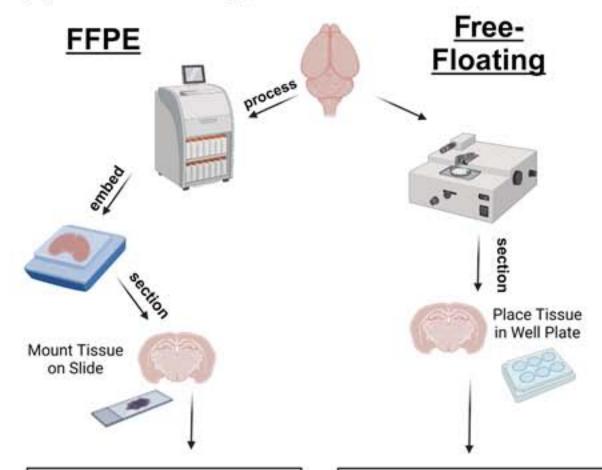
Supplemental Figure S1

Niedowicz, et al.



- 1. Deparaffinize
- 2. H₂O₂
- 3. Pepsin, 20 min., 37°C
- 4. Block (goat serum)
- 5. Primary Antibody
- 6. Secondary Antibody
- 7. ABC Reagent
- 8. DAB
- 9. Counterstain
- 10. Dehydrate
- 11. Coverslip

- 1. H₂O₂
- 2. pH9, 20 min., 80°C
- 3. Block (goat serum)
- 4. Primary Antibody
- 5. Secondary Antibody
- 6. ABC Reagent
- 7. DAB
- 8. Mount on slides
- 9. Dry
- 10. Coverslip



Supplementary Figure S1. Collagen IV Staining Workflow. Though there are some similarities between the FFPE and free-floating procedures, there are differences. For FFPE, mouse brains are fixed in formalin, processed, and embedded in paraffin. Tissue is sectioned coronally, and mounted on glass slides for staining. For free-floating sections, the brains are cut coronally using a freezing, sliding microtome, and the sections placed in a microplate for staining. Another big difference is the antigen retrieval method used: pepsin-mediated retrieval is used for FFPE, while high pH, heat-mediated retrieval is used for free-floating sections. Once the stained sections are mounted and coverslipped, the slides are scanned and analyzed using the same microvessel algorithm.

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