

1 **Supplemental Article S1**

2 Cardiac perfusion and brain extraction

3 Brain Tissue Cryosectioning

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19 Cardiac perfusion and brain extraction

20 1. Mouse anesthesia (if anesthesia is good, can enter the next step): Administer 1%
21 sodium pentobarbital intraperitoneally (i.p.) at 0.1 mL/20 g body weight (50 mg/kg).
22 Assess anesthesia efficacy 5–10 minutes post-injection by pinching the hind limb with
23 hemostatic forceps. If the anesthetic effect is insufficient, administer an additional
24 dose of 0.03mL/20 g (equivalent to 15mg/kg), reassess after 5 minutes, and repeat the
25 process as needed.

26 2. Liquid preparation: 20ml perfusion salt solution and 20ml paraformaldehyde were
27 extracted using a 20ml syringe.

28 3. Exposure of Mouse Heart and Liver: The abdomen of the mouse was upward.
29 Make an inverted V-shaped incision through the skin to expose the abdomen,
30 diaphragm, and thoracic cage. Gently separate the diaphragm from the thoracic wall
31 to avoid cardiac injury. Cut the ribs bilaterally and reflect them upward. Use
32 ophthalmic forceps to carefully dissect the pericardial fat, fully exposing the heart.

33 4. Create the perfusion channel : Insert a disposable intravenous infusion needle
34 approximately 3–4 mm into the left ventricular apex and secure it (avoid penetrating
35 the left ventricle completely). Using ophthalmic scissors, make an incision at the
36 darkened region superior to the right ventricle (right atrial appendage) to allow
37 drainage of dark red venous return blood (Supplementary Fig. 1I–J).

38 5. Anticoagulant perfusion with saline solution: The assistant connects the perfusion
39 saline syringe to the infusion needle and injects slowly. During this process, the liver
40 color will transition to a pale hue and effluent from the right auricle will progressively
41 lighten. After the outflow was completely clarified, the heparinization was essentially
42 completed (Supplementary Fig. 1J–K).

43 6. Paraformaldehyde Perfusion Fixation: Connect the paraformaldehyde syringe to the
44 infusion needle and administer slowly. In this process, the limbs of the mice can be
45 observed twitching and the tail swinging, which indicates that the perfusion channel is
46 well constructed. Upon completion of perfusion, the mouse body becomes rigid.
47 When lifting by the tail, the torso exhibits complete stiffness, indicating successful
48 fixation (Supplementary Fig. 1L).

49 7. Brain tissue exposure: Sever the connection between the cervical vertebrae and
50 skull, then remove the head. Incise the scalp to fully expose the skull. Using forceps,
51 carefully peel the skull outward starting from the foramen magnum, taking care to
52 avoid damaging brain tissue.

53 8. Brain tissue isolation: Gently lift the brain tissue upward from the cerebellum
54 region, carefully sever the cranial nerves, and transfer the isolated brain into 4%
55 paraformaldehyde solution for overnight fixation.

56 Brain Tissue Cryosectioning

57 1. Sucrose Immersion: Remove the brain tissue from the fixative and immerse it in
58 30% sucrose solution at 4°C for 2 hours.

59 2. OCT Embedding: Select an appropriately sized embedding mold or shape
60 aluminum foil to create a suitable container. First, add a small amount of OCT
61 embedding medium. After absorbing excess sucrose solution from the brain tissue
62 surface, place the tissue horizontally in the mold. Then, add more OCT medium until
63 the tissue is completely submerged.

64 3. Flash Freezing: Place the embedding mold on a pre-cooled freezing stage. After the
65 OCT compound turns white, apply a small amount of OCT to the sample holder.
66 Transfer the embedded sample from the mold to the holder and position it on the
67 freezing stage.

68 4. Trimming Sections: Install the blade at a 5-7° angle and mount the sample holder
69 onto the microtome head. Set the section thickness to approximately 50 μm and trim
70 until reaching the desired tissue plane.

71 5. Slicing : Adjust the thickness of a single slice to about 5-15μm, put down the anti-
72 roll plate, slice (when cut to the corresponding level, take a picture to record the
73 position of the dye), lift the anti-roll plate, and stick the slice with a glass slide.

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