SUPPLEMENTARY MATERIAL

MATERIALS AND METHODS

Rat model of cerebral ischemia
All surgical procedures were performed under sevoflurane anesthesia (6% induction and 4% maintenance in a mixture of 70% NO₂ and 30% O₂). Rectal temperature was maintained at 37 ± 0.5°C in all animals during surgery using a thermostat-controlled electric pad (Neos Biotec, Pamplona, Spain). The animals’ heads were placed on a porexpan plate in order to avoid direct contact between pad and head. Transient focal ischemia (45 min) was induced by intraluminal MCA occlusion as previously described (Fernandez-Susavila et al., 2017) using commercially available sutures with silicone-rubber-coated heads (350 μm in diameter and 1.5 mm long; Doccol, Sharon, MA, USA). Cerebral blood flow was monitored with a Periflux 5000 laser Doppler perfusion monitor (Perimed AB, Järfälla, Sweden) by placing the Doppler probe (model 411; Perimed AB) under the temporal muscle at the parietal bone surface, near the sagittal crest. Once artery occlusion had been achieved, as indicated by Doppler signal reduction, each animal was carefully moved from the surgical bench to the MR system for baseline ischemic lesion assessment using ADC maps. MRA was also performed to ensure that the artery remained occluded throughout the MR procedure and to detect arterial malformations. After basal MR analysis, animals were returned to the surgical bench and the Doppler probe was repositioned. Reperfusion was performed 45 minutes after occlusion onset.

The following exclusion criteria were used: (1) <70% reduction in relative cerebral blood flow; (2) arterial malformations, as determined by MRA; (3) baseline lesion volume <25% or >45% of the ipsilateral hemisphere, as measured using ADC maps; (4) absence of reperfusion or prolonged reperfusion (>10 minutes until achieving ≥50% of baseline cerebral blood flow) after filament removal; and (5) failure to complete treatment. All excluded or deceased animals were replaced until the total number of animals indicated for each group was attained.

Sacrifice and tissue preservation for qPCR and WB
To ensure correct preservation of nucleic acids and proteins, and similar anesthetic dosages before sample collection, all animals undergoing RNA or protein extraction were sacrificed following the same methodology. Briefly, anesthesia was induced with 6% sevoflurane in a gas mixture of 70% NO₂ and 30% O₂ for approximately 1 minute, enough to render the animals unconscious and block voluntary motor response. The animals were then sacrificed by decapitation with a guillotine (Fine Science Tools, USA). Rat heads were rapidly positioned on an ice plate, and brains were dissected and positioned in sterile Petri plates for section in three transversal slices, in which the ventral region and lateral cortex were removed (Supplementary Fig. 1). Finally, in each slice, brain hemispheres were separated by cutting through the interhemispheric line. All brain pieces (approx. 100 mg/each) were inserted into sterile RNase-free cryovials and homogenized in liquid N₂. Frozen samples were stored at -80°C for subsequent analysis by qPCR or WB.

Functional analysis of ischemic animals
A researcher blinded to the experimental groups measured functional deficit using the grip test for muscle strength and rotarod test for motor coordination and balance, at baseline 2 days after surgery (28 days after viral infection) and at 48 hours after ischemia induction.
MRI assessment and data analysis
All MR studies were conducted using a 9.4 T MR system (Bruker BioSpin, Billerica, MA, USA) with 440 mT/m gradients and a combination of a linear birdcage resonator (7 cm in diameter) for signal transmission and a 2 × 2 surface coil array for signal detection. An initial MRI study was performed during the occlusion period to measure baseline lesion volumes. Time-of-flight MRA was first performed to confirm that the MCA remained occluded after moving the animal from the surgery bench. MRA images were acquired using a three-dimensional fast low-angle shot (3D-FLASH) sequence with TE = 2.5 ms, TR = 21 ms, FA = 20º, and NA = 1. One slice was recorded (12 mm thick) with FOV = 19.2 × 19.2 mm² and a resolution of 0.120 mm/pixel (160 × 160 × 100-point matrix). To measure lesion volumes, ADC maps were obtained 8 minutes after occlusion but before removing the occluding filament. The area of ischemic damage was determined by counting pixels with ADC values below a threshold in the ipsilateral brain hemisphere. ADC values in healthy rat brain normally do not fall below 0.55 × 10⁻³ mm²/s; therefore, this threshold provides a convenient means of segmenting abnormal tissue. Diffusion-weighted images (DWI) used to calculate ADC maps were acquired using diffusion-tensor imaging with a spin-echo echo-planar imaging sequence (DTI-EPI) with the following parameters: FOV = 24 × 16 mm², matrix = 96 × 64 points (in-plane resolution = 0.25 mm/pixel), 14 consecutive slices (1 mm thick), TE = 27 ms, TR = 4 s, NA = 4, and diffusion b values of 300, 600, 900, 1200, 1600 and 2000 s/mm² applied in the z direction. To analyse lesion volume evolution, a multi-slice multi-spin-echo (MSME) sequence was performed with the following acquisition parameters: TE = 9 ms, TR = 3 s, 16 echos, NA = 2, 14 consecutive slices (1 mm thick), FOV = 19.2 × 19.2 mm² and an in-plane resolution of 0.1 mm/pixel (192 × 192-point matrix). These data were also used to calculate T2 relaxation maps. All images were processed using ImageJ (Rasband WS, National Institutes of Health, Bethesda, MD, USA) on an independent workstation. A treatment-blinded researcher determined infarct volumes from quantitative ADC maps and T2-weighted images averaged between the 4th to 8th echoes. Edema formation was measured using midline deviation (MD) calculated as the ratio between ipsilateral and contralateral hemispheric volumes; lesion volumes were then calculated in mm³/MD. Lesion volume as a percentage of the ipsilateral hemispheric volume was calculated as (lesion volume [mm³/MD]/ipsilateral hemispheric volume [mm³]) × 100. Images were evaluated by a treatment-blinded researcher.

RBM3 mRNA quantification by qPCR
RBM3 mRNA expression was evaluated by quantitative polymerase chain reaction (qPCR) assay. Homogenized sections (around 100 mg) stored at -80ºC were directly submerged in lysis buffer avoiding previous thawing and extracted using a commercial column-based method (PureLinkTM RNA Mini Kit, Invitrogen, USA). Final elution was in 50 μL RNase-free water. RNA quantity and quality were determined by microvolume spectrophotometry (NanoDrop, Thermo Fisher Scientific, USA). To evaluate the purity of RNA extract, the ratios 260:280 and 260:230 were studied, and only samples with ratios over 2 and 1.7 were included in the study. A total amount of 1 μg of RNA was used for retrotranscription into cDNA using the GoScript™ Reverse Transcription System (Promega, USA) and a thermocycler (Biometra, Germany). Briefly, the volume corresponding to 1 μg RNA was diluted in RNase-free water to a total final volume of 11 μL and 1 μL of random primers (0.5 μg in total) was added. This mix was incubated for 5 minutes at 70ºC allowing unwinding of secondary RNA structures before rapid cooling in ice for 10 minutes. Reverse transcription mix was composed of 4 μL reaction buffer (5X), 2 μL of MgCl₂ (2.5 mM in the final volume), 1
μL dNTPs (0.5 mM of each nucleotide in the final volume), and 1 μL GoScript™ reverse transcriptase. The final reaction mix, totaling 20 μL, was incubated for 5 minutes at 25°C to allow annealing, and 60 minutes at 42°C to allow extension of the cDNA strand. After reverse transcription, the product was diluted in 1:5 in RNase-free water. Finally, RBM3 expression and reference genes β-actin and RPL13 were studied by qPCR using the GoTaq qPCR masterMix (Promega, USA) and Mx3005P qPCR system (Agilent Technologies, USA). Briefly, a total volume of 2 μL diluted cDNA was mixed with 3.5 μL nuclease-free water, 0.5 μL of 10 mM specific primers (0.25 μL for each sense), and 6 μL GoTaqq® qPCR Master Mix (2X), to a total final reaction volume of 12 μL. Reactions were performed in triplicate in 96-well plates. Table 1 shows the primer sequences and Table 2 the qPCR thermal cycling protocol. Quantitative PCR results were analyzed using MxPro Mx3005P v4.10. The average of three Ct values obtained for RBM3 in each sample was relativized to the average of three Ct values obtained for β-actin or Rpl13 expression in the three replicates of each sample. Relative expression was then averaged across all samples in each group, and control samples were used as a baseline to normalize expression in the various groups.

**RBM3 quantification by western blot**

RBM3 expression was quantified by western blot (WB). Cold buffer lysis was added to brain slices in an approximate 10:1 (volume:weight) proportion and the tissue disrupted using a TissueLyser II (Qiagen, Switzerland); lysates were then centrifuged for 30 minutes at 20,000 g and the supernatants collected for WB. Lysate protein content was quantified using the Bradford method (BioRad, Germany). The requisite volume of cell lysate containing a total 30 μg protein was subjected to SDS-PAGE in 12% Criterion™ TGX™ Precast Midi Protein Gel (BioRad, USA) using a fixed voltage of 180V. Proteins were then electrotransferred to a PVDF membrane (Millipore, Ireland) using a Trans-Blot semi-dry system (Bio-Rad, USA) with a limited voltage of 25V and 180 milliamps for 2 hours.

Post-blot membranes were blocked for 45 minutes with 3% bovine serum albumin (BSA) in tris-chloride buffer containing 0.1% Tween 20 (TBST). Once blocked, target proteins were detected by incubation with primary antibodies against RBM3 (rabbit monoclonal anti-RBM3 ab134946, Abcam, UK) and β-actin (rabbit polyclonal anti-β-actin, Abcam, UK) diluted 1:1000 and 1:2000 respectively in TBST with 3% BSA. Primary antibodies were incubated overnight at room temperature with agitation, then the membranes were washed three times in PBST to remove excess primary antibody. Secondary antibody was HRP-conjugated goat anti-rabbit IgG (Dako, Denmark) diluted to 1:5000 in TBST with 3% BSA and incubated for 1 hour at room temperature with agitation. Finally, after washing in TBST, HRP activity was revealed with Pierce™ _ECL Western Blotting Substrate (Thermo Fisher, USA) and detected manually with X-ray film. WB results were analyzed using ImageJ (Rasband WS, USA) by measuring the mean gray value of protein bands delimited in ROIs. Relative expression of RBM3 to β-actin was calculated for each sample, and each group sample average was normalized to the control.

**RBM3 levels in ischemic stroke patients**

RBM3 levels were analyzed in two cohorts of IS patients: one from the Clinical Hospital of Santiago de Compostela (‘Santiago’), the other from Dr. Josep Trueta Clinical Hospital in Girona (‘Girona’), after approval by the respective ethics committees and in accordance with the STROBE Statement. Recruitment ran from January to December 2017. We began by studying 20 patients with acute IS to determine sample size and RBM3 sampling times: levels were analyzed on admission.
(baseline) and 2, 24 and 72 hours after admission for IS patients. In view of these preliminary results (Supplementary Fig. 2 and 3), we selected RBM3 levels on admission and at 24 hours after stroke onset for the whole sample. Sample size was calculated using EPIDAT statistical software based on the difference of RBM3 values at 24 hours in relation to outcome at 3 months (Supplementary Fig. 4). Minimum sample size (n=198) was calculated on the basis of 5% alpha and 80% power. Two independent cohorts totaling 301 patients were included in our study, 172 from Santiago and 129 from Girona, of whom 215 were finally eligible (113 and 102, respectively) according to the inclusion and exclusion criteria (Tables 3 and 4). Stroke Unit patients in both university hospitals were treated in accordance with the guidelines of the Spanish Society of Neurology’s Cerebrovascular Diseases Study Group. The primary clinical endpoint was hyperthermia, defined as an axillary temperature ≥37.5°C measured by the Stroke Unit nursing staff. An axillary temperature <37.5°C was defined as normothermia. The highest temperature measured in the first 24 hours was selected as the variable. The second clinical endpoint was 3-month outcome, ‘good’ being defined as a modified Rankin Scale (mRS) score <3. Stroke severity was assessed using the National Institute of Health Stroke Scale (NIHSS) score at admission, 24, 48 and 72 hours. Both scores were evaluated by internationally accredited neurologists.

**RBM3 analysis in human blood samples**

Blood samples were collected in test tubes, centrifuged at 3,000 g for 15 minutes, frozen immediately, and stored at -80°C. RBM3 levels were measured using commercial ELISA kits following manufacturer instructions (Biotez RBM3 ELISA). RBM3 was analyzed over the range 31.25 pg/mL to 2000 pg/mL, with a sensitivity of 10 pg/mL. Intra- and inter-assay coefficients of variation were 1.9% and 2.7%, respectively. We defined percentage variation in RBM3 levels in the first 24 hours as

\[
\Delta RBM3 = \frac{(RBM3 \text{ at } 24h - RBM3 \text{ on admission})}{RBM3 \text{ at } 24h} \times 100
\]
**SUPPLEMENTARY TABLES**

| Primer | Forward               | Reverse               |
|--------|-----------------------|-----------------------|
| Rbm3   | CTTCAGCAGCTTCGGGCCTA  | CCCATCCAGGGACTCTCCAT  |
| β-actin| GCTATGAGCTGCTGACGTTA  | GTTTGATGGATGCCACAGGA  |
| Rpl13  | CGGAGGCGAGGTTCTAGTA   | GTACAACCACCACCTTTCGG  |

**Supplementary Table 1. Primer sequences used for qPCR**
**Supplementary Table 2. Thermal protocol used for qPCR**

| Step                        | Temp/Time     |
|-----------------------------|---------------|
| Hot-start enzyme activation | 95°C for 2 min|
| Denaturation                | 95°C for 15 sec|
| 40 cycles                   |               |
| Denaturation                | 95°C for 1 min|
| Annealing/extension         | 60°C for 40 sec|
| Denaturation                | 55°C for 30 sec|
| Dissociation curve          | Gradual increase from 55°C to 95°C |
| Inclusion criteria                                      | Exclusion criteria                                      |
|--------------------------------------------------------|---------------------------------------------------------|
| • Ischemic stroke < 6 hours post-onset (all Girona patients had thrombolysis) | • Wake-up stroke                                         |
| • Confirmation by neuroimaging                          | • Loss to follow-up ≥ 3 months                          |
| • Previous mRS score ≤ 1                                | • No blood sample at admission and/or at 24 hours       |
| • Stroke Unit neurologist care since inclusion          | • Systemic hypothermia treatment                        |
| • Signed informed consent                               | • Comorbidity with life expectancy < 6 months           |

**Supplementary Table 3.** Inclusion and exclusion criteria.
|                      | TOTAL SAMPLE | GIRONA | SANTIAGO |
|----------------------|--------------|--------|---------|
| **Patients included**| 301          | 129    | 172     |
| **Patients excluded**|              |        |         |
| Wake-up stroke       | 7            | 0      | 7       |
| Loss to follow-up    |              |        |         |
| ≥ 3 months           | 35           | 12     | 23      |
| No blood sample      |              |        |         |
| admission and/or at  | 40           | 15     | 25      |
| 24 hours             |              |        |         |
| Systemic hypothermia | 0            | 0      | 0       |
| treatment            |              |        |         |
| Comorbidity with life| 4            | 0      | 4       |
| expectancy < 6 months|              |        |         |
| **Eligible patients**| 215          | 102    | 113     |

Supplementary Table 4. Number of patients included and excluded per center (Girona and Santiago).
### Supplementary Table 5. RBM3 mRNA expression studied by qPCR.

RBM3 expression was relativized to β-actin, then normalized to control expression, defined as 100%. Data are shown as mean ± SEM (n=3 per group). SC, systemic control; SH, systemic hypothermia; FC, focal control; FH, focal brain hypothermia; I, ischemic animal.

|                      | Ipsilateral | Contralateral |
|----------------------|-------------|---------------|
| Control              | 104.2 ± 4.2 |               |
| SC–4h                | 239.6 ± 9.1 |               |
| SH–4h                | 333.3 ± 9.0 |               |
| I + SC–4h            | 152.9 ± 15.9| 164.0 ± 17.0  |
| I + SH–4h            | 192.9 ± 6.1 | 226.3 ± 2.2  |
| FC–24h               | 62.5 ± 6.3  | 66.7 ± 10.4  |
| FH–24h               | 387.5 ± 69.6| 100.0 ± 14.4 |
| I + FC–24h           | 108.3 ± 5.5 | 75.0 ± 9.5   |
| I + FH–24h           | 237.5 ± 15.7| 120.8 ± 34.7 |
| Systemic treatment | SC–4h  | SH–4h  | I + SC – 4h | I + SH – 4h | FC – 24h | FH – 24h | I + FC – 24h | I + FH – 24h |
|---------------------|--------|--------|-------------|-------------|----------|----------|-------------|-------------|
|                     | 87.5 ± 18.0 | 162.5 ± 34.3 | 143.1 ± 41.28 | 368.3 ± 40.1 | 151.6 ± 32.5 | 418.2 ± 79.4 | 105.0 ± 24.6 | 245.1 ± 39.6 |
| Control             | 100.5 ± 21.4 |                  | 204.8 ± 10.9 | 561.3 ± 187.5 |          |          | 134.3 ± 40.5 |          |
| Ipsilateral         |         |          |             |              |          |          |             |              |
| Contra lateral      |         |          |             |              |          |          |             |              |

**Supplementary Table 6.** RBM3 protein expression studied by WB. RBM3 expression was relativized to β-actin, then normalized to control expression, defined as 100%. Data are shown as mean ± SEM (n=3 per group). SC, systemic control; SH, systemic hypothermia; FC, focal control; FH, focal brain hypothermia; I, ischemic animal.
| Sample Description                        | Total sample n = 215 | Girona n = 102 | Santiago n = 113 | P *  
|-----------------------------------------|----------------------|----------------|------------------|-------
| Age, years                              | 75.9 ± 10.2          | 76.6 ± 12.4    | 75.2 ± 7.8       | 0.786 |
| Women, %                                | 45.1                 | 44.1           | 46.0             | 0.681 |
| History of hypertension, %              | 63.3                 | 65.7           | 61.1             | 0.576 |
| History of diabetes, %                  | 25.6                 | 23.5           | 27.4             | 0.535 |
| Smoking, %                              | 14.9                 | 17.6           | 12.4             | 0.339 |
| Dyslipidemia, %                         | 33.5                 | 38.2           | 29.2             | 0.193 |
| Atrial fibrillation, %                  | 30.2                 | 22.5           | 37.2             | 0.025 |
| Ischemic heart disease, %               | 7.4                  | 7.8            | 7.1              | 0.517 |
| Previous stroke, %                      | 10.7                 | 12.7           | 8.8              | 0.384 |
| Peripheral arterial disease, %          | 5.6                  | 4.9            | 6.2              | 0.772 |
| Systolic blood pressure, mmHg           | 151.3 ± 28.8         | 153.9 ± 28.2   | 149.1 ± 29.3     | 0.221 |
| Diastolic blood pressure, mmHg          | 80.6 ± 17.6          | 81.2 ± 18.0    | 80.1 ± 17.3      | 0.624 |
| Glycemia, mg/dL                         | 147.3 ± 62.4         | 138.6 ± 57.5   | 155.1 ± 65.7     | 0.053 |
| Leukocytes, x 10⁹/mL                    | 9.2 ± 3.1            | 9.0 ± 3.1      | 9.4 ± 3.1        | 0.373 |
| Fibrinogen, mg/dL                       | 491.0 ± 152.6        | 502.6 ± 156.9  | 480.6 ± 148.5    | 0.292 |
| Platelets, x 10⁹/mL                     | 222.1 ± 71.3         | 222.8 ± 72.1   | 221.6 ± 70.9     | 0.899 |
| Axillary temperature on admission, °C   | 36.4 ± 0.6           | 36.4 ± 0.4     | 36.3 ± 0.7       | 0.162 |
| Maximum temperature in first 24 hours, °C | 37.3 ± 0.7           | 37.3 ± 0.6     | 37.2 ± 0.7       | 0.338 |
| Temperature in first 24 hours ≥37.5°C, % | 36.7                 | 33.3           | 39.8             | 0.395 |
| Systemic fibrinolysis, %                | 74.0                 | 100            | 50.4             | <0.0001 |
| Endovascular treatment, %               | 10.7                 | 1.0            | 19.5             | <0.0001 |
| TOAST                                    |                      |                |                  | <0.0001 |
| - Atherothrombotic, %                   | 20.0                 | 14.7           | 24.8             |       |
| - Cardioembolic, %                      | 45.6                 | 52.0           | 39.8             |       |
| - Lacunar, %                            | 9.3                  | 0              | 17.7             |       |
| - Indeterminate, %                      | 25.1                 | 33.4           | 17.7             |       |
| Previous Rankin                         | 0 [0, 1]             | 0 [0, 1]       | 0 [0, 0]         | 0.008 |
| NIHSS on admission                      | 16 [9, 20]           | 16 [10, 20]    | 14 [8, 17]       | 0.139 |
| NIHSS at 24 hours                       | 8 [2, 25]            | 11 [2, 21]     | 7 [2, 20]        | 0.163 |
| NIHSS at 48 hours                       | 6 [2, 17]            | 9 [2, 20]      | 3 [0, 13]        | <0.0001 |
| NIHSS at discharge                      | 4 [0, 13]            | 6 [1, 17]      | 2 [0, 10]        | 0.006 |
| Rankin at 3 months                      | 2 [0, 5]             | 3 [1, 5]       | 1 [0, 2]         | <0.0001 |
| Good outcome at 3 months, %             | 57.2                 | 47.1           | 66.4             | 0.006 |
| RBM3 on admission, pg/mL                | 288.1 ± 231.7        | 292.5 ± 231.7  | 292.5 ± 231.7    | 0.722 |
| RBM3 at 24 hours, pg/mL                 | 471.1 ± 523.5        | 523.5 ± 523.5  | 523.5 ± 523.5    | 0.050 |

**Supplementary Table 7. Sample description.** *P* values between hospital samples.
|                      | RBM3 on admission | RBM3 at 24 hours |
|----------------------|-------------------|-----------------|
| **GIRONA**           |                   |                 |
| Temperature on admission | 0.180, $P=0.070$ |                 |
| Temperature at 24 hours | -                 | -0.541, $P<0.0001$ |
| **SANTIAGO**         |                   |                 |
| Temperature on admission | -0.339, $P<0.0001$ |                 |
| Temperature at 24 hours | -                 | -0.692, $P<0.0001$ |

**Supplementary Table 8. Correlation (Spearman coefficient)** between RBM3 levels and temperature on admission and after 24 hours in the two study centers.
### Supplementary Table 9. Clinical and biochemical characteristics in normothermic vs hyperthermic patients.

|                          | < 37.5°C | ≥37.5°C | \(P\)  |
|--------------------------|----------|---------|--------|
| Age, years               | 76.4 ± 10.6 | 74.9 ± 9.7 | 0.301 |
| Women, %                 | 48.5     | 41.8    | 0.495 |
| History of hypertension, % | 64.7   | 60.8    | 0.562 |
| History of diabetes, %   | 25.7     | 25.3    | 0.940 |
| Smoking, %               | 11.0     | 21.5    | 0.057 |
| Dyslipidemia, %          | 31.6     | 36.7    | 0.457 |
| Atrial fibrillation, %   | 36.8     | 39.2    | 0.771 |
| Ischemic heart disease, %| 5.9      | 10.1    | 0.287 |
| Previous stroke, %       | 10.3     | 11.4    | 0.822 |
| Peripheral arterial disease, % | 6.6 | 3.8 | 0.542 |
| Systolic blood pressure, mmHg | 155.4 ± 29.6 | 144.4 ± 26.3 | 0.057 |
| Diastolic blood pressure, mmHg | 82.3 ± 18.0 | 77.8 ± 16.7 | 0.072 |
| Glycemia, mg/dL          | 136.5 ± 48.0 | 165.8 ± 78.3 | 0.001 |
| Leukocytes, x 10^3/mL    | 8.6 ± 2.7 | 10.4 ± 3.4 | <0.0001 |
| Fibrinogen, mg/dL        | 461.7 ± 122.2 | 541.4 ± 184.3 | <0.0001 |
| Platelets, x 10^3/mL     | 215.9 ± 60.2 | 233.0 ± 86.5 | 0.090 |
| Axillary temperature at admission, ºC | 36.2 ± 0.5 | 36.6 ± 0.7 | <0.0001 |
| Systemic fibrinolysis, % | 81.6     | 60.8    | 0.001 |
| Endovascular treatment, %| 7.4      | 16.5    | 0.042 |
| TOAST                    |          |         | 0.056 |
| - Atherothrombotic, %    | 15.4     | 27.8    |        |
| - Cardioembolic, %       | 44.9     | 46.8    |        |
| - Lacunar, %             | 13.2     | 2.5     |        |
| - Indeterminate, %       | 26.5     | 22.8    |        |
| Previous Rankin          | 0 [0, 0] | 0 [0, 1] | 0.004 |
| NIHSS at admission       | 12 [8, 18] | 17 [10, 20] | <0.0001 |
| NIHSS at 24 hours        | 4 [1, 12] | 16 [6, 21] | <0.0001 |
| NIHSS at 48 hours        | 4 [0, 12] | 14 [3, 20] | <0.0001 |
| NIHSS at discharge       | 2 [0, 9] | 10 [2, 18] | <0.0001 |
| Rankin at 3 months       | 1 [0, 3] | 3 [1, 4] | <0.0001 |
| RBM3 at admission, pg/mL | 320.7 ± 194.6 | 231.8 ± 177.6 | 0.001 |
| RBM3 at 24 hours, pg/mL  | 641.4 ± 421.9 | 177.7 ± 158.3 | <0.0001 |
| RBM3 at 24 hours ≥300 pg/mL, % | 74.3 | 15.2 | <0.0001 |
| ΔRBM3, %                 | 25.2 ± 67.3 | -43.3 ± 66.0 | <0.0001 |
| ΔRBM3 ≥10%, %            | 76.5     | 19.0    | <0.0001 |
|                          | Poor outcome n = 92 | Good outcome n = 123 | P      |
|--------------------------|---------------------|----------------------|--------|
| Age, years               | 77.6 ± 9.4          | 74.5 ± 10.7          | 0.028  |
| Women, %                 | 53.3                | 40.7                 | 0.073  |
| History of hypertension, %| 59.8                | 65.9                 | 0.393  |
| History of diabetes, %   | 29.3                | 22.8                 | 0.343  |
| Smoking, %               | 15.2                | 14.6                 | 0.527  |
| Dyslipidemia, %          | 40.2                | 28.5                 | 0.081  |
| Atrial fibrillation, %   | 40.2                | 35.8                 | 0.570  |
| Ischemic heart disease, %| 6.5                 | 8.1                  | 0.795  |
| Previous stroke, %       | 10.9                | 10.6                 | 0.557  |
| Peripheral arterial disease, % | 5.6               | 9.8                  | 0.164  |
| Systolic blood pressure, mmHg | 150.6 ± 26.2       | 151.9 ± 30.8         | 0.755  |
| Diastolic blood pressure, mmHg | 80.6 ± 17.1        | 80.7 ± 18.0          | 0.980  |
| Glycemia, mg/dL          | 154.4 ± 64.7        | 141.9 ± 60.3         | 0.150  |
| Leukocytes, × 10^3/mL    | 9.6 ± 2.9           | 9.0 ± 3.2            | 0.186  |
| Fibrinogen, mg/dL        | 501.4 ± 152.7       | 483.2 ± 152.6        | 0.388  |
| Platelets, × 10^3/mL     | 217.6 ± 58.9        | 225.5 ± 79.4         | 0.421  |
| Axillary temperature at admission, ºC | 36.5 ± 0.7         | 36.3 ± 0.4           | 0.009  |
| Maximum temperature in first 24 hours, ºC | 37.6 ± 0.7         | 37.1 ± 0.5           | <0.0001 |
| Temperature in first 24 hours <37.5ºC, % | 45.7               | 76.4                 | <0.0001 |
| Systemic fibrinolysis, % | 70.7                | 76.4                 | 0.351  |
| Endovascular treatment, %| 9.8                 | 11.4                 | 0.825  |
| TOAST                    |                     |                      | 0.001  |
| - Atherothrombotic, %    | 13.0                | 25.2                 |        |
| - Cardioembolic, %       | 53.5                | 39.8                 |        |
| - Lacunar, %             | 2.2                 | 14.6                 |        |
| - Indeterminate, %       | 31.5                | 20.3                 |        |
| TOAST categorized        |                     |                      | <0.0001 |
| - Not cardioembolic, %   | 15.2                | 39.8                 |        |
| - Probably cardioembolic, % | 84.8               | 60.2                 |        |
| Previous Rankin          | 0 [0, 2]            | 0 [0, 0]             | <0.0001|
| NIHSS at admission       | 17 [12, 21]         | 11 [8, 17]           | <0.0001|
| NIHSS at 24 hours        | 20 [12, 22]         | 2 [1, 8]             | <0.0001|
| NIHSS at 48 hours        | 18 [13, 22]         | 2 [1, 13]            | <0.0001|
| NIHSS at discharge       | 6 [1, 17]           | 2 [0, 7]             | <0.0001|
| Rankin at 3 months       | 4 [3, 6]            | 1 [0, 1]             | <0.0001|
| RBM3 at admission, pg/mL | 308.6 ± 207.8       | 272.7 ± 180.3        | 0.177  |
| RBM3 at 24 hours, pg/mL  | 296.5 ± 286.1       | 601.6 ± 447.1        | <0.0001|
| RBM3 at 24 hours ≥300 pg/mL, %  | 33.7               | 66.7                 | <0.0001|
| ΔRBM3                    | -46.2 ± 73.8        | 34.6 ± 53.3          | <0.0001|
| ΔRBM3 ≥10%, %            | 19.6                | 82.1                 | <0.0001|

Supplementary Table 10. Clinical and biochemical characteristics in patients with poor vs good outcome at 3 months.
Supplementary Figure 1. Brain dissection diagram for measuring RBM3 expression by qPCR and WB.
Supplementary Figure 2. Correlation between hyperthermia (maximum temperature in the first 24 hours ≥37.5°C) and mean RBM3 levels on admission and at 2, 24 and 72 hours.
Supplementary Figure 3. Mean RBM3 levels on admission and after 2, 24 and 72 hours in the first 20 IS patients included in the study.
Supplementary Figure 4. Clinical outcome at 3 months and RBM3 levels on admission and after 24 hours in the first 20 IS patients included in the study.
• Full-size WB gels corresponding to the Fig.1b, d and Fig. 2b, c.

Complementary to Fig 1A-B

Complementary to Fig 1C-D

Complementary to Fig 2B

Complementary to Fig 2C
REFERENCES

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