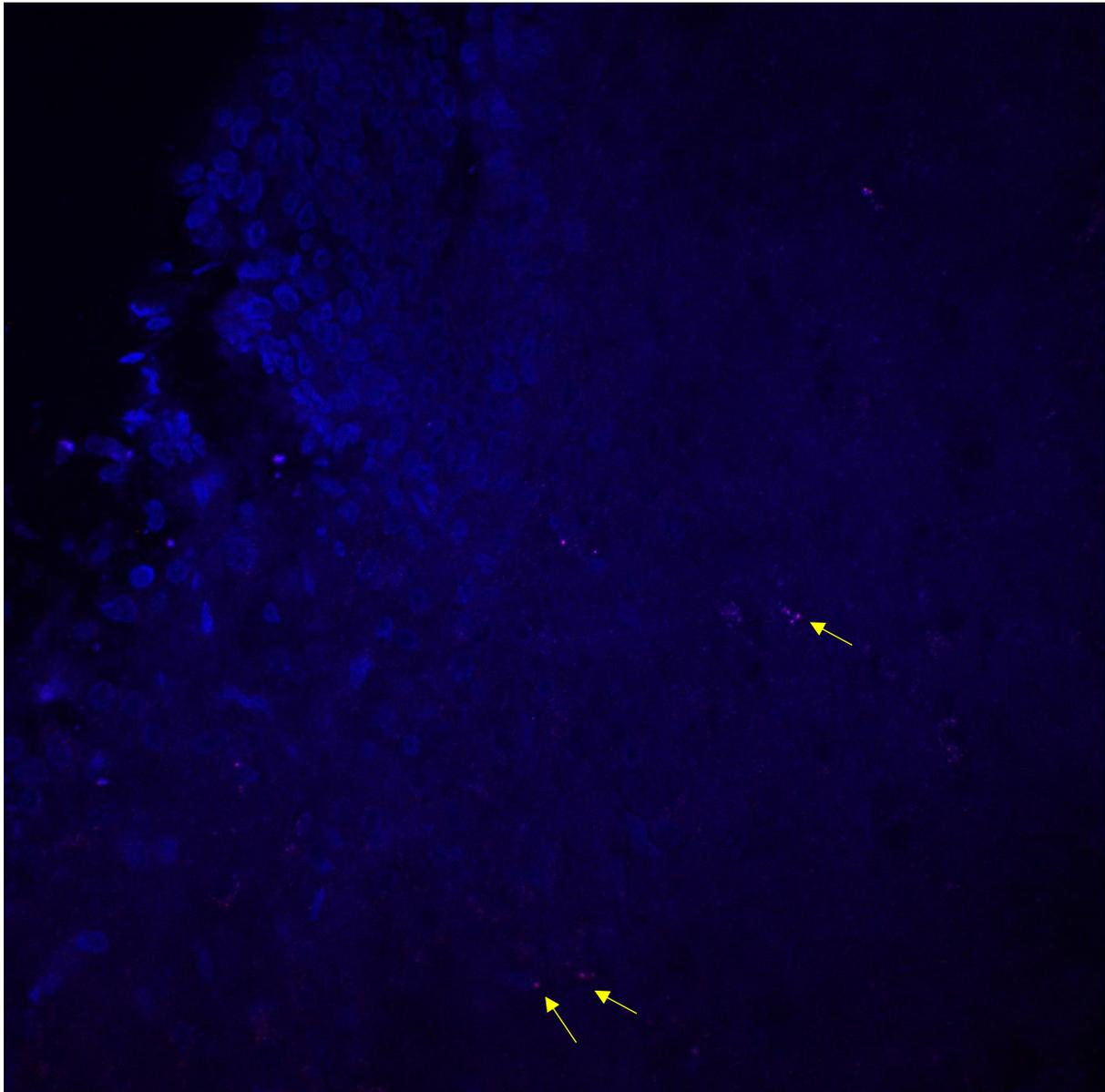


Figure S9: CLSM image (with the nucleus stained blue with DAPI and Cy<sup>3</sup>-siRNA-GAPDH stained red) of the rat mid brain 1 hour after the intranasal administration of 4APPA- Cy<sup>3</sup>-

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