SUPPLEMENTAL DATA

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Mouse thrombolysis stroke model

Supplemental Methods

Surgical preparation of animals, post-operative recovery and temperature regulation

i) Surgical Preparation: All procedures were carried out under a surgical microscope. Anaesthesia was induced with 5% Isoflurane and maintained throughout the procedure at 1.5-2.5% (1-1.2 L/min) oxygen via a nose cone. A topical local anaesthetic was applied at incision sites (Xylocaine 1%, AstraZeneca, NSW, Australia or bupivacaine 2mg/kg; 100µl; Bupivacaine-Claris, Lyppards). Rectal temperature was maintained at 37°C with a feedback-controlled thermoblanket (Harvard Apparatus Ltd., Kent, UK). Poly Visc^{TM} Lubricating Eye Ointment was applied prior to procedures (Alcon Laboratories Pty Ltd, NSW, Australia). Mice were positioned in a stereotaxic frame to allow for rotation between prone and supine positions, for imaging and surgical manipulation of the carotid artery, respectively (SGM-4 head holder for mice, Narishige Scientific Instrument Laboratory, Japan).

A medial incision was made to the ventral side of the neck to access the left and right CCAs, where either one or both carotid arteries were isolated from the vagus nerve and surrounding connective tissue. An ultrasound Doppler flow probe was attached to the left CCA to monitor blood flow (0.5 mm i.d, MC0.5PSB-NH-JN-WC60-CRA10-GA, Transonic Systems Inc, NY, USA). The flow probe was connected to a Doppler flow meter (TS420, Transonic Systems Inc., NY, USA) with data recorded using a *PowerLab* data acquisition system (ADInstruments, NSW, Australia). Mean blood flow was recorded with *LabChart* software (version 7.0, ADInstruments, NSW, Australia) and corrected for body weight (ml/min/100g).

ii) Post-operative recovery & temperature regulation: Mice were allowed to recover for 24-hours post-occlusion to allow for assessment of functional deficits and cerebral infarction. In preliminary studies, we noted a significant reduction in core body temperature, with initial studies revealing that up to 33% of mice failed to develop cerebral infarction, despite persistent CCA occlusion. Based on these preliminary observations and extensive literature consistent with the neuroprotective effects of hypothermia[64-66], we adhered to a rigorous regimen to control body temperature post-surgery. Mice were recovered post-surgery in a warmed environment (26-28°C) from 2-24 hours post-recovery, with cages placed partially half-on, half-off on a feedback-controlled heat mat (37°C) to allow the animal to self-regulate. A heat lamp was positioned over the cage to provide additional warmth for the first post-operative hour. This alone resulted in an improvement in the incidence of cerebral infarction from 65%

to >80%. Mice in their cages were placed in a warming cabinet (Cat# ASSWC24; Able Scientific, NSW, Australia) during overnight recovery to control post-operative ambient temperature.

Ambient intraoperative temperature was also found to impact infarct development. The infarcts generated by the iCAT model were typically smaller cortical and hippocampal infarcts (mean ± SEM: 30 ± 8mm^3; n=8) when the studies were conducted at an alternate site with a lower ambient temperature (<2-4°C) (Figure 1B-Ci), compared to the studies presented in Figure 1Bii which were conducted at 22-24°C.

Transient bilateral common carotid artery occlusion (tBCCO)

Surgical preparation was conducted as described in *Surgical preparation of animals, post-operative recovery and temperature regulation (Supplemental Methods).* To achieve bilateral carotid artery occlusion, the ipsilateral carotid artery was occluded with an electrolytic-induced thrombotic occlusion, and the contralateral carotid artery was mechanically occluded with a haemostatic clamp (Micro serrefine clamp, Cat #18055-05, Fine Science Tools, Canada). Bilateral occlusion was maintained for 20, 25 or 30 minutes, as specified in the results section. At the end of the bilateral occlusion period, the haemostatic clamp was removed from the contralateral carotid artery, and animals monitored for 30 minutes following clamp removal, prior to recovery.

Cerebral Perfusion Monitoring

Cerebral perfusion monitoring was conducted with laser speckle contrast imaging (LSCI) and LDF through the mouse skull. A longitudinal incision was made along the scalp, above the midline and over bregma, with a crosswise incision made between the ears to allow the skin to be retracted.

(i) Laser Speckle Contrast Imaging (LSCI): LSCI of cerebral perfusion (designated flux units) was obtained using a moorFLPI-2 blood flow imager and associated software (Moor FLPI-2 Measurement V1.1, Moor Instruments, UK). Imaging was performed prior to manipulations of the carotid arteries (3 minutes, "baseline"), from 15-30 minutes after contralateral stenosis removal ("post-bilateral occlusion/stenosis"), and at 24 hours post-occlusion (3 minutes, "24 hour"). Images were captured at 30-second intervals for each recording period, with a temporal filter of 250 frames (1 frame/10 seconds), and 20 milliseconds exposure. Gain was adjusted for the "Baseline" reading per mouse and used for each subsequent reading (Gain range: minimum 145, maximum 180). Analysis was performed

with Moor FLPI-2 Review V4.0 and V5.0 software (Moor Instruments, UK). Hemispheric regions of interest (ROI) were created from the generated colour image of the skull by tracing along the sagittal suture between the eye sockets to lambda, along the lambdoid suture, and the temporalis muscle border. All flux values were calculated from 3 minutes of recording and post-occlusion/stenosis and 24-hour flux values were expressed as a percentage of the mean baseline flux for each hemisphere.

ii) Laser doppler flowmetry (LDF): For perfusion monitoring during surgical procedures, LDF was used as previously described with modifications[19]. Following baseline LSCI measurements, two fibre-optic laser doppler probes (P10d and VP10M200ST, Moor Instruments, UK) in custom-made 2x2x2 mm silicone probe holders were affixed to the skull surface. The probes were positioned in each hemisphere over the MCA territory: 1 mm posterior to bregma and 1 mm lateral to the temporalis muscle border. Regional cerebral blood flow (rCBF) was analyzed using moorVMS-LDF2 (Moor Instruments, UK), connected to *PowerLab* and *LabChart* software, allowing direct comparison of rCBF with carotid blood flow data. "Baseline" rCBF was calculated as the mean flow of a 5-minute period prior to electrolytic injury and all subsequent recordings were expressed as a percentage of baseline. Continuous LDF measurements were acquired during the procedure until 10 minutes after the right carotid clamp or stenosis was removed. The probe holders were then removed for post-occlusion LSCI.

Assessment of cerebral infarction

For the quantification of infarct volume, mice were deeply anaesthetized with isoflurane and transcardially perfused with cold saline. The brain was removed and sliced into 4 x 2 mm coronal brain sections using a Kopf Mouse brain block, discarding the occipital bulbs and cerebellum. Brain slices were incubated at 37°C in 1% 2,3,5-triphenyl-tetrazolium chloride (TTC, Sigma Aldrich, MO, USA) in saline for 14 minutes (7 minutes each side). After overnight fixation in 10% neutral buffered formalin, brain sections were imaged on a flatbed scanner (Epson Perfection V700) and infarct analysis conducted with Image J software. An investigator blinded to the experimental groups conducted quantification of infarct volume. Total area and infarct area were calculated for the top and bottom of each section, and then averaged to provide total volume and lesion volume for each section. Infarct volume is presented as the summation of lesion volume for the whole brain. Infarct volume was not analysed in mice deceased prior to the predesignated endpoint.

Dialysis of tissue plasminogen activator

Actilyse® (Boehringer Ingelheim Pty Ltd) was dialyzed and the activity was assessed as previously described[30]. Removal of arginine components is required to eliminate the potent vasodilatory response induced by intravenous injection of Actilyse® directly into the mouse jugular vein. Preliminary studies demonstrated that direct injection of Actilyse® into the jugular vein resulted in high mortality in C57BL/6 mice, whereas mortality rate was reduced when Actilyse® was dialyzed prior to injection (ALS and SAS, Personal communication). Actilyse®, purchased from Boehringer Ingelheim (50 mg alteplase), was dialyzed (10 kDa molecular weight cut off dialysis membrane; ThermoScientific Cat#66810) against 0.35 M Hepes-NaOH pH 7.4 at 4°C for 24 hours. Proteolytic activity of the dialyzed rtPA was determined using rtPA substrate (US Biological Life Sciences Cat#T5600-19) and read on the ClariostarPlus plate reader (BMG Labtech, Victoria, Australia) as per manufacturer's instructions. Dialyzed rtPA was stored at -80°C and thawed at 4°C prior to use. For intravenous infusion, rtPA was diluted in sterile saline immediately prior to injection.

Administration of antithrombotic agents

A clinical preparation of argatroban (Argatra/Exembol®) was purchased from Mitsubishi Tanabe Pharma (Germany) and prepared in sterile saline with 25% vol/vol of propylene glycol. Argatroban (80ug/kg bolus; 40 ug/kg/min 60-minute infusion) was delivered intravenously with rtPA therapy using a Harvard apparatus pump (Cat# 704504; Pump Elite 11 I/W Single Syringe Pump, NSW, Australia). Carotid artery blood flow and LDF were monitored concurrently for 60 minutes following treatment onset. To maintain persistent anticoagulation in iCAT recovery experiments, an ALZET osmotic minipump (2001D) was loaded with argatroban (dose equivalent: 40 ug/kg/min) and implanted subcutaneously in the mid-scapular region at 1 hour after commencement of intravenous therapy, as per manufacturer's instructions. The minipump remained in place until the 24-hour endpoint.

Assessment of recanalization

Carotid artery blood flow and recanalization was measured using the ultrasound doppler flow probe attached the left common carotid artery, as described in Supplemental Methods (*ii) Surgical Preparation).* Recanalization was defined as a measurable return of blood flow (>0ml.min) within the carotid artery following treatment administration, and classified as;

stable (steady flow), *unstable* (fluctuating flow), *transient with reocclusion* or *none* based on the LabChart flow trace.

Supplementary Figures

Supplementary Figure I. Schematic overview of the procedural manipulations of the CCAs when performing electrolytic injury alone, tBCCO and iCAT surgery in mice. For all procedures (**A-C**), the left (L) carotid artery (*ventral perspective*) was isolated, and an ultrasound doppler flow probe attached to monitor baseline blood flow (*Blood flow*). To induce thrombotic occlusion of the left (ipsilateral) carotid artery, the flow probe was removed, and the carotid artery subjected to electrical current delivered through a platinum electrode (*electrolytic injury*), coupled with distal application of a haemostatic clamp to maintain blood stasis (*stasis*). The flow probe was replaced at the completion of electrolytic injury and blood flow monitored for occlusive clot formation, indicated by reduction of blood flow to 0ml/min/100g. To induce transient bilateral common carotid artery occlusion (tBCCO) (**B**), thrombotic occlusion of the left carotid artery was coupled with transient mechanical occlusion of the right (R) contralateral carotid artery using a haemostatic clamp (*transient clamp*) for 20-30 minutes (**Bi**). tBCCO induced bilateral hypoperfusion within the MCA territory, as measured with LDF (**Bii; between (a)-(b)**). In the *in-situ* carotid artery thrombolysis (iCAT) model (**C**), graded cerebral ischemia was induced by transient stenosis of the right carotid artery (*transient stenosis*) for 25, 45 or 60 minutes (**Ci-ii; between (a)-(b)**). In all models (A-C), intravenous drug delivery (*Treatment IV*) was administered via the jugular vein post-thrombotic occlusion, and effects on left carotid artery blood flow assessed via the attached flow probe (A-C). Cerebral perfusion was assessed with laser doppler flowmetry concurrently with carotid artery blood flow, and LSCI conducted at 90 minutes post-ischemia induction (**B, Ciii**). In both tBCCO and iCAT models, animals were recovered to 24 hours for assessment of cerebral perfusion and end-organ damage (B/Ciii).

Supplementary Figure II. Schematic overview of the iCAT model.

The iCAT model incorporates thrombotic occlusion of the common carotid artery induced by electrolytic injury to allow for real-time monitoring of occlusion and recanalization events (**A**). Following thrombotic occlusion of the carotid artery, transient stenosis of the contralateral carotid artery induces ipsilateral cerebral hypoperfusion sufficient to induce infarction (<25% baseline flow), measurable with laser doppler flowmetry over the MCA territory (**B**). Cerebral perfusion analysis with laser speckle contrast imaging at 90 minutes post stroke onset is predictive of 24-hour outcome (**C**), including behavioural deficit, infarct progression and

mortality (**D**). Note – elements of this image were created with “BioRender.com" and exported under a paid subscription. (**E**) Timeline for the induction of electrolytic injury and cerebral ischemia/stroke, including the relative timing of cerebral perfusion measurements pre- and post-stroke and delivery of treatment(s) post-carotid occlusion. Baseline LDF, LSCI and carotid artery blood flow readings are collected prior to electrolytic injury to the carotid artery. Following electrolytic injury (3 minutes), the ultrasound doppler carotid artery flow probe is replaced to monitor for carotid artery occlusion. Following 10 minutes of observed stable thrombotic occlusion, transient stenosis of the contralateral carotid artery is performed and maintained in place for 60 minutes prior to release. At 15 minutes after stable thrombotic occlusion, intravenous (IV) treatment is delivered via the jugular vein. A 90-minute LSCI reading is obtained, prior to recovery of the animal to 24 hours.

Supplementary Figure III. Unilateral occlusion of the carotid artery causes varied changes in cerebral perfusion, producing inconsistent cerebral infarction at 24 hours. Thrombotic bilateral common carotid artery occlusion (tBCCO) results in bilateral cerebral infarction and high mortality in C57BL/6 mice. (**A**) C57BL/6 mice underwent electrolytic-induced thrombotic occlusion of the left (ipsilateral) carotid artery (thrombotic occlusion) or complete ligation of the carotid artery with a silk suture (ligation), as described under “Methods”. Cerebral infarct volume was quantified 24-hours post thrombotic occlusion using TTC staining, as described in the Supplemental Data section (*Assessment of cerebral infarction)*. Infarct volume (mean ± SEM) represents the summation of lesion volume for the whole brain, with quantification performed using ImageJ, where each symbol represents an individual mouse. The inset depicts an example of infarcted (white) tissue indicated by arrowhead. (**B**) In a separate cohort of mice, laser doppler flowmetry (LDF) was employed over the ipsilateral MCA territory to assess baseline cerebral perfusion (a), as well as perfusion during injury (b) and perfusion changes immediately following electrolytic injury (c) and thrombotic occlusion (d). Each trace (mouse 1-6) represents an individual mouse. Open symbols/solid lines indicate mice with no infarct. Closed symbols/broken lines indicate mice with infarct development. (**C-E**) C57BL/6 mice underwent the tBCCO procedure (as described under *Supplementary Methods* and summarised in *Supplementary Figure I),* with the period of clamp duration varied between 20, 25 and 30 minutes, and animals recovered to 24 hours to assess cerebral infarction. Cerebral infarct was assessed with TTC-staining of excised brain sections. (**C**) The infarct area of individual mice subjected to 30 minutes tBCCO (quantified in D) was demarcated and overlaid onto a single image of the relevant brain section, with layers

colour-coded to reflect the number (n) of animals presenting with cerebral infarction within the denoted region (as indicated by the colour Key) (*using The Mouse Brain in Stereotaxic Coordinates*[67]). [Total number of animals assessed n=9]. (**D**) Dot plot depicting infarct volumes quantified for each mouse undergoing tBCCO for either 20, 25 and 30 minutes (left y-axis; mm^3), with mortality rate (N) for each cohort depicted in red (right y-axis, % cohort). *Inset* - Representative image of cerebral infarct on posterior brain slices at 24 hours following tBCCO for 30 min. (**E**) Average cerebral perfusion of the ipsilateral (left; i) and contralateral (right; ii) hemispheres over duration of the stroke induction period in mice undergoing 25-minute tBCCO, where average cerebral perfusion is presented as a percentage (%) of baseline. Temporal perfusion for mice demonstrating stroke (closed circles) or no stroke (open circles) outcomes, as well as perfusion experienced by mortalities (☠) and sham (closed squares) mice are indicated. Where two values exist for a particular timepoint (Injury or BCCO-stop), they represent perfusion values immediately prior to and immediately following the indicated timepoint. The threshold for ischemia induction was denoted as 25% of baseline flow. Results represent the mean ± SEM.

Supplementary Figure IV: Carotid artery recanalisation rates in mice undergoing iCAT, recovered to 24 hours (Figure 4C).

Figure 4A depicts carotid artery flow data results from multiple cohorts undergoing electrolytic injury (see legend - Figure 4). A subset of animals (one cohort) was recovered to 24-hours for assessment of stroke outcomes (control n=8, rtPA n=8, or rtPA-Argatroban n=14), with cerebral perfusion of this cohort presented in Figure 4C. The carotid artery blood flow and recanalisation rates of this specific cohort are depicted here.

Supplementary Figure V: Early recanalization restores cerebral perfusion and protects against stroke development. C57BL/6 mice were subjected to sham, the iCAT stroke model (60 minutes ischemia; $iCAT^{[occlusion]}$) or mechanical recanalization at 60 minutes ($iCAT^{[clamp-recan]}$). Animals were recovered to 24-hours for assessment of stroke outcomes. (**A**) In $iCAT^{[clamp-recan]}$ experiments, carotid artery occlusion was induced with the application of vascular clamp (1). Stroke was induced with stenosis of the right carotid artery (2) to reduce left hemispheric cerebral blood flow (L CBF) to below 25% of baseline, whilst maintaining right cerebral blood flow (R CBF) above 25% of baseline perfusion. Following 60-minutes of occlusion, the vascular clamp was removed from the left carotid artery and stenosis removed from the right carotid artery (3). (**B**) Cerebral perfusion (LSCI) was assessed at 90 minutes

post-stroke onset in sham (n=4), iCAT$^{[occlusion]}$(n=8) or iCAT$^{[clamp-recan]}$ (n=6). Animals that died prior to the 90-minute timepoint were not included in cerebral perfusion assessment. Quantification of ipsilateral cerebral perfusion at 90 minutes post-stroke onset is presented in a bar graph, with all data points shown for clarity. (**C**) Cerebral infarct was assessed with TTC-staining of excised brain sections from studies (**B**). Animals that died prior to the 24-hour point were not included in the infarct assessment. (**D**) At 24-hour post recovery, functional evaluation of animals conducted with open-field assessment using MouseMove. The histogram depicts quantification of travel distance (m) over 15-minute assessment period. Statistical analysis was performed using an ordinary one-way ANOVA with Tukey's or Sidak's multiple comparisons test, where $^{****}p<0.0001$; $^{***}p<0.001$ and $^{ns}p>0.05$.

Supplementary Figure VI: rtPA-mediated thrombolysis leads to platelet-rich reocclusion. C57BL/6 male mice underwent the electrolytic procedure with concomitant real-time imaging of thrombolysis (A) and blood flow measurement (**B**) conducted with the mouse carotid artery transilluminator and doppler flow probe, respectively (as described under 'Methods"). In (**A**) following thrombotic occlusion, the transilluminator was placed directly underneath the left carotid artery to illuminate platelets (white) and erythrocytes (red). Thrombolytic therapy (rtPA) was delivered intravenously at 15 minutes after thrombotic occlusion and imaging conducted for 60 minutes after treatment onset. Images presented at 10-minute intervals for 60 minutes following treatment onset. Timepoints at 20 and 30 minutes represent recanalization events and platelet-remediated reocclusion, respectively. Treatment dosing regimens: rtPA - 10 mg/kg (1/9 mg/kg bolus/infusion over 30 minutes). (**B**) The flow trace depicts blood flow through the carotid artery following electrolytic injury, and administration of rtPA (as described under methods). Images/data are taken from one experiment representative of eight independent experiments.

Supplementary Video I: Transillumination of the mouse carotid artery to visualize thrombolysis with rtPA and argatroban therapy. C57BL/6 male mice underwent the electrolytic procedure. Following electrolytic injury, the mouse carotid artery transilluminator was placed directly underneath the left carotid artery to illuminate platelets (white) and erythrocytes (red). Thrombolytic therapy (rtPA/Argatroban) was delivered intravenously at 15 minutes after thrombotic occlusion and imaging conducted for 60 minutes after treatment onset. Video demonstrates lysis and embolization events of the platelet thrombus approximately 48 minutes following treatment administration. Treatment dosing regimens: *rtPA - 10 mg/kg (1/9*

mg/kg bolus/infusion over 30 minutes); argatroban - 80 ug/kg bolus; 40 ug/kg/min infusion over 60 minutes. A panel of still images of these events are presented in Figure V(B).

Development of a Mouse Carotid Artery Thrombolysis (iCAT) Model to Assess the Impact of Adjunctive Antithrombotic Therapies on Stroke

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Appendix: Standard operating procedure for *in situ* carotid artery thrombolysis (iCAT) model.

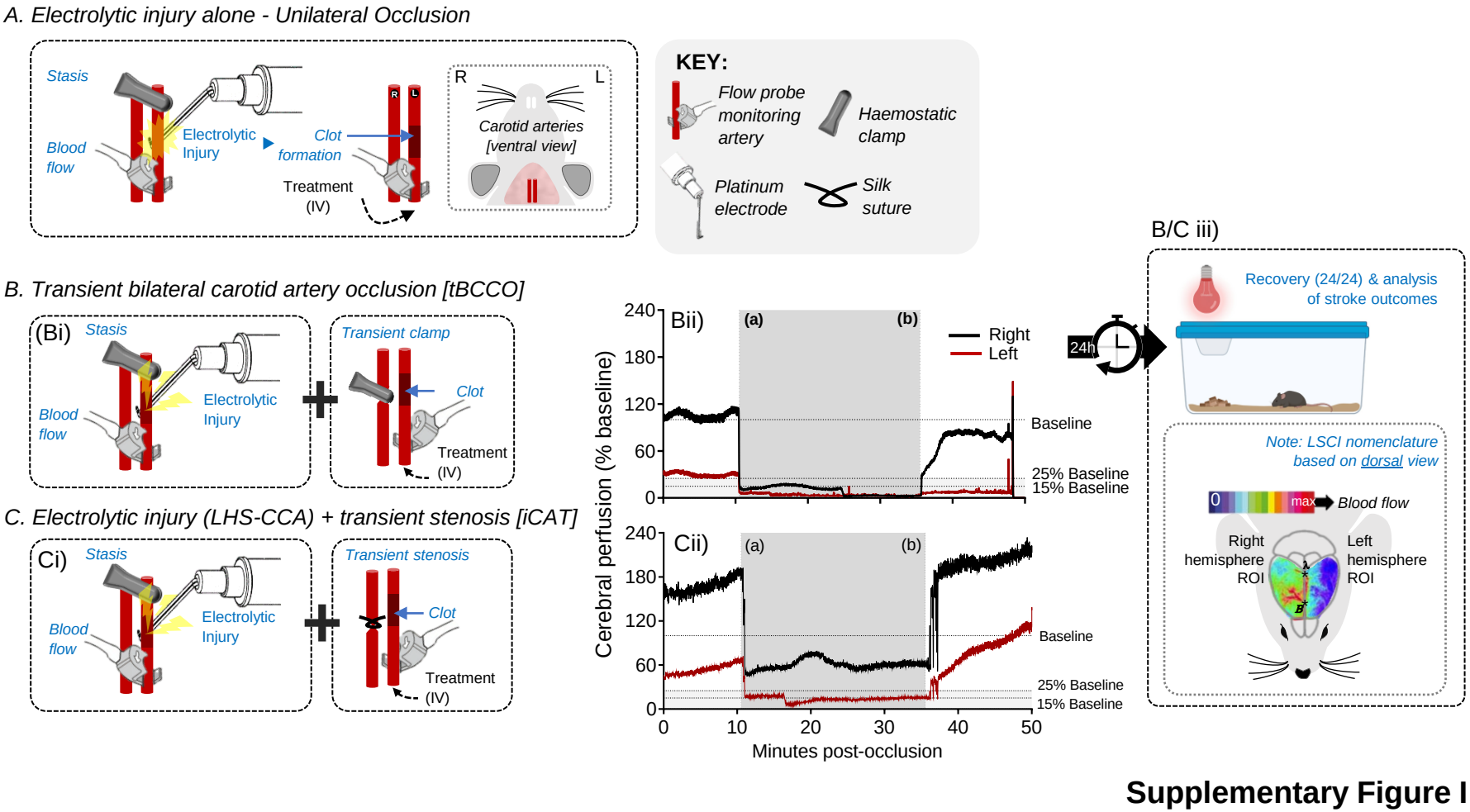
This SOP is designed to provide an overview of the procedural requirements of the iCAT stroke induction method. Refer to *Methods Section* in Maclean *et al*, (2022) for specific methodological details, such as equipment details.

1. Weigh and anaesthetize mouse with inhalation anesthetic and oxygen supplementation. Maintain anesthesia for the duration of the procedure, in line with the ethical requirements of the overseeing institution. *Refer to Methods Section: Animal and surgical preparation.*
2. Transfer mouse to a feedback temperature heat mat to ensure maintenance of physiological temperature for the duration of the procedure.
3. Position mouse in a stereotaxic headframe as per manufacturer's instructions, to provide stability of the head and neck region for the duration of the procedure.
4. Apply eye lubricant to prevent drying out of the eyes.
5. Clean the scalp with chlorhexidine, shave the scalp and administer local analgesic as per ethical requirements of the project.
6. Perform a 'T' shaped incision in the scalp, from between the eyes up along the sagittal suture to the lambdoid suture and make a perpendicular incision between the ears to expose the dorsal skull.
7. Retract the scalp to expose the lambdoid and coronial sutures, and the temporalis border. Clean fluid and hair from the skull. In accordance with manufacturer's instructions, obtain a 3-minute (30s intervals) baseline reading of cerebral perfusion with the laser speckle contrast imager. Imaging is performed through the intact dorsal skull. *Refer to Methods Section and Online Supplement: Cerebral Perfusion Monitoring.*

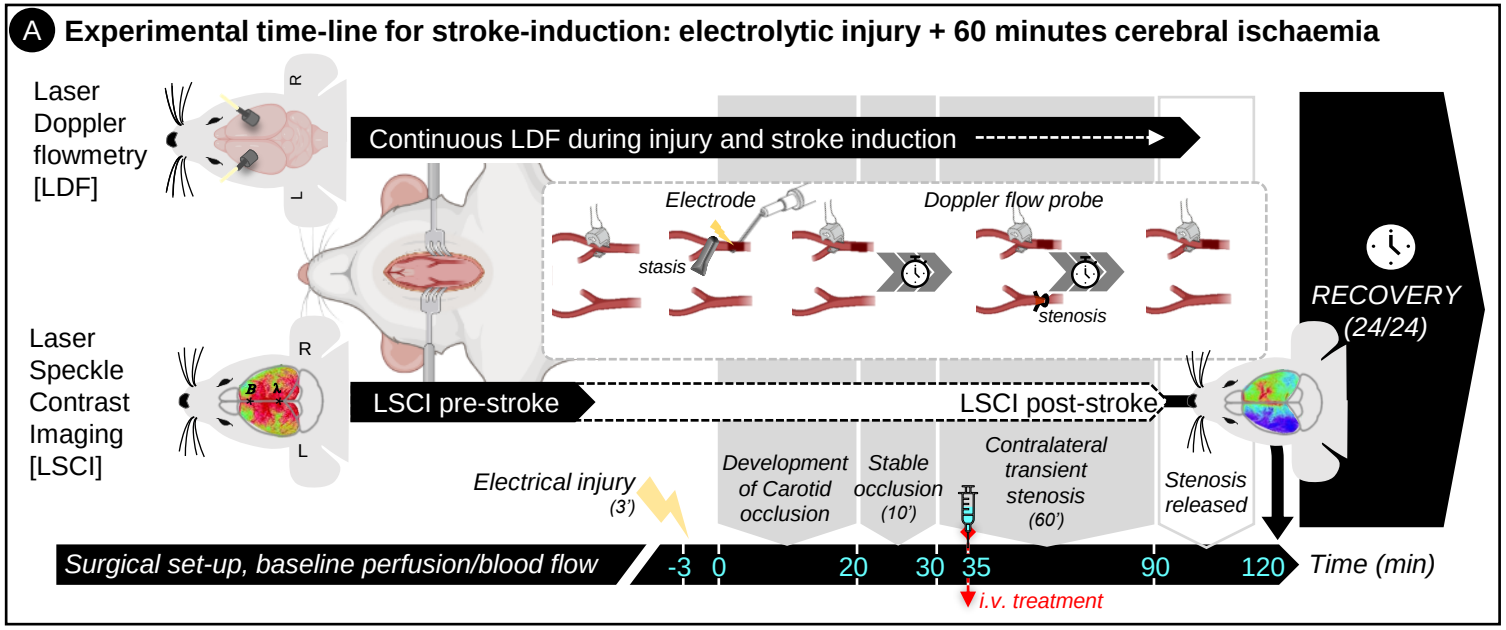
8. As per manufacturer's instructions, attach laser doppler flowmetry probes to the intact mouse skull, directly over the left and right MCA territories. Probes are positioned approximately 1mm posterior to Bregma and 1mm medial to the temporalis muscle border. *Refer to Methods Section and Online Supplement: Cerebral Perfusion Monitoring.*
9. Rotate the mouse to the supine position, ensuring the LDF probes remain securely attached. Clean the ventral neck with chlorhexidine, shave neck to allow surgical access to both carotids and right jugular. Administer local analgesia as per ethical requirements of the project.
10. Perform a medial incision to the ventral side of the neck to isolate the left and right common carotid arteries from the vagus nerve and surrounding connective tissue. Isolate the left common carotid artery. Isolate the right jugular vein to allow for intravenous treatment administration.
11. Attach an ultrasound Doppler flow probe to the left CCA to monitor and record blood flow through a Doppler Flowmeter and PowerLab data acquisition system. Wait 10 minutes to ensure blood flow and breathing has stabilized prior commencing the next step.
12. Isolate the right CCA from the vagus nerve and surrounding connective tissue. Place a 1cm 6-0 silk suture in a double overhand loop *loosely* around the right CCA. Do not tighten. *Refer to Methods Section: Thrombotic occlusion of the carotid artery & stroke induction.*
13. Ensure surgical site is irrigated with sterile saline for remainder of procedure.
14. Obtain a 5-minute baseline reading for rCBF using laser doppler flow probes and the corresponding carotid artery blood flow. *NB:* Ensure this baseline reading is stable, as this value is critical to inform stenosis application.
15. Remove flow probe from the left CCA and perform electrolytic injury to the left CCA. Record start and end times on LabChart. At the end of injury, remove the clamp and platinum electrode. Monitor flow for thrombus formation and subsequent occlusion. If required, insert catheter to right jugular vein for intravenous treatment delivery. *Refer to Methods Section: Thrombotic occlusion of the carotid artery & stroke induction.*
16. Following 10-minutes of stable occlusion of the left CCA, commence stenosis of the contralateral CCA by tightening the 6-0 silk suture loosely places in Step 12. Manually tighten the stenosis to reduce ipsilateral (left) rCBF to <25% of baseline, whilst maintaining contralateral perfusion >25% of baseline for the duration of ischemia (60-

minutes). *Refer to Methods Section: Thrombotic occlusion of the carotid artery: in situ carotid artery thrombolysis.*

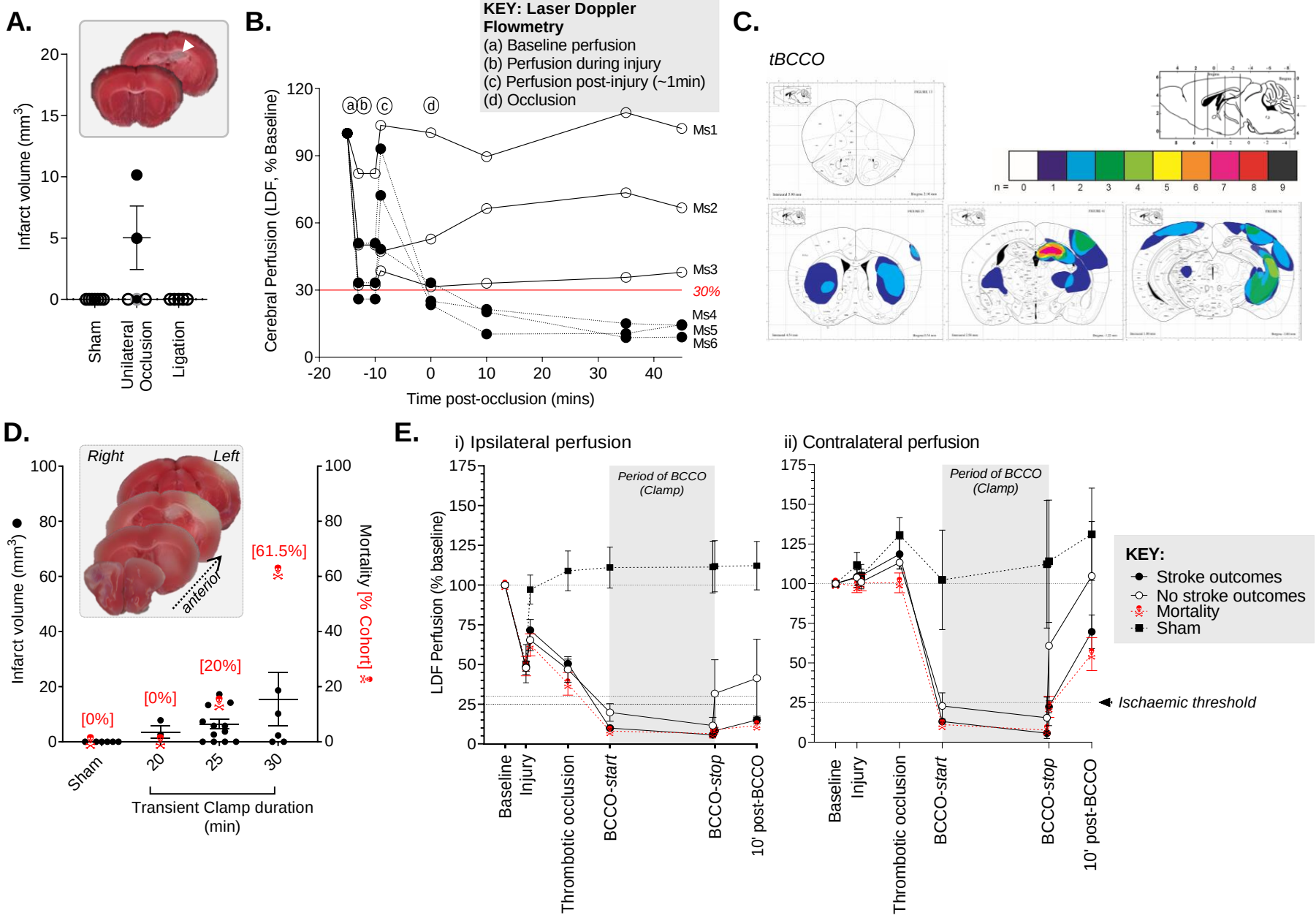
17. Monitor and record rCBF and carotid artery blood flow for the duration of ischemia.
18. At the completion of ischemia induction, completely remove the 6-0 stenosis to restore maximal blood flow through the right CCA. Continue monitoring carotid artery blood and rCBF to 10-minutes following stenosis removal or 1-hour post treatment onset (whichever is latest).
19. Remove flow probe from left CCA. Remove catheter/s and ligate the jugular vein to prevent bleeding from the insertion site. Administer local anesthetic and suture the neck incision.
20. Rotate mouse to prone position and remove the LDF probes. In accordance with manufacturer's instructions, obtain a 3-minute (30s intervals) LSCI reading of cerebral perfusion with the laser speckle contrast imager. Imaging is performed through the intact dorsal skull. The final LSCI image is obtained at 90-minutes post-stenosis application.
21. Administer local analgesic and suture scalp incision site.
22. Turn off anesthetic and supplement mouse with oxygen for 5-10 minutes prior to transfer to recovery cage.
23. Transfer mouse to recovery cage under infra-red heat lamp for one hour, monitoring mouse closely in accordance with ethics approval. At 1-hour post recovery, transfer mouse a warmed recovery unit, and monitor as per the ethical requirements of the overseeing institution. *Refer to Methods section and Online Supplement: Post-operative recovery.*



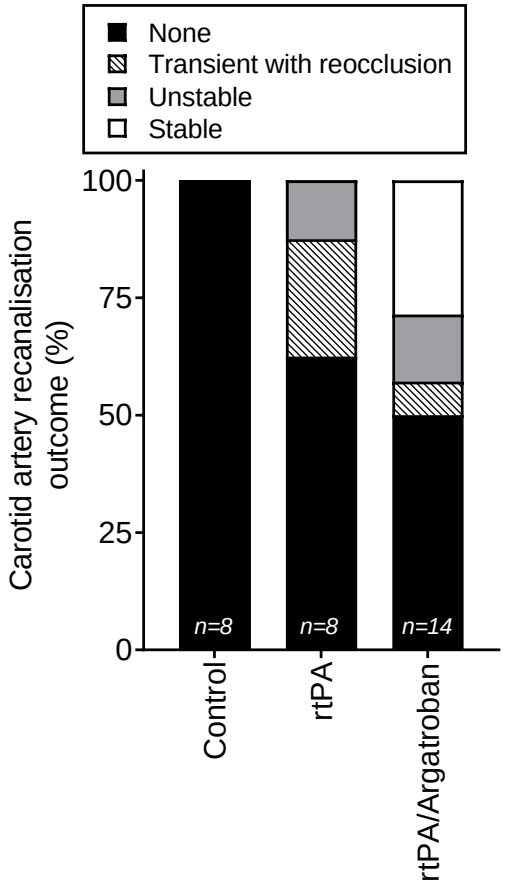
Supplementary Figure I



Supplementary Figure II

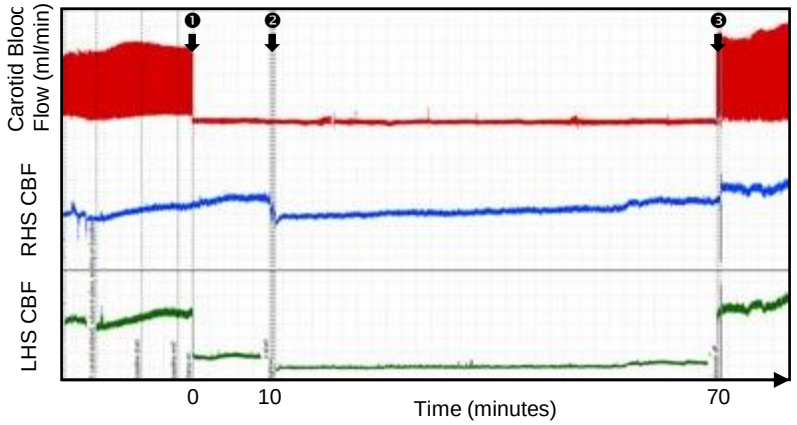


Supplementary Figure III



Supplementary Figure IV

A.



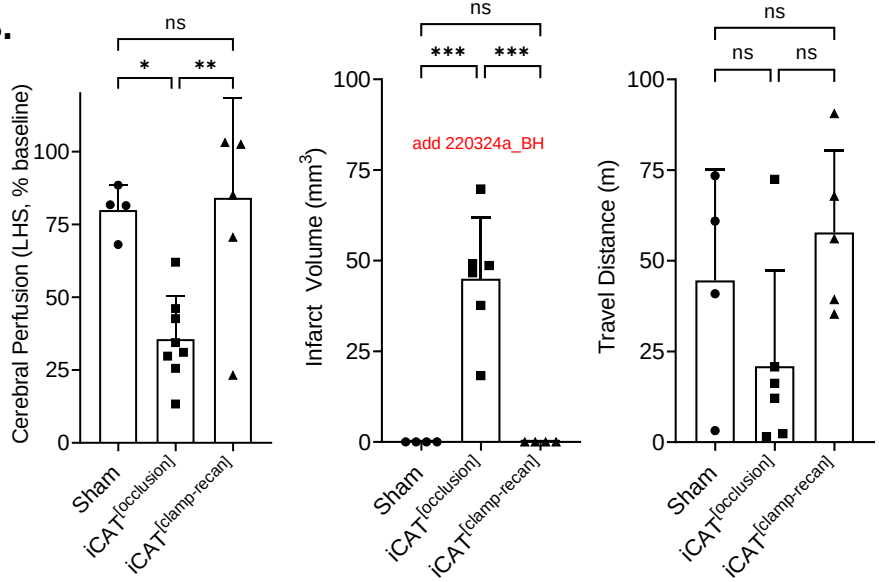
KEY:

❶ Clamp on:
100% carotid occlusion

❷ Stenosis on:
LHS<25% baseline
RHS>25% baseline

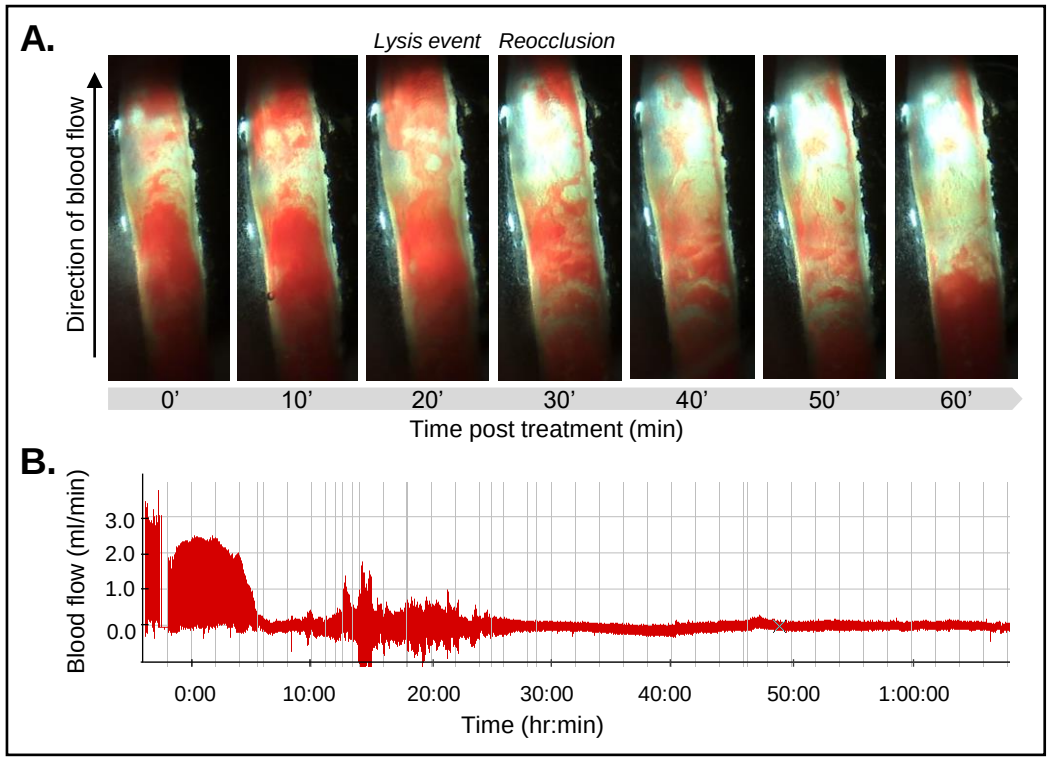
❸ Clamp/stenosis off:
100% reperfusion

B.



Supplementary Figure V

Recanalisation with rtPA alone



Supplementary Figure VI