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Supplementary Materials for

High-resolution fluorescence-guided transcranial ultrasound mapping in the live mouse brain

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The PDF file includes:

Other Supplementary Material for this manuscript includes the following:

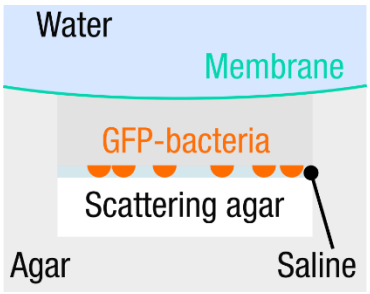

Fig. S1. Experimental setup for simultaneous sonication and fluorescence imaging of GFP expressing bacteria.

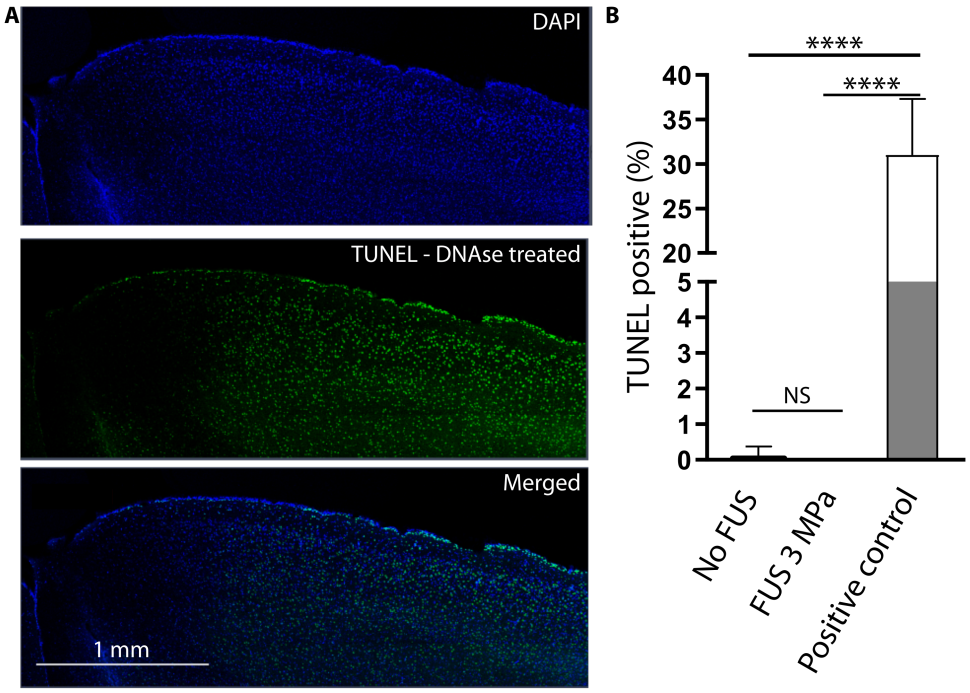

Fig. S2. TUNEL staining validation and analysis of thermal injury for repetitive 3 MPa sonication. (A) Positive control DNAse treated cortex with TUNEL staining (green) and DAPI (blue). (B) Percentage of apoptotic cells is unchanged between untreated and 3MPa-stimulated mice. Comparison was performed by counting TUNEL-positive cells in the cortex of untreated age-matched C57BL/6 mice, in the treated cortical region of 3MPa-stimulated and in DNAse-treated brain slices (positive control). TUNEL-positive cells were normalized to the total number of cells, stained with DAPI. (1-way ANOVA with Bonferroni post-hoc test. $P<0.0001$ ****).

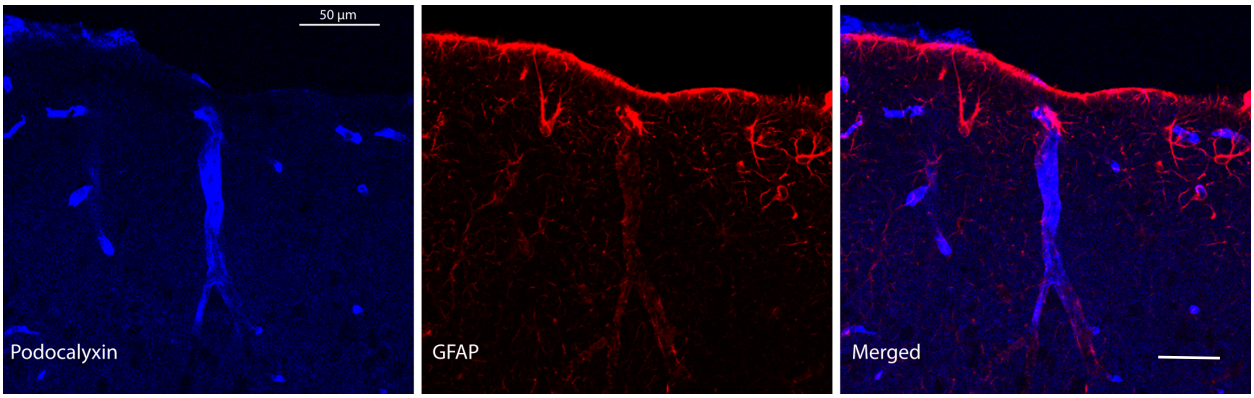

Fig. S3. Vessels and astrocytes after single 3.9 MPa sonication. Sonication with 3 MHz, 3.9 MPa over 100 ms generated astrocytic gliosis in an area of 100-200 µm around the US focus. Astrocytes are immunolabeled with an anti-GFAP (glial fibrilliary astrocytic protein) antibody, revealing the typical morphological changes (enlarged cell body and processes) of activated astrocytes. Podocalyxin labels vessels.

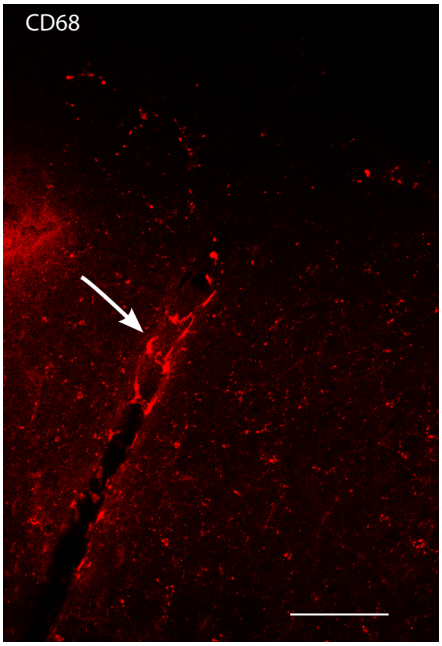

Fig. S4. Microglia after single 3.9 MPa sonication. Tissue sample sonicated with 3 MHz, 3.9 MPa over 100 ms was immunolabeled with CD68, which stains microglia. Enlarged microglia (white arrow) undergoing gliosis are seen in a vessel at the US focus, indicative of rupture of the blood brain barrier. Scale bar 100 μm.

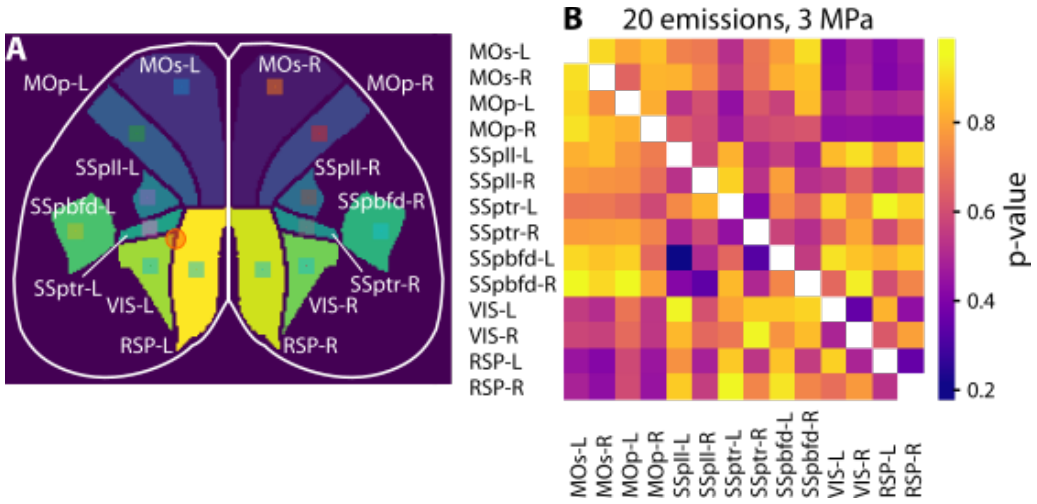

Fig. S5. Functional connectivity for repetitive 3 MPa sonication. (A) Schematic of the mouse brain. (B) Paired t-test of repeated 20 sonications using sequence 1 (3 MPa, 3 MHz, 150 ms continuous sonication) showing no significant changes from the baseline. Minimum p = 0.18. Experiment performed on 3 mice.

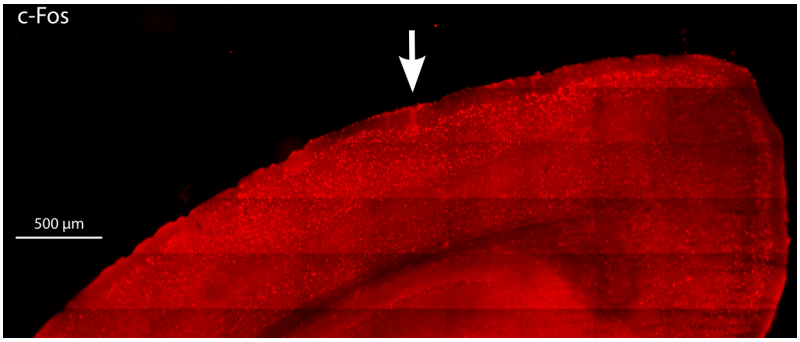

Fig. S6. Lesion after single 4.5 MPa sonication. Sonicating tissue sample with 3 MHz, 4.5 MPa over 150 ms resulted in a lesion in the upper cortical layer, evidenced by accumulation of IgGs (arrow). Strong c-Fos immmunoreactivity, reflecting high cellular activity due to the lesion and, presumably, the ensuing cortical spreading depolarisation (CSD), can be observed across the entire cortex.

Data S1. (separate file)

Functional connectivity (z-score) calculated as described in the material and methods section. The file contains connectivity matrices before and after sonication with a single 100 ms, 3MHz, 3.9 MPa pulse (Sheet 1) and 150 ms, 3 MHz, 3 MPa pulse delivered 20 times (Sheet 2).