

## Supplementary Figures and Tables

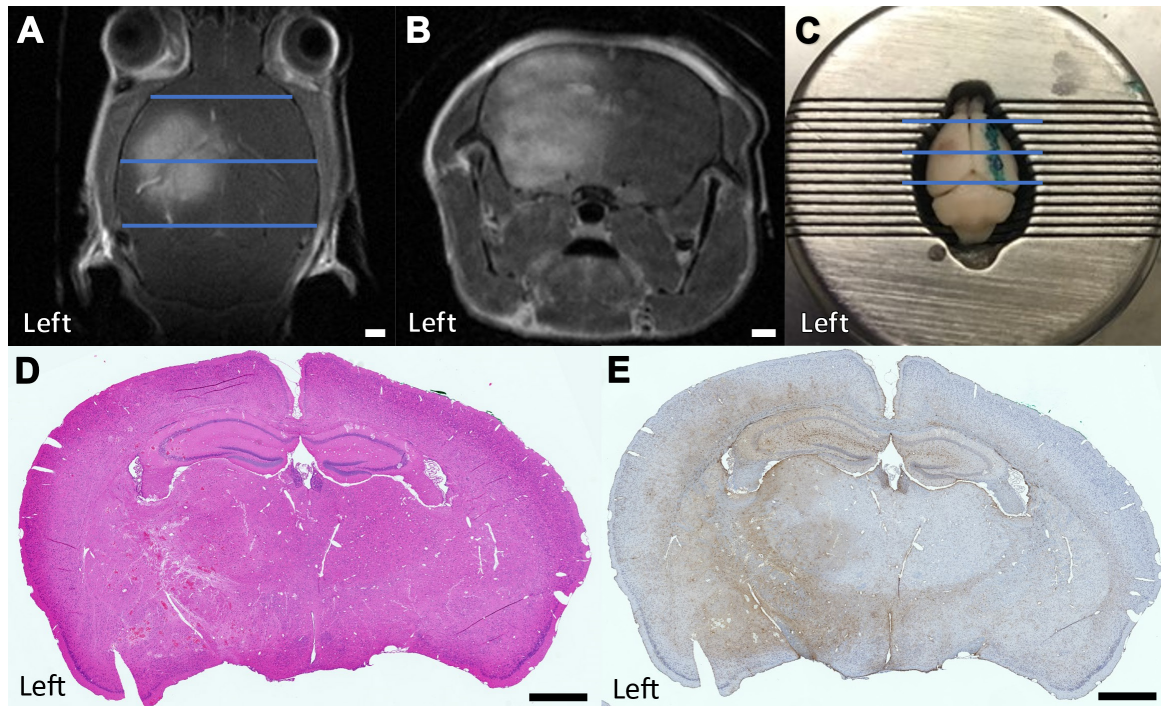


Figure S1. (A) Protocol used to obtain brain slices for histology. MR images were flipped from radiological convention such that the left hemisphere corresponded to the left of the image. Axial (A) and coronal (B) CE-MRI slices were used alongside hemorrhage visible on the surface of the brain (C), if present, to help guide the slicing process. Following perfusion, the harvested brain (with green dye applied to identify the untreated hemisphere) was positioned in a brain slicing mold (C), with blue lines denoting where cuts were made to yield 3 mm thick coronal slices. Brain slices were routinely processed for H&E and IHC (D, E). All scalebars represent 1 mm.

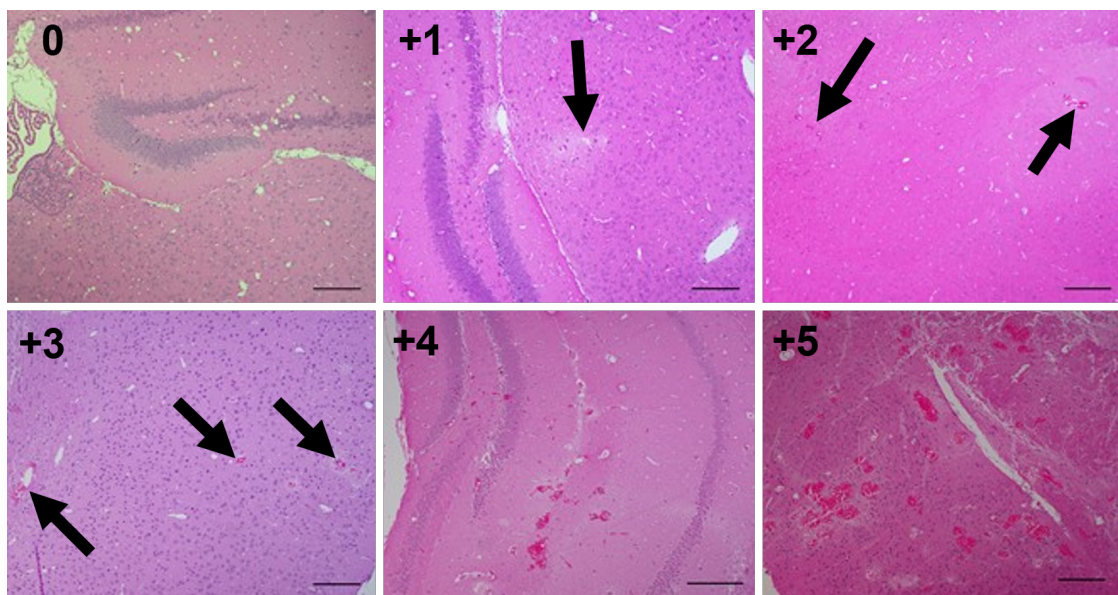


Figure S2. Representative H&E-stained histology images (10× magnification) for each histopathological grade: 0 (none), +1 (minimal), +2 (mild), +3 (moderate), +4 (marked), and +5 (severe). Scalebar = 100 μm.

*Table S1. Percentage of mice with BBB opening on MRI and the average histopathology grade observed at applied PNP. Signal enhancement on CE-MRI resulting from BBB opening was observed with both doses of MB at a PNP of 0.15 MPa. Changes in the parenchyma were always more severe at high doses of MB at the same PNP. No pathological findings were observed in the control (non-sonicated) contralateral hemispheres.*

| PNP (MPa) | MB dose ( $\mu\text{L}/\text{kg}$ ) | Proportion of animals with BBB opening on CE-MRI | Average histopathology grade |
|-----------|-------------------------------------|--|------------------------------|
| 0         | 0 (saline)                          | 0/5  | 0                            |
| 0.35      | 0 (saline)                          | 0/5  | 0                            |
| 0.15      | 10                                  | 2/5  | 0                            |
| 0.25      | 10                                  | 5/5  | 1.8                          |
| 0.35      | 10                                  | 5/5  | 3                            |
| 0.45      | 10                                  | 5/5  | 4.4                          |
| 0.10      | 250                                 | 0/5  | 0                            |
| 0.15      | 250                                 | 3/5  | 0.2                          |
| 0.20      | 250                                 | 4/5  | 1                            |
| 0.25      | 250                                 | 5/5  | 3.6                          |
| 0.30      | 250                                 | 5/5  | 3.8                          |
| 0.35      | 250                                 | 5/5  | 3.8                          |

*Table S2. Summary statistics of the number of activated microglia and astrocytes per  $\text{mm}^2$  observed in each 1.32 mm by 0.72 mm field of view. Means and standard deviations were computed across all brain regions (two regions in each hemisphere per animal) for each treatment group.*

| PNP (MPa) | MB dose ( $\mu\text{L}/\text{kg}$ ) | Number of activated microglia (Iba1+) per $\text{mm}^2$ |                      |                 | Number of activated microglia (TMEM119+) per $\text{mm}^2$ |                      |                 | Number of activated astrocytes (GFAP+) per $\text{mm}^2$ |                      |                 |
|-----------|-------------------------------------|---|----------------------|-----------------|--|----------------------|-----------------|--|----------------------|-----------------|
|           |                                     | Control hemisphere                                      | Sonicated hemisphere | <i>p</i> -value | Control hemisphere   | Sonicated hemisphere | <i>p</i> -value | Control hemisphere                                       | Sonicated hemisphere | <i>p</i> -value |
| 0         | 0 (saline)                          | 18.31 $\pm$ 4.30  | 17.99 $\pm$ 3.66     | 0.376           | 9.36 $\pm$ 2.19  | 9.47 $\pm$ 2.22      | 0.639           | 6.21 $\pm$ 1.26  | 6.42 $\pm$ 1.35      | 0.441           |
| 0.35      | 0 (saline)                          | 34.72 $\pm$ 4.32  | 38.19 $\pm$ 5.04     | 0.795           | 32.93 $\pm$ 3.58   | 36.09 $\pm$ 4.89     | 0.005           | 12.52 $\pm$ 6.40   | 12.63 $\pm$ 4.94     | 0.032           |
| 0.15      | 10                                  | 17.36 $\pm$ 4.01  | 27.99 $\pm$ 7.09     | 0.007           | 10.21 $\pm$ 4.76   | 17.47 $\pm$ 8.24     | 0.005           | 6.84 $\pm$ 2.29  | 8.94 $\pm$ 2.68      | 0.005           |
| 0.25      | 10                                  | 22.20 $\pm$ 5.28  | 39.04 $\pm$ 8.48     | 0.005           | 13.89 $\pm$ 5.67   | 24.62 $\pm$ 6.86     | 0.005           | 6.84 $\pm$ 1.51  | 14.20 $\pm$ 3.30     | 0.005           |
| 0.35      | 10                                  | 24.31 $\pm$ 4.61  | 43.35 $\pm$ 5.15     | 0.005           | 13.68 $\pm$ 2.53   | 27.78 $\pm$ 3.82     | 0.005           | 6.84 $\pm$ 2.99  | 15.26 $\pm$ 5.60     | 0.005           |
| 0.45      | 10                                  | 22.10 $\pm$ 4.78  | 44.72 $\pm$ 8.74     | 0.005           | 12.73 $\pm$ 2.50   | 28.41 $\pm$ 6.16     | 0.005           | 7.58 $\pm$ 4.17  | 14.94 $\pm$ 4.70     | 0.005           |
| 0.1       | 250                                 | 30.41 $\pm$ 5.42  | 37.46 $\pm$ 6.20     | 0.049           | 24.20 $\pm$ 5.15   | 30.41 $\pm$ 6.48     | 0.005           | 20.83 $\pm$ 5.99   | 23.88 $\pm$ 7.41     | 0.005           |
| 0.15      | 250                                 | 36.41 $\pm$ 10.05                                       | 49.56 $\pm$ 10.71    | 0.005           | 21.89 $\pm$ 4.75   | 34.20 $\pm$ 4.61     | 0.005           | 13.47 $\pm$ 4.08   | 23.67 $\pm$ 6.49     | 0.005           |
| 0.2       | 250                                 | 30.62 $\pm$ 7.83  | 52.19 $\pm$ 8.42     | 0.005           | 19.89 $\pm$ 3.76   | 35.77 $\pm$ 7.08     | 0.005           | 16.10 $\pm$ 4.33   | 32.30 $\pm$ 7.36     | 0.005           |
| 0.25      | 250                                 | 26.41 $\pm$ 4.56  | 46.09 $\pm$ 9.12     | 0.005           | 17.36 $\pm$ 2.23   | 36.20 $\pm$ 6.26     | 0.005           | 12.73 $\pm$ 4.39   | 28.20 $\pm$ 7.76     | 0.005           |
| 0.3       | 250                                 | 32.30 $\pm$ 8.78  | 51.14 $\pm$ 12.68    | 0.005           | 19.89 $\pm$ 3.08   | 40.40 $\pm$ 4.84     | 0.005           | 10.31 $\pm$ 3.96   | 28.72 $\pm$ 7.70     | 0.005           |
| 0.35      | 250                                 | 29.04 $\pm$ 12.02                                       | 55.66 $\pm$ 15.37    | 0.005           | 16.41 $\pm$ 5.25   | 43.14 $\pm$ 10.40    | 0.005           | 10.94 $\pm$ 6.60   | 35.04 $\pm$ 10.87    | 0.005           |