Supporting Information

Aptamer-Conjugated Framework Nucleic Acids for the Repair of Cerebral Ischemia-Reperfusion Injury

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Experimental Section

Materials & Methods

Materials

All DNA oligonucleotides were purchased from Integrated DNA Technologies Inc. (www.IDTDNA.com) and were directly used for self-assembly of FNA. Bipyramidal FNA was assembled by annealing six single-strained DNA with designated sequences in 1 x TAE-Mg^{2+} buffer (12.5 mM magnesium acetate, 2 mM EDTA, 20 mM acetic acid, and 40 mM Tris-base, pH 8.0) using a previously established method.[1] A maximum of six overhangs can be extended from the FNA edges for hybridization with aC5a.

Atomic force microscopy (AFM) imaging of FNA

Freshly cleaved mica was used for loading samples. After loading 3 μL of a FNA sample on the mica for 1 min, 200 μL of 1×TAE/Mg^{2+} buffer (200 μL) was added. Imaging was performed using the fluid DFM scanning mode with a BL-AC40TS tip (Olympus) AFM.

Zeta-potential and DLS Measurements

The FNA was prepared at a final concentration of 1 μM in 1×TAE/Mg^{2+} buffer (pH 8.0). Zeta potentials and dynamic light scattering measurements were performed using a Malvern Zetasizer Nano ZS90 (Malvern, UK).

^{89}Zr-labeling of FNA

Deferoxamine (DFO) was first conjugated to amine-functionalized ssDNAs and the obtained DFO-ssDNA was purified using a PD-10 desalting column with 1 x PBS as the elution buffer. ^{89}Zr was produced by using a PETtrace cyclotron (GE Healthcare) employing the ^{89}Y (p, n) ^{89}Zr reaction. For the radiolabeling of DFO-ssDNA, ^{89}Zr (37 MBq)

was diluted with 500 μL of HEPES buffer (0.1 M, pH 7.5) and mixed with DFO-ssDNA. Following 1 h incubation at 37 ºC with constant shaking, ^{89}Zr-labeled ssDNA fractions were collected using a PD-10 column and fractions with the highest radioactivity were further mixed with FNA to obtain ^{89}Zr-labeled FNA.

In vivo magnetic resonance imaging (MRI)

All MRI experiments were performed using a 4.7 T small animal scanner (Agilent technologies, Santa Clara, CA). In vivo imaging was performed 3 days after cerebral IRI. To quantify infarcted volume, in vivo T2*-weighted images were collected using a multi-slice gradient echo sequence with the following parameters: TR = 500 ms; TE = 12 ms; flip angle = 20°; FOV = 40×40 mm^2; matrix 256×256; NEX = 8; slice thickness = 1 mm for axial brain images.

PET imaging of FNA in rats

100 μCi of ^{89}Zr-labeled FNA or aC5a-FNA were injected intrathecally into SD rats with cerebral IRI. A Siemens Inveon microPET/CT (Siemens Medical Solutions, Erlangen, Germany) was used for longitudinal PET scans at 15 min, 6 h, and 9 h after injection. Based on the calibration coefficient of the scanner, region-of-interest (ROI) data was generated from counts/pixel and the volume for determining time-activity curves in different organs, which was then divided by the injected dose to acquire the final ROI uptake in %ID/g.

Superoxide anion scavenging with FNA in solution

The superoxide anion scavenging activity was determined using an SOD assay kit-WST (Sigma-Aldrich, USA). FNA (20 μL) at different concentrations (100 and 200 nmol/L) were

mixed with 160 μL of a 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H tetrazolium sodium salt (WST-1) working solution, and 20 μL of a xanthine oxidase solution was added to each microplate well. After incubating for 20 min at 37 °C, the absorbance of these samples was measured at 450 nm.

Hydroxyl radical scavenging with FNA in aqueous solutions

A hydroxyl radical antioxidant capacity (HORAC) assay kit (Cell Biolabs, Inc., USA) was used to test the hydroxyl radical scavenging activity of FNA. After 20 μL of each sample with different concentrations (100 and 200 nmol/L) was mixed with 140 μL of fluorescent solution, 20 μL of the hydroxyl radical initiator and 20 μL of Fenton reagent was added to each microplate well. After incubating for 30 min at 25 °C, the fluorescence was measured using a multiple plate reader.

Free radical scavenging with FNA in solution

The free radical scavenging capacity of FNA was studied using the 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) radical cation decolorization assay, based on the reduction of ABTS+• radicals by FNA. To acquire ABTS radical cations (ABTS+•),[2] ABTS was solubilized and reacted with 2.45 mM potassium persulfate. The reaction mixture was kept in a dark room for 12-16 h before use. A UV/Vis spectrophotometer was used to detect the absorbance of pure ABTS+• solution (AB), and ABTS+•solution with 100 or 200 nmol/L of FNA at 734 nm.

Isolation of PMN and microglia

According to previous reports, rat polymorphonuclear leukocytes (PMNs) were isolated from rat peripheral blood.[3] Blood was drawn from rats with or without cerebral I/R injury

and then kept in the collection tubes. PMNs were isolated using a discontinuous Ficoll-Paque gradient centrifugation. PMNs were washed and suspended in Dulbecco's phosphate buffered saline and adjusted to the desired concentration for different experiments.

Primary microglia were obtained from cortices of neonatal SD rats (Charles River) as previously described.[4] Briefly, cells were dissociated from cerebral hemispheres using trypsin (0.25% trypsin at 37°C for 15 min), and plated in 25 cm^2 culture flasks and cultured in DMEM containing 10% heat-inactivated fetal calf serum (FCS, Gibco, Carlsbad, CA). The media was changed every 3 days. After 12 days of culture, the cells were collected and filtered through a nylon mesh. The cells were then plated in a petri dish and allowed to attach for 30 min at 37 °C and followed by washing with culture medium. More than 95% of the adherent cells were microglia.

Binding of [^{89}Zr]aC5a to PMNs and microglia

To assess the binding of aC5a with C5a receptors (C5aR), binding assays were performed with PMNs or microglia seeded in six-well plates (1×10^6 cells/well). DFO-conjugated C5a was prepared following previous reports.[5] Cell-binding assays were performed using ^{89}Zr-aC5a as the radioligand. C5aR binding affinities of C5a were evaluated by PMNs from rats with IRI and activated microglia. The IC50 values were calculated using nonlinear regression with the GraphPad Prism Software. Experiments were performed with triplicates.

Tissue immunostaining

Brain tissues were fixed with 4% paraformaldehyde for 24 h, then cut into serial 12 μm

thick coronal slices. After blocking with 10% donkey serum for 1 h at room temperature, sections were incubated with monoclonal anti-Iba-1, anti-CD11b, anti-CD68, and anti-cleaved caspase-3 antibodies at 4 °C. Nissl staining was used for counterstaining with anti-cleaved caspase-3 antibodies. Sections were washed and incubated with secondary antibodies for 1 h. Images were captured using a Nikon A1R confocal machine. Three brain coronal sections per rat (+1.20, +0.00 and -1.32 mm from bregma, George Paxinos and Charles Watson, The Rat Brain in Stereotaxic Coordinates, Academic Press), were used to quantify the stained area. $Iba^+/CD68^+$, $CD11b^+$ or cleaved-$Caspase\text{-}3^+/Nissl^+$ cells per mm^2 within areas measured from ten different images using the ImageJ software (National Institutes of Health).[6]

Enzyme-linked immunosorbent assay

Brain tissues isolated from rats in different groups were placed in ice-cold lysis buffer. The ipsilateral hemisphere was homogenized in RIPA buffer (10 μl/mg brain). Total protein content was determined using the bicinchoninic acid Protein Assay kit (Pierce, Appleton, WI, USA). According to the manufacturer's instruction, cytokines were quantified using commercially available ELISA kits for detection of IL-1β and TNF-α. The commercially available kit (MyBioSource, San Diego, USA) was used for quantitative analysis of C5a. Levels of C5a in plasma were expressed as nanograms per milliliter of sample and calculated from the standard curve and run for each experiment. Levels of C5a in the brain were expressed as nanograms of C5a per microgram of protein.

Rat models of cerebral IRI

Cerebral IRI rat model was induced using the intraluminal filament model with minor modifications.[7] Briefly, rats were anesthetized with inhaled isoflurane (3-5 %). Under an

operating microscope (Carl Zeiss, Oberkochen, Germany), a midline neck incision was created to expose the right carotid artery. Middle cerebral artery occlusion was performed by advancing 4-0 nylon suture with silicon-coated tip from the left external carotid artery into the left internal carotid artery and advanced to the circle of Willis so that the origin of the left middle cerebral artery was occluded. Following 60 min of ischemia, the nylon suture was withdrawn to establish reperfusion injury.

Evaluation of neurological deficits and infarct sizes

SD rats were randomly divided into 5 groups: sham group, rat IRI models treated with PBS (PBS group), rat IRI models treated with FNA (0.1 mg/kg), rat IRI models treated with aC5a (0.1 mg/kg), and rat IRI models treated with aC5a-FNA (0.1 mg/kg). Rats were treated with the different agents via intrathecal injection after the onset of IRI. The modified Neurological Severity Score (mNSS) test is consisted of motor, sensory, reflex, and balance assessments: a score of 13–18 indicates severe injury, 7–12 indicates moderate injury, and 1–6 indicates mild injury. A score of zero represents normal and a higher score represents injury that is more significant. Rats were excluded from analysis when failure to conduct the test or lack of reflex were observed.

Three days after reperfusion, the animals were euthanized and their brains were removed. The sections were stained with 2% 2, 3, 5-triphenyltetrazolium chloride staining (TTC, Sigma, UK) at 37 °C in the dark. The infarct area and the bilateral hemispheric area were quantified using an image-analysis system (ImageJ software, NIH). The infarct volumes and total volumes were calculated for each brain slice.

Table S1. Oligonucleotides used for preparation of FNA and aC5a-FNA.

Oligonucleotides	Sequence
S1	**TTTTTTTTTTTTTTTTTTTT**AGGCAGTTGAGACGAACATTCCTAAGTCTGAAATTTATCACCCGC CATAGTAGACGTATCACC
S2	**TTTTTTTTTTTTTTTTTTTT**CTTGCTACACGATTCAGACTTAGGAATGTTCGACATGCGAGGGT CCAATACCGACGATTACAG
S3	**TTTTTTTTTTTTTTTTTTTT**GGTGATAAAACGTGTAGCAAGCTGTAATCGACGGGAAGAGCATG CCCATCCACTACTATGGCG
S4	**TTTTTTTTTTTTTTTTTTTT**CTGCGCGGATGACTCAACTGCCTGGTGATACGATCTAGTCTCTA CGTCAAGTAAGAACCTTAG
S5	**TTTTTTTTTTTTTTTTTTTT**CCTCGCATGACATCCGCGCAGCTAAGGTTCAAAGTTCCTGCCGC TTCACGGACGGTATTGGAC
S6	**TTTTTTTTTTTTTTTTTTTT**CTCTTCCCGACCGTGAAGCGGCAGGAACTTATACTTGACGTAGA GACTAGAAGGATGGGCATG
A20-NH_2	AAAAAAAAAAAAAAAAAAAA-NH_2
A20-aC5a*	**AAAAAAAAAAAAAAAAAAAA**CmGCCGCmGmGUCUCmAmGmGCGCUmGmAmGUCUmGm AmGUUUACCUmGCmG

* mG and mA stand for 2'-OMe modified DNA bases

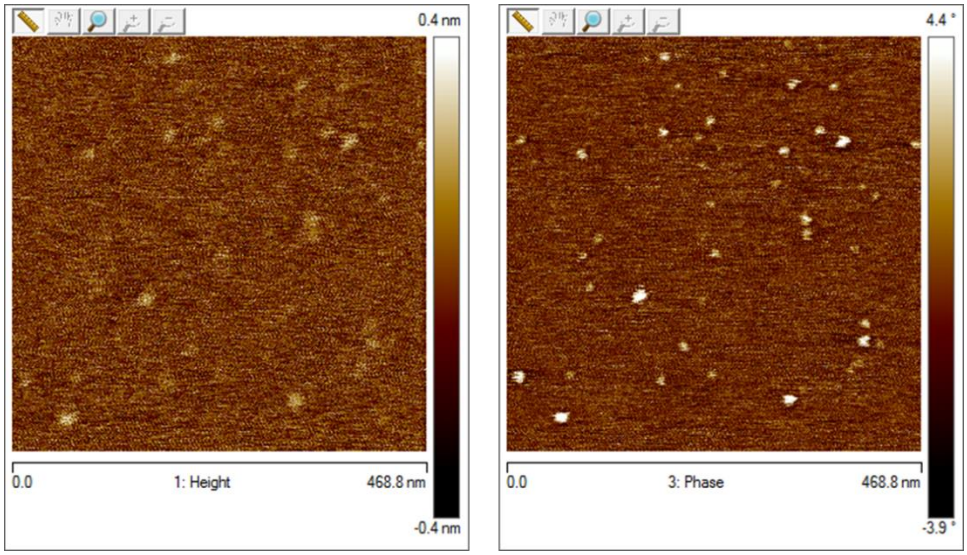

Figure S1. Atomic force microscopy images of FNA in both height mode (Left) and phase mode (Right).

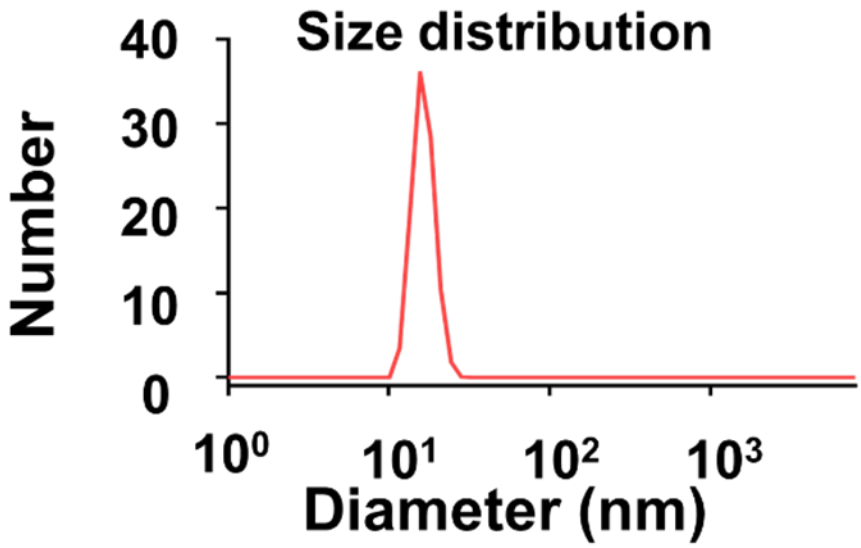

Figure S2. Dynamic light scattering measurement of FNA.

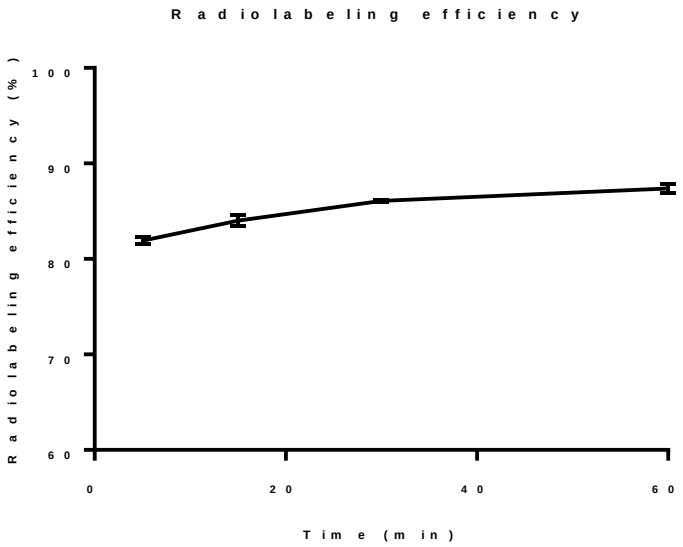

Figure S3. Radiolabeling efficiency of single-stranded DNA with Zr-89.

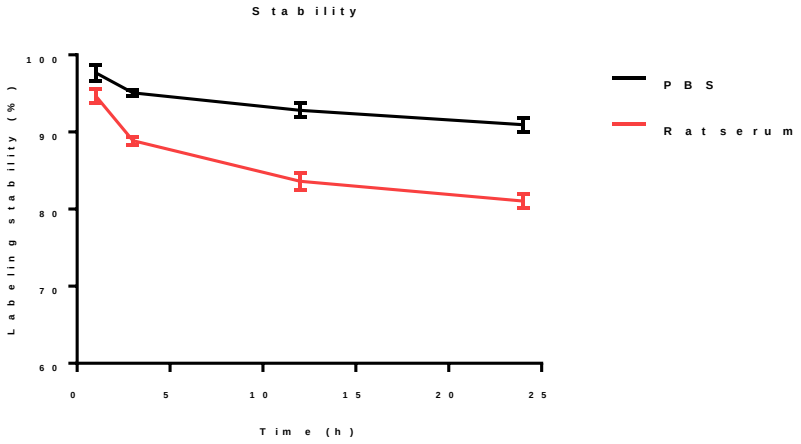

Figure S4. Radiolabeling stability of ^{89}Zr-labeled aC5s-FNA in both 1 x PBS buffer and 80% rat serum.

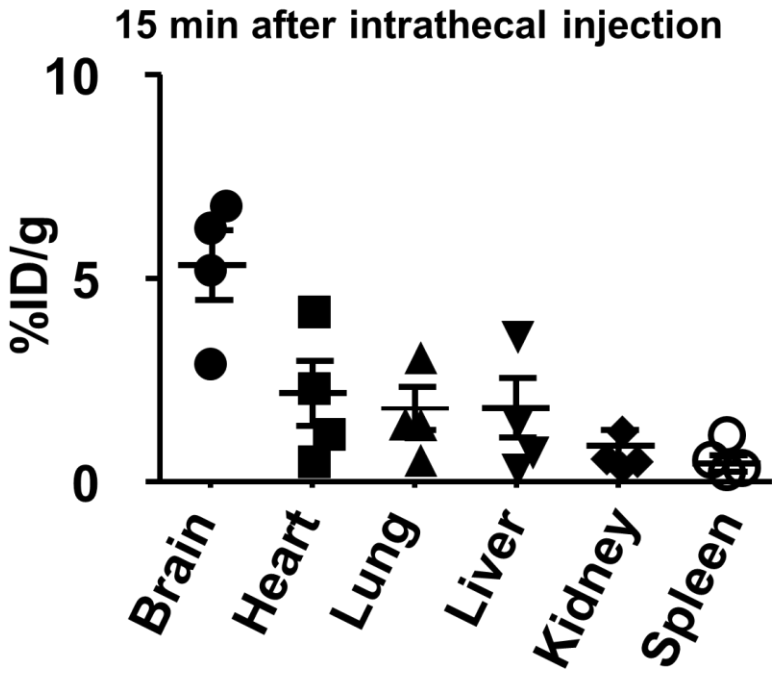

Figure S5. Quantification of ^{89}Zr-aC5a-FNA in the brain, heart, lung, liver, kidneys, and spleen at 15 min after intrathecal injection. Data represents Mean ± SD from four independent replicates.

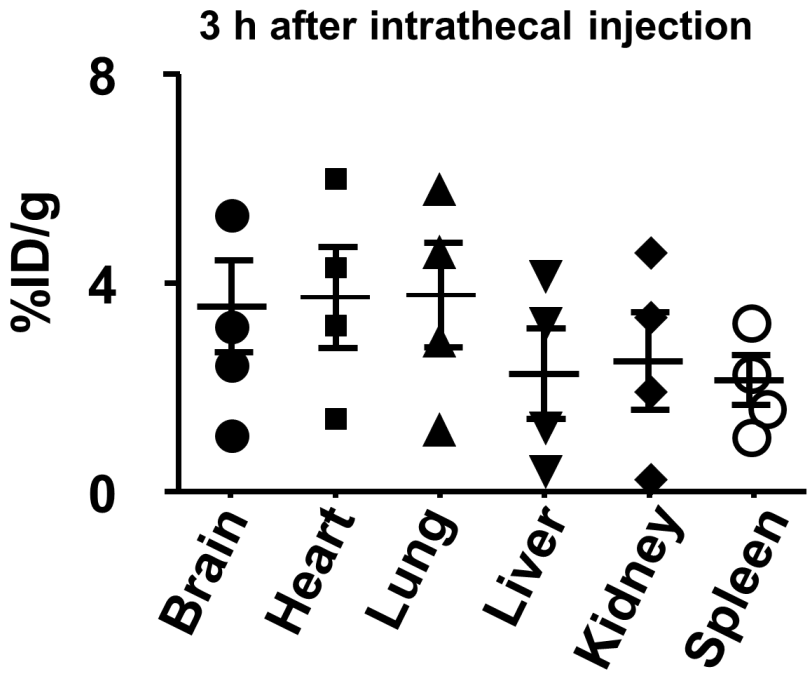

Figure S6. Quantification of ^{89}Zr-aC5a-FNA in the brain, heart, lung, liver, kidneys, and spleen at 3 h after intrathecal injection. Data represents Mean ± SD from four independent replicates.

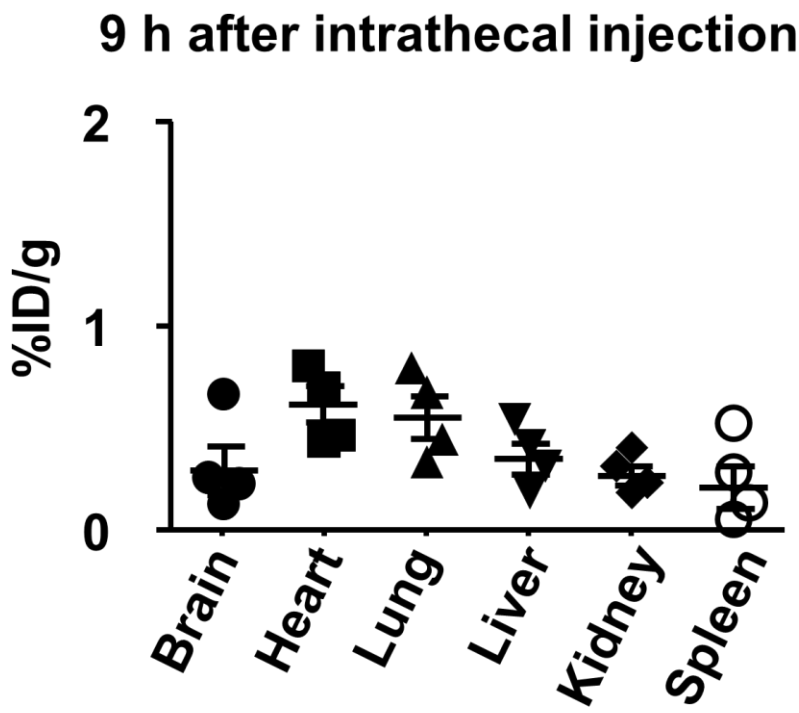

Figure S7. Quantification of ^{89}Zr-aC5a-FNA in the brain, heart, lung, liver, kidneys, and spleen at 9 h after intrathecal injection. Data represents Mean ± SD from four independent replicates.

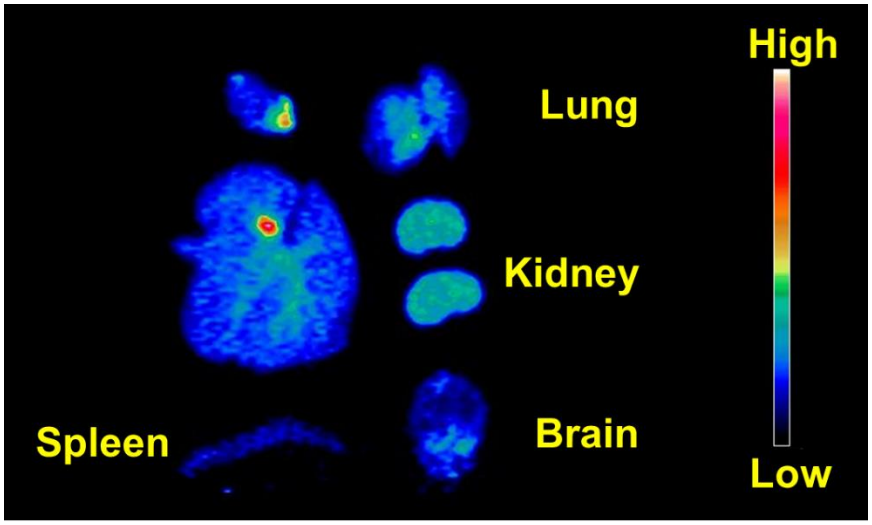

Figure S8. PET imaging of major organs (the heart, liver, spleen, lung, kidneys, and brain) ex vivo at 1 h after intrathecal injection of ^{89}Zr-labeled aC5a-FNA.

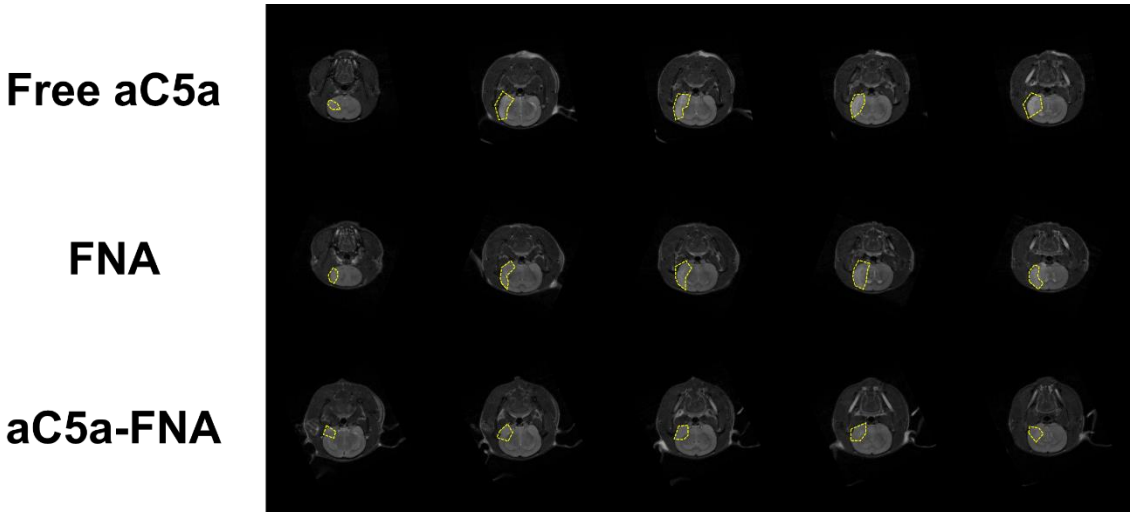

Figure S9. Representative MRI images of stroke rats treated with free aC5a, FNA and aC5a-FNA. Yellow circles denote damaged brain tissue.

References

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