Supporting Information

Targeting the Extracellular Matrix in Traumatic Brain Injury Increases Signal Generation from an Activity-Based Nanosensor

*Rebecca M. Kandel[‡], Julia A. Kudryashev[‡], and Ester J. Kwon**

Department of Bioengineering, University of California, San Diego, La Jolla, California 92093, United States

[‡]These authors contributed equally.

*Corresponding author. Email: ejkwon@ucsd.edu

Table S1. Immunostaining reagents and their dilutions used in this study.

Target and supplier	dilution	product #
fluorescein/Oregon Green (ThermoFisher)	2.5 μg/mL	A-889
fluorescein/Oregon Green *(alternate)* (ThermoFisher)	5.0 μg/mL	A-11095
NeuN (Millipore Sigma)	0.6 μg/mL	MAB377
CD31 (BD)	2.5 μg/mL	553370
biotinylated hyaluronic acid binding protein (bHABP) (Sigma)	5.0 μg/mL	385911

Table S2. Concentration measurements of calpain substrate peptide[a] and HApep[b] on TBI-ABNs in PBS.

TBI-ABN	Ratio of HApep to calpain substrate
Non-Targeted	N/A
Moderate-targeted	3.2
High-Targeted	8.5

[a]Calpain substrate absorbance measured at $\lambda = 646$ nm, $\varepsilon_{646\ nm} = 112{,}783.33\ (M*cm)^{-1}$

[b]HApep absorbance measured at $\lambda = 495$ nm, $\varepsilon_{495\ nm} = 75{,}000\ (M*cm)^{-1}$

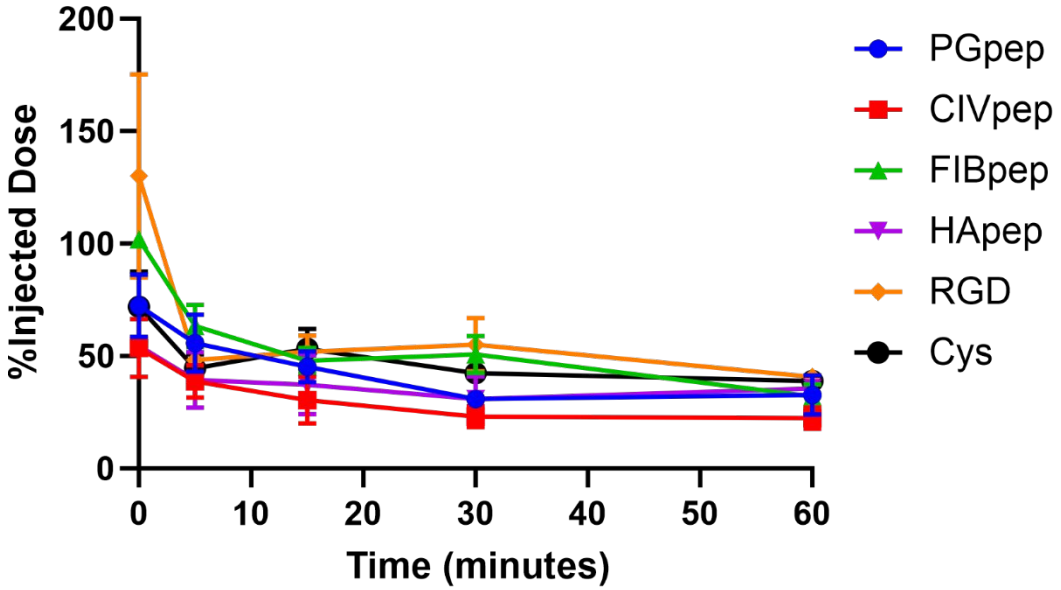

Figure S1. Percent injected dose of ECM-targeted nanomaterials in the blood at 0, 5, 15, 30, and 60 minutes after intravenous administration (n = 3, mean ± SEM).

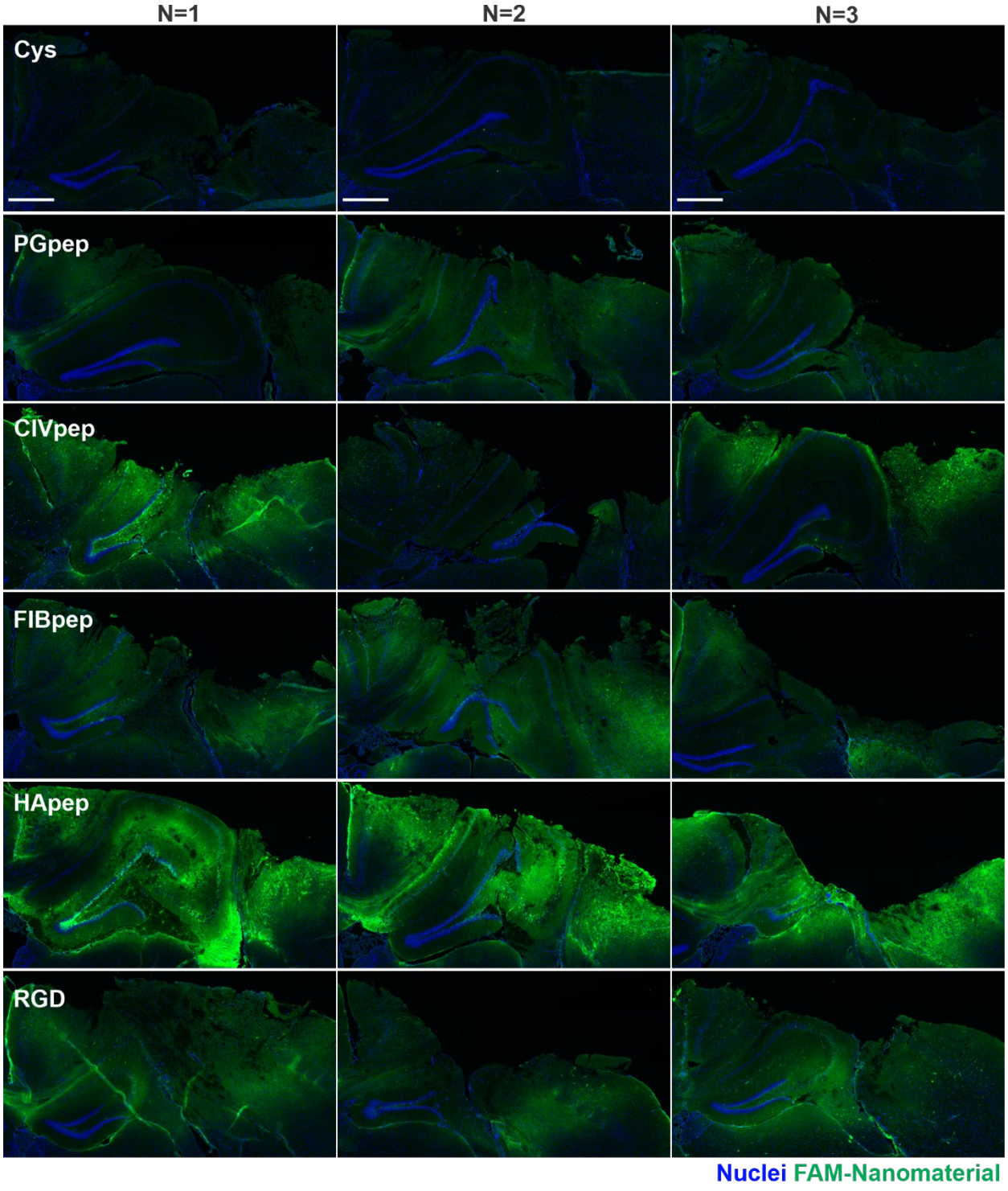

Figure S2. Triplicate histology in coronal brain slices of ECM-targeting peptides used in the *in vivo* screen (blue, nuclei; green, FAM-labeled ECM-targeting peptide on nanomaterial; scale bar = 500 μm).

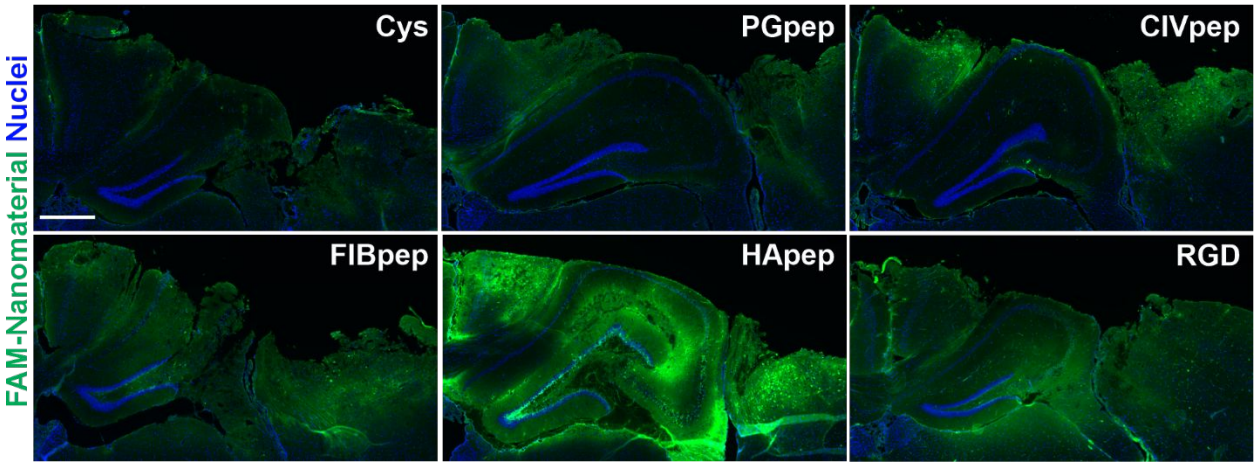

Figure S3. Coronal brain slices stained with an alternate anti-FAM antibody, ThermoFisher A-11095 (blue, nuclei; green, FAM-labeled ECM-targeting peptide on nanomaterial; scale bar = 500 μm).

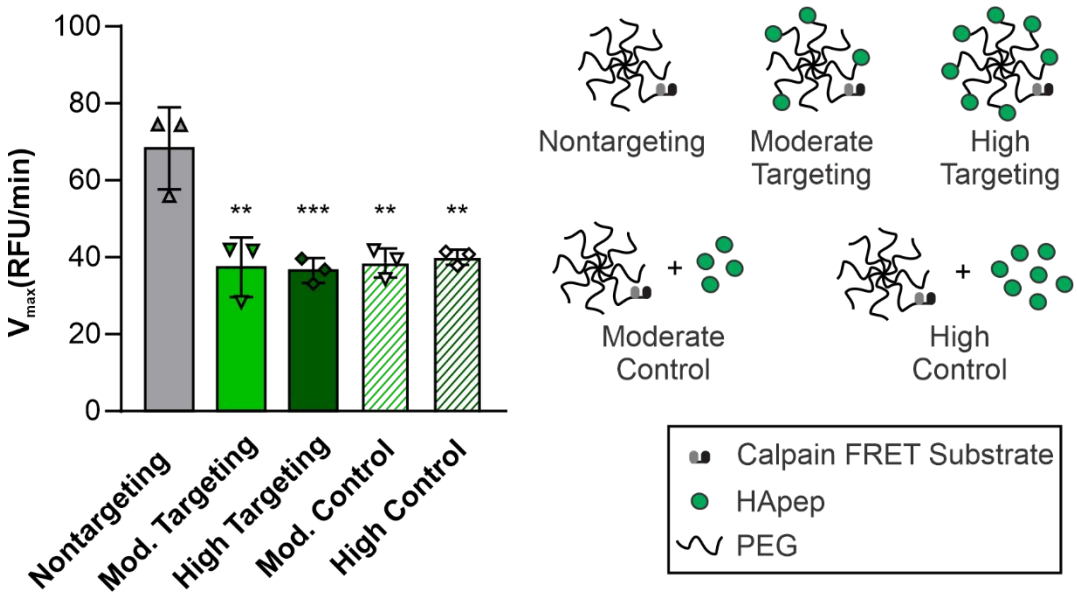

Figure S4. Maximal cleavage velocities of TBI-ABNs (8 μM quantified by calpain FRET substrate peptide) incubated with human calpain-1 and different levels of conjugated HApep (for targeting conditions) or unconjugated HApep (for control conditions) (n = 3, mean ± SD, **p ≤ 0.01, ***p ≤ 0.001, ordinary one-way ANOVA and Tukey's post-hoc test).

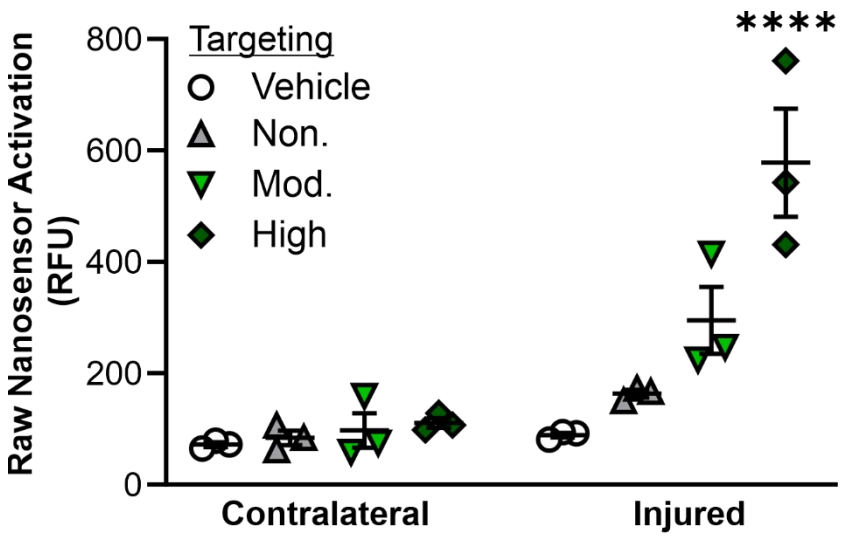

Figure S5. Raw activated nanosensor signal for non-, moderate-, and high-targeted TBI-ABN measured in cortical brain tissue lysate collected from contralateral and injured hemispheres, including background tissue signal from vehicle control (n=3, mean ± SEM, ****$p \leq 0.0001$, two-way ANOVA with Sidak's multiple comparisons post-hoc test compared to non-targeted; each data point represents one mouse).

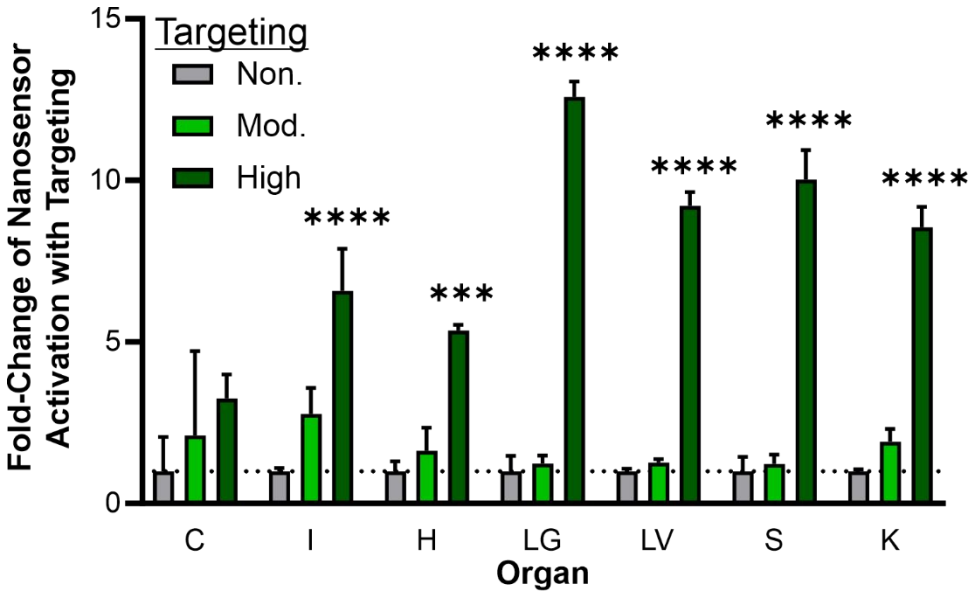

Figure S6. Relative fold-change of TBI-ABN activation in brain (C = contralateral cortical tissue; I = injured cortical tissue) and off-target organs (H = heart; LG = lungs; LV = liver; S = spleen; K = kidneys) compared to non-targeted TBI-ABNs (n = 3, mean ± SEM, ***p ≤ 0.001, ****p ≤ 0.0001, two-way ANOVA with Dunnett's multiple comparisons post-hoc test compared to non-targeted groups within each organ).

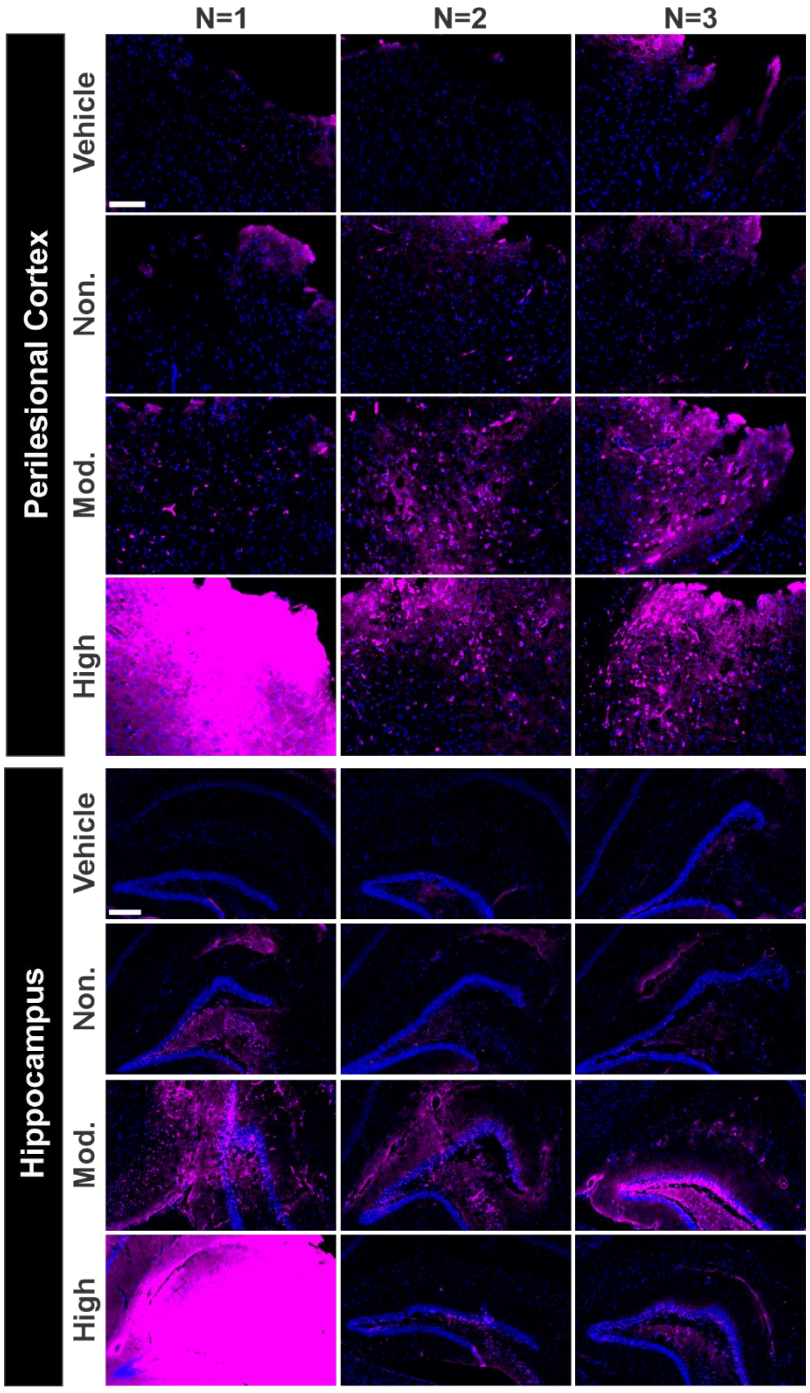

Figure S7. Nanosensor activation in triplicate brains for vehicle, non-targeted, moderate HApep-targeted, and high HApep-targeted TBI-ABN in the perilesional cortex and hippocampus (scale bar = 100 μm for perilesional cortex, scale bar = 200 μm for hippocampus; blue, nuclei; magenta, activated nanosensor).

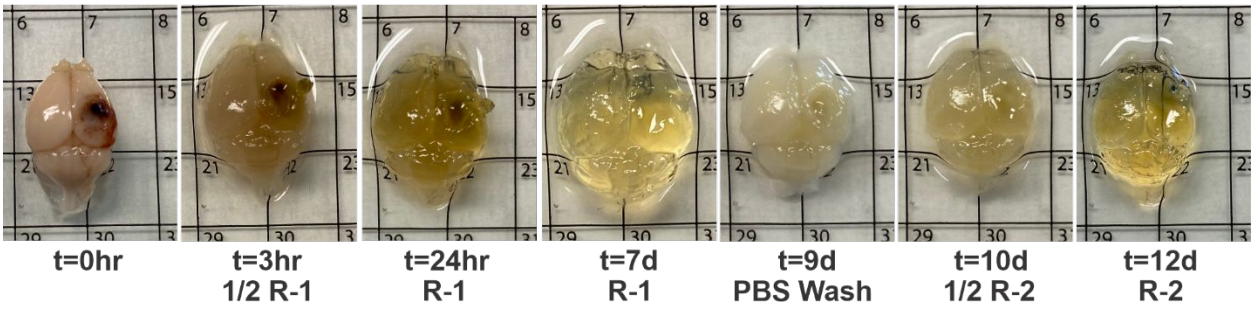

Figure S8. CUBIC clearing progress for excised injured brain demonstrating progressive clearing over 12 days following sequential incubations with reagent-1 (R-1) and reagent-2 (R-2).

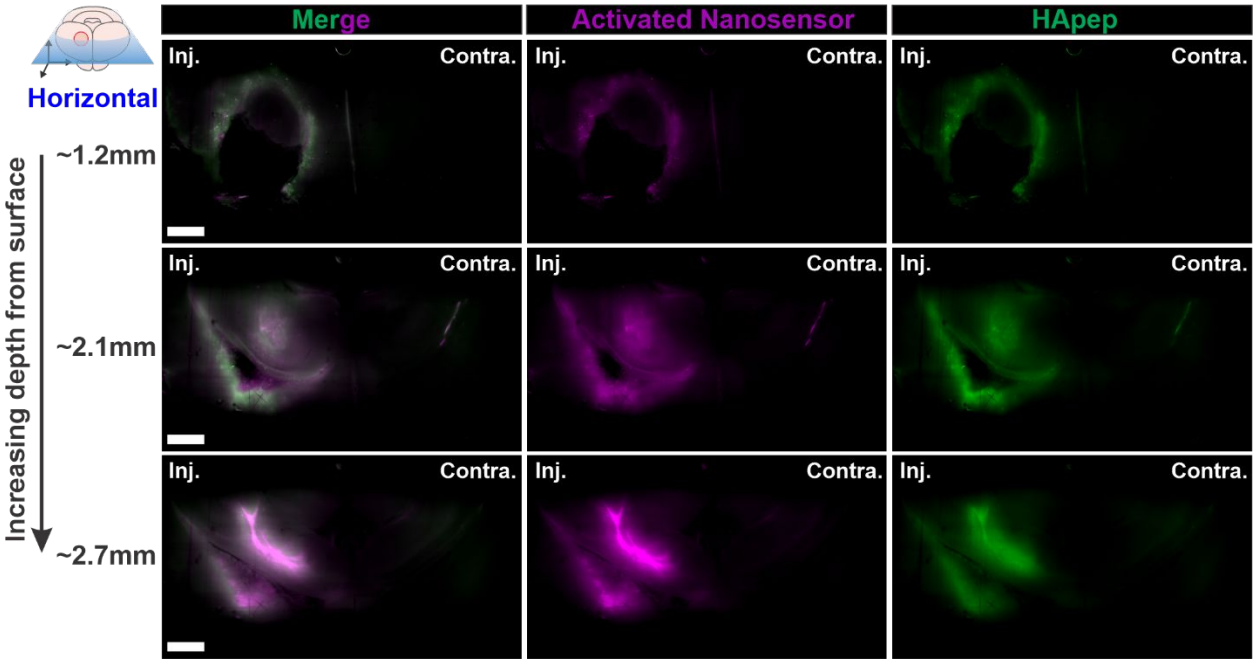

Figure S9. Light sheet fluorescence microscopy (LSFM) horizontal cross sections of cleared brain over three imaging depths from the cortical surface (1.2 mm, 2.1 mm, 2.7 mm) (magenta, activated nanosensor; green, HApep on nanosensor; scale bar = 1 mm; Inj. = injured hemisphere; Contra. = contralateral hemisphere).

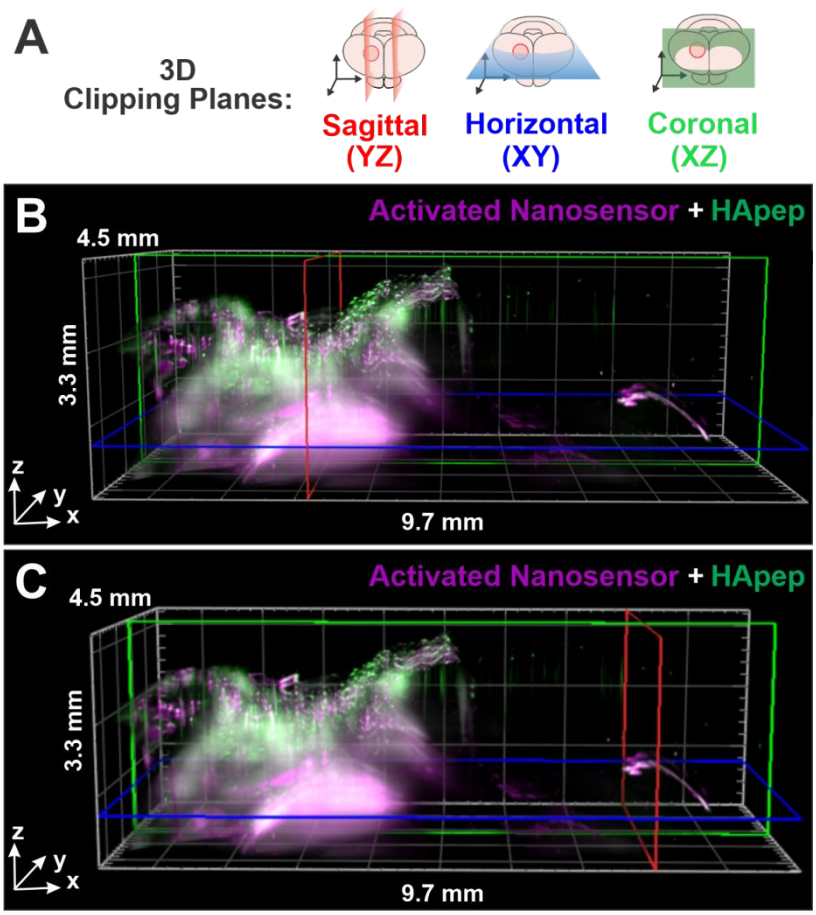

Figure S10. 3D rendering of TBI-ABN in the injured brain with clipping planes. (A) Schematic of sagittal (red, YZ), horizontal (blue, XY), and coronal (green, XZ) planes. (B) 3D render showing the three clipping planes with the sagittal plane located in the injured cortex. (C) 3D render showing the same clippling planes but with the sagittal plane in the contralateral cortex. (magenta, activated nanosensor; green, HApep on nanosensor).