SUPPLEMENTARY MATERIAL

Direct Imaging of Cerebral Thromboemboli Using Computed Tomography and Fibrin-targeted Gold Nanoparticles

Kim et al. CT-based direct cerebral thrombus imaging

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Table S1. Results of neurobehavioral tests over 3 weeks after intravenous injection of fib-GC-AuNPs (120 mg/kg) in C57Bl/6 mice (n = 6)

	0 week						1 week						2 week						3 week					
Behavior \ Animal ID	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6
Body position	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Touch escape	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Finger approach	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Tail pinch	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Tail elevation	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Abdominal tone	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Grip strength	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ataxic gait	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Tremors	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Palpebral closure	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lacrimation	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Skin color	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Acute death	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Values are neurobehavioral scores;[1] and, 0 indicates no abnormal sign.

Table S2. Results of complete blood counts and liver function test at 3 weeks after intravenous injection of fib-GC-AuNPs (120 mg/kg) in C57Bl/6 mice (n = 6)

Parameter	Normal Range	Values (n=6)
WBC ($\times 10^3$ cells/μl)	1.8 ~ 10.7	4.5 ± 0.1
RBC ($\times 10^6$ cells/μl)	6.4 ~ 9.4	6.4 ± 0.2
Hemoglobin (g/dL)	11.0 ~ 15.1	10.9 ± 0.2
Hematocrit (%)	35.1 ~ 45.4	33.4 ± 1.3
MCV (fL)	45.4 ~ 60.3	48.0 ± 0.4
MCH (pg)	14.1 ~ 19.3	16.8 ± 0.4
MCHC (g/dL)	30.2 ~ 34.2	33.9 ± 0.4
CHCM (g/dL)	25.0 ~ 30.0	28.6 ± 0.3
RDW (%)	12.4 ~27.0	13.4 ± 0.2
HDW (g/dL)	2.2 ~ 2.4	2.2 ± 0.03
MPV (fL)	5.0 ~ 20.0	28.5 ± 0.3
Neutrophil (%)	6.6 ~ 38.9	15.6 ± 5.6
Lymphocyte (%)	55.8 ~ 91.6	67.3 ± 6.6
Monocyte (%)	≤ 7.5	15.2 ± 2.7
Eosinophil (%)	≤ 3.9	0.8 ± 0.1
LUC (%)	0.6 ~ 1.3	0.4 ± 0.1
Basophil (%)	≤ 0.2	0.5 ± 0.2
Reticulocyte (%)	5.6 ~ 9.9	5.3 ± 0.2
Platelet ($\times 10^3$ cells/μl)	592 ~ 2972	1323.3 ± 137.4
AST (IU/L)	70 ~ 120	110.8±16.3
ALT (IU/L)	≤ 45	43.8 ± 15.6
ALP (IU/L)	30 ~ 120	36.4 ± 23.0
Total protein (mg/dL)	5.4 ~ 5.8	5.4 ± 0.4
Total bilirubin (mg/dL)	0.1 ~ 0.12	0.1 ± 0.01

Values are mean ± standard error or as frequency (percentage).
WBC: white blood cell
RBC: red blood cell
MCV: mean corpuscular volume
MCH: mean corpuscular hemoglobin
MCHC: mean corpuscular hemoglobin concentration
CHCM: cellular hemoglobin concentration mean
RDW: red cell distribution width
HDW: hemoglobin distribution width
MPV: mean platelet volume
LUC: large unstained cell
AST: aspartate aminotransferase
ALT: alanine aminotransferase
ALP: alkaline phosphatase

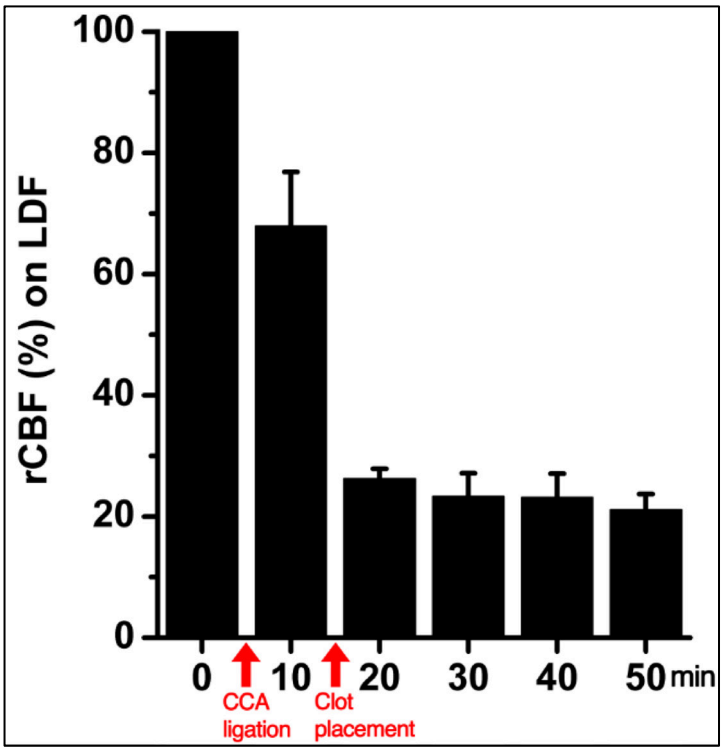

Figure S1. Results of laser Doppler flowmetry monitoring of cerebral blood flow (relative to the baseline, rCBF) in mice (n = 76) that underwent embolic middle cerebral artery occlusion (clot placement in the middle cerebral artery – anterior cerebral artery bifurcation area of the distal internal carotid artery). CCA denotes common carotid artery.

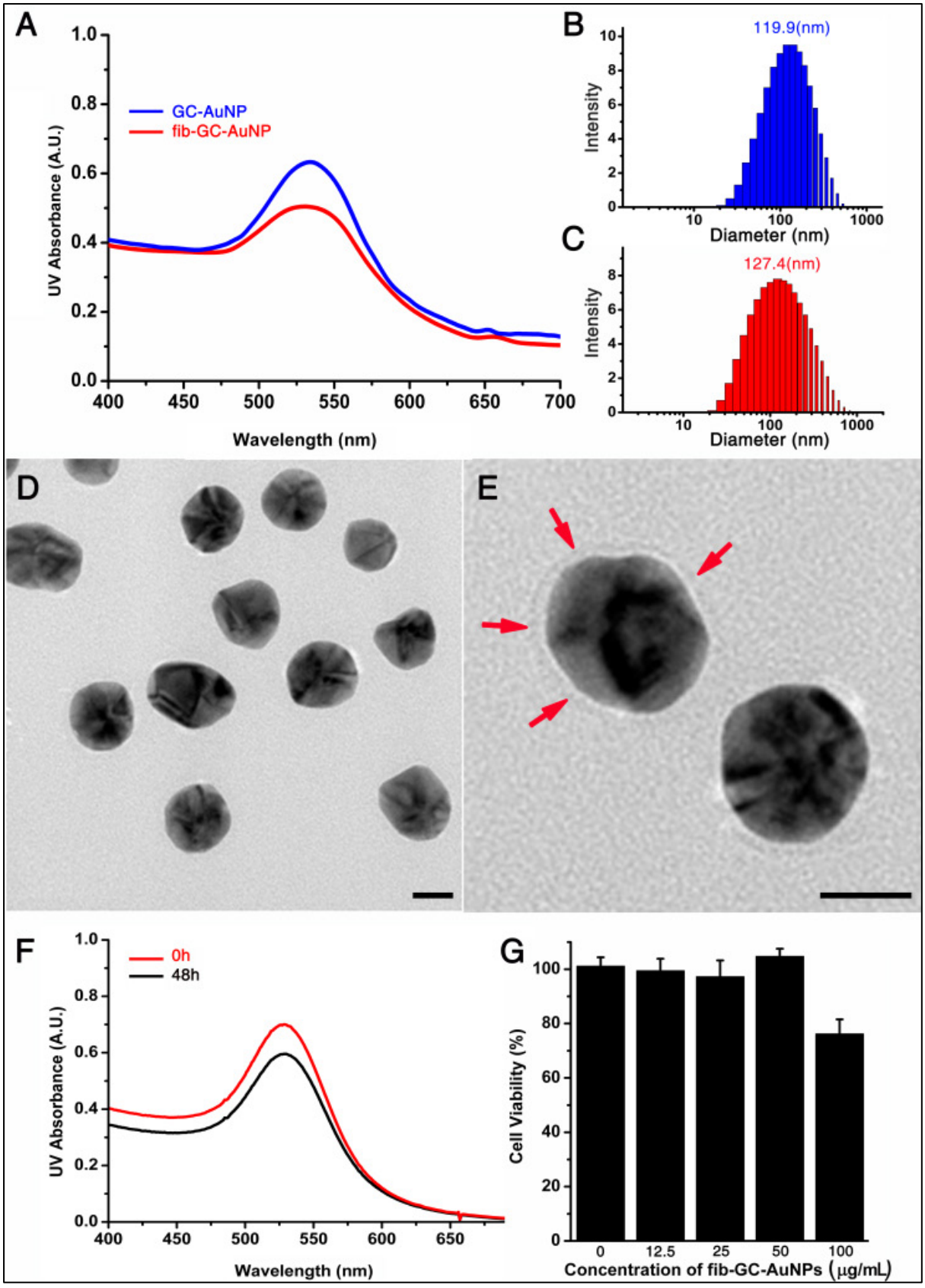

Figure S2. Characterization of the physicochemical properties of fibrin-targeted gold

nanoparticles.

A, Ultraviolet-visible (UV-vis) light absorption spectra of glycol chitosan-coated gold nanoparticles (GC-AuNPs) before (blue) and after (red) the conjugation of fibrin-targeting peptides used in EP-2104R.[1] The surface plasmon resonance peak of GC-AuNPs and fibrin-targeted fib-GC-AuNPs appear similarly at 533 nm, indicating that the fibrin-targeting peptides on GC-AuNPs are not aggregated. **B** and **C**, Size distribution of GC-AuNPs (**B**) and fib-GC-AuNPs (**C**) as measured with dynamic light scattering (DLS). This indicates hydrodynamic diameters (mean ± SEM) of 119.9 ± 2.8 nm and 127.4 ± 2.7 nm, respectively, with a mono-modal size distribution. **D** and **E**, Transmission electron microscographs show monodispersed and spherical fib-GC-AuNPs with a diameter of approximately 30 nm, indicating the non-hydrated metallic core size. Red arrows indicate the GC coating layer, which is collapsed under the non-hydrated conditions required by transmission electron microscopy. Size differences between the two methods of transmission electron microscopy measurement (**D** and **E**) and DLS (**B** and **C**) reflect the hydrophilic coat (i.e. GC) expanding in solution. **F.** UV-vis light absorption spectra of fib-GC-AuNPs in PBS buffer at 0 vs. 48 hours, reflecting the stability of the imaging agent. **G.** No noticeable cytotoxic effects of fib-GC-AuNPs (up to 50 μg Au / ml) on HeLA cells for 24 hours. Scale bars = 20 nm.

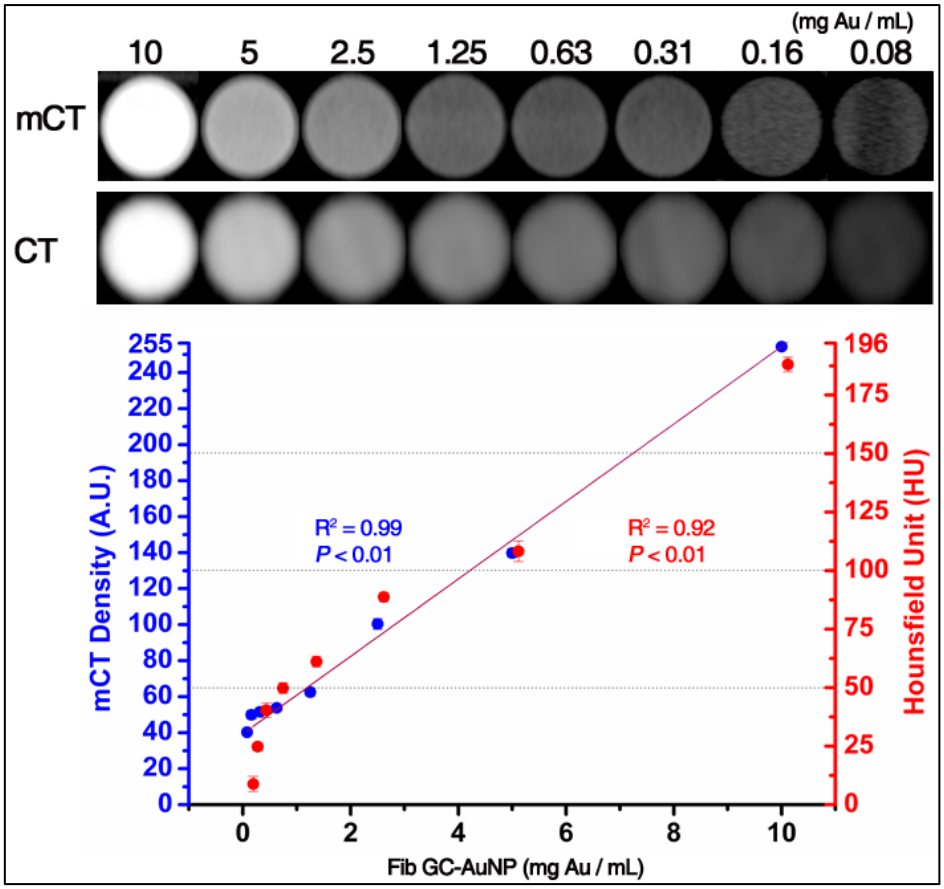

Figure S3. X-ray attenuation property of fibrin-targeted glycol chitosan-coated gold nanoparticles (fib-GC-AuNPs).

In vitro imaging studies (n = 3) were performed using a clinical positron emission tomography (PET) / computed tomography (CT) scanner (Gemini; Philips Medical Systems, Cleveland, OH; values in Hounsfield Unit) as well as a microCT (mCT) scanner (NFR Polaris-G90; NanoFocusRay, Jeonju, Korea; values in arbitrary unit: A.U.).

PET / CT parameters: 120 kVp, 41 mA, 600 × 600 mm field of view, 0.390 x 0.390 x 0.390 mm^3 voxel size, 360 views, 512 × 512 reconstruction matrix, 236 slices, scanning time 4.11 seconds. mCT parameters: 65 kVp, 60 μA, 26.7 × 26.7 mm field of view, 0.053 × 0.053 × 0.054 mm^3 voxel size, 360 views, 512 × 512 reconstruction matrix, 600 slices, 500 milliseconds per frame.

Pearson correlation analysis was used to calculate the R^2 and *P* values.

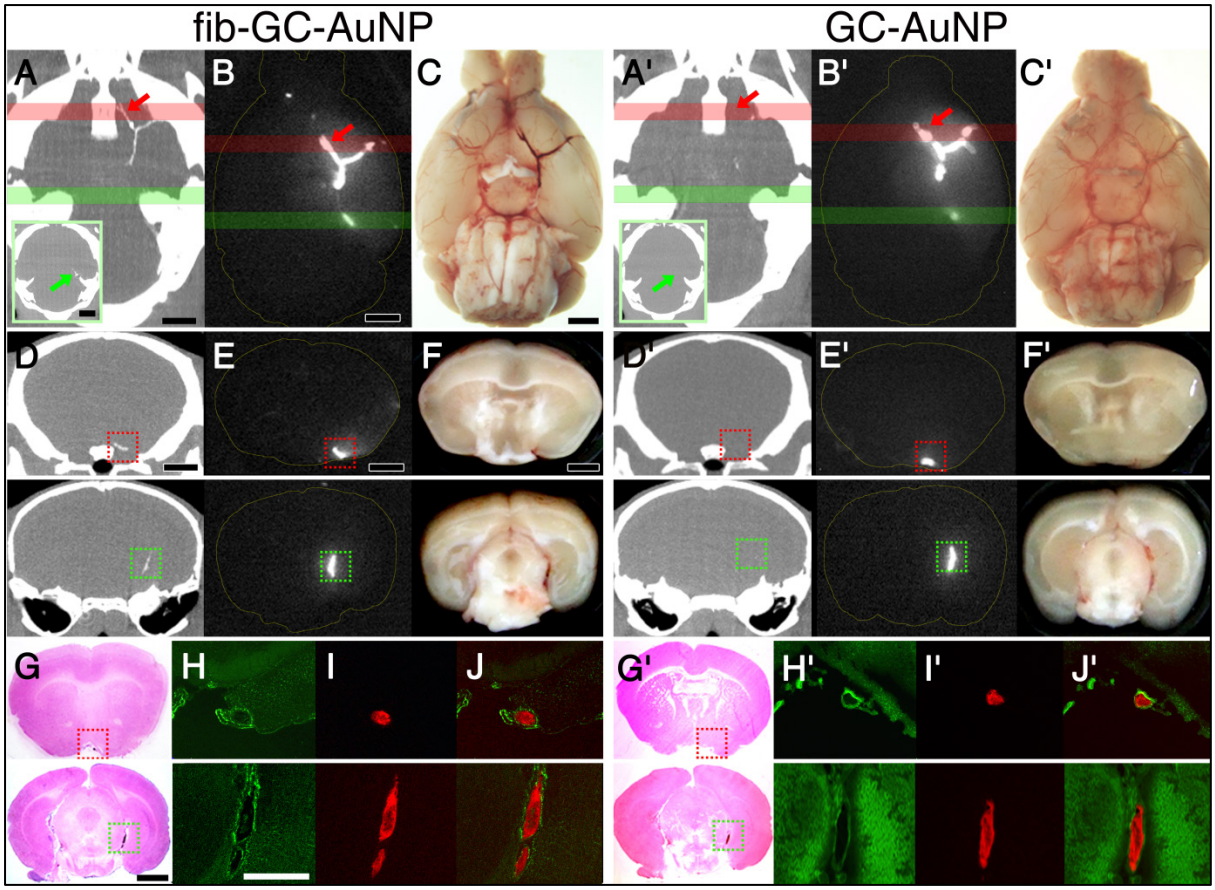

Figure S4. Imaging-histology co-localization study for fibrin-targeted glycol chitosan-coated gold nanoparticles (fib-GC-AuNPs) vs. non-targeted GC-AuNPs.

A–**C**, Axial micro-computed tomography (mCT) images (**A**), *ex vivo* Cy5.5 near-infrared fluorescent (NIRF) thrombus image (**B**) and visible light image (**C**) of a representative C57Bl/6 mouse brain with embolic clot at the left distal internal carotid artery bifurcation area after injection with fib-GC-AuNP. Compared with the larger axial mCT image in **A**, the smaller image in the green inlet of **A** is about 2 mm higher (toward the vertex). Red arrows indicate the thromboembolus in the anterior cerebral artery, and the green arrow indicates the thromboembolus in the posterior cerebral artery. **D**–**F**, Coronal reformations of the mCT (**D**) in the same plane as the sectioned brain (at red- and green-colored brain regions with arrows pointing the locations of thromboembolism in **A** and **B**) imaged optically for Cy5.5 (**E**), and gross brain digitally photographed (**F**). Red arrows in **A** and **B** match with the red-dotted

reticles in **D** and **E**, and the green arrow in the inlet of **A** matches with the green-dotted reticles in **D** and **E**. **G–J**, Cryosections (10 μm thickness) stained with hematoxylin and eosin (**G**), autofluorescence image in the green (fluorescein isothiocyanate) channel showing the vessel walls at a higher magnification view (**H**) obtained in the areas indicated by the red- and green-dotted reticles in **G**, Cy5.5 image showing the thrombus (**I**), and merged images (**J**) showing both. **A'–J'**, The same for an animal injected with GC-AuNPs. Please, note that fluorescently marked embolic clot is equivalent, but that targeted nanoparticles are much better seen on CT than the non-targeted version. Scale bars = 2 mm. (**A-G**); 500 μm (**H**).

References

1. Irwin S. Comprehensive observational assessment: Ia. A systematic, quantitative procedure for assessing the behavioral and physiologic state of the mouse. Psychopharmacologia. 1968; 13: 222-57.
2. Overoye-Chan K, Koerner S, Looby RJ, et al. EP-2104R: a fibrin-specific gadolinium-Based MRI contrast agent for detection of thrombus. J Am Chem Soc. 2008; 130: 6025-39.