SINGLE CELL IMMUNO-LASER MICRODISSECTION COUPLED TO QUANTITATIVE LABEL-FREE PROTEOMICS TO REVEAL THE PROTEOTYPES OF HUMAN BRAIN CELLS AFTER ISCHEMIA

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Supplementary Data S1. Protein and peptide identifications in neurons and BBB.

Please, look at the Excel file with the same title.

Supplementary Table S1. List of proteins identified with ≥2 unique peptides in neurons and/or BBB.

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Supplementary Table 2. Demographic and clinical characteristics of the cohorts included in this study.

Factors	Ischemic strokes (N=45)	Stroke-mimicking conditions (N=13)	Controls (N = 8)	p-value
Age, years median (IQR)	81 (71-83)	66 (52-78)	68 (66.5-69)	**0.005**
Sex (Male) % (n)	46.7 (21)	53.8 (7)	50 (4)	0.897
Admission NIHSS score median (IQR)	10 (5-17)	5 (4-7)	-	0.145
Previous mRS score median (IQR)	1 (1-2)	1 (0-3)	-	0.891
Glycemia, mg/dL mean ± SD	135.2 ± 39.6	131.8 ± 35.7	145 ± 86.9	0.850
SBP, mm Hg mean ± SD	146.2 ± 24.8	145.3 ± 29.3	151.2 ± 18.6	0.860
DBP, mm Hg mean ± SD	77.4 ± 15.2	72.5 ± 13.9	76.9 ± 8.5	0.616
Smokers % (n)	11.4 (4)	18.2 (2)	12.5 (1)	0.844
Arterial hypertension % (n)	77.8 (35)	53.8 (7)	100 (8)	**0.048**
Diabetes mellitus % (n)	26.7 (12)	30.8 (4)	25 (2)	0.947
Dyslipidemia % (n)	42.2 (19)	38.5 (5)	50 (4)	0.945
Atrial fibrillation % (n)	27 (10)	0 (0)	12.5 (1)	0.121
Cardiopathy % (n)	46.7 (21)	15.4 (2)	12.5 (1)	**0.039**
Previous stroke % (n)	20 (9)	46.2 (6)	0 (0)	**0.037**
SAHH2 pg/mL median (IQR)	1369.5 (993-2054)	1530.5 (1278-1978)	1382.4 (1289-1724)	0.728
Z-SRSF1 median (IQR)	1.6 (1.6-2.0)	1.6 (1.6-1.6)	1.6 (1.5-3.3)	0.443

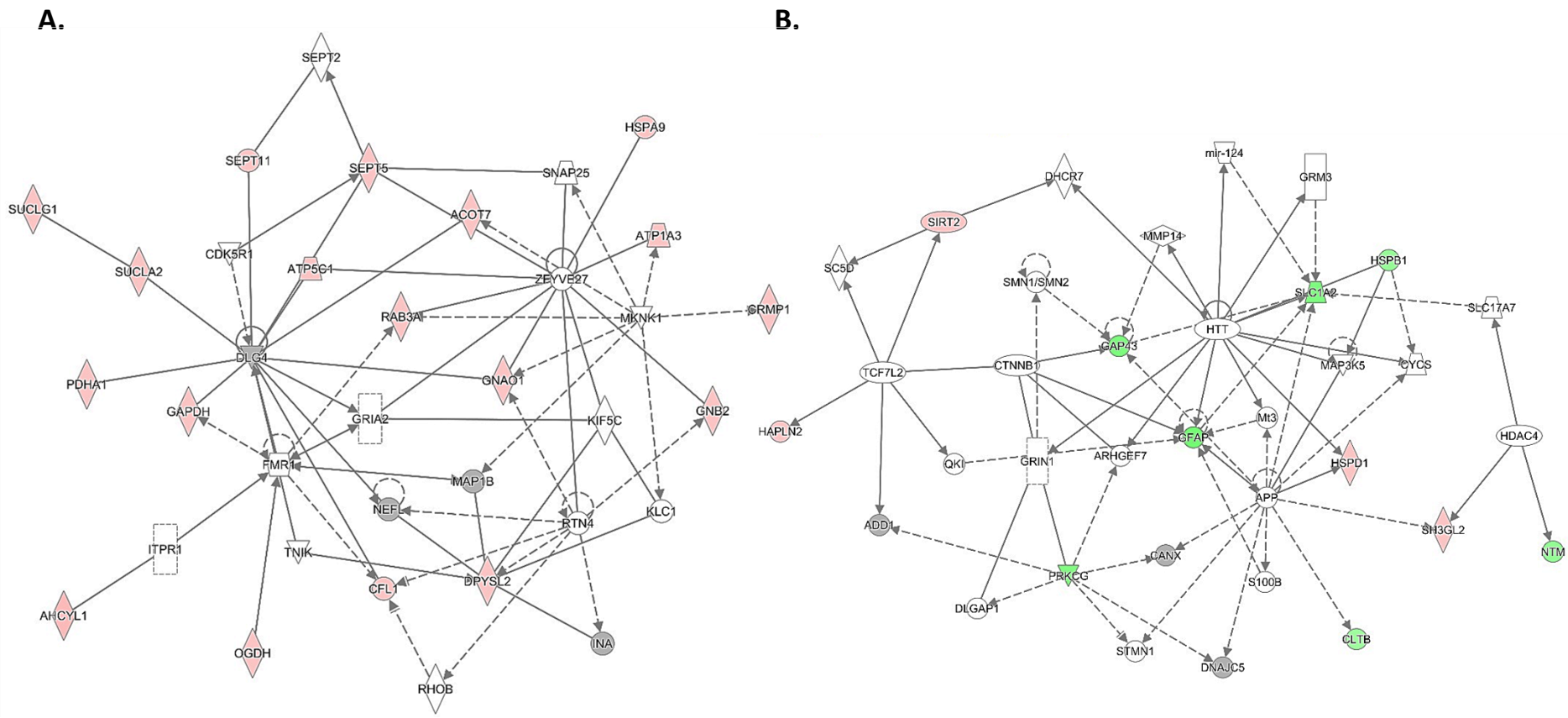
NIHSS: National Institutes of Health stroke scale; mRS: modified Rankin scale; SBP: systolic blood pressure; DBP: diastolic blood pressure; SAHH2: adenosylhomocysteinase-2 ; Z-SRSF1: standardized values for serine/arginine-rich splicing factor-1.
Continuous variables are expressed as mean ± standard deviation (SD) while semi-continuous variables and non-normal distributed biomarkers are expressed as median (interquartile range, IQR). Categorical variables are expressed as frequencies: percentage (sample size, n).
Statistically significant differences between studied groups are highlighted as bold p-values.

Supplementary Table 3. Univariate analysis: Demographic and clinical characteristics associated with neurological improvement at 48h in ischemic stroke patients.

Factors	Neurological improvement (N=20)	No neurological improvement (N=25)	p-value
Age, years median (IQR)	78.5 (71-82)	81 (75-84)	0.166
Sex (Male) % (n)	50.0 (10)	44.0 (11)	0.688
Admission NIHSS score median (IQR)	12 (9.5-18)	7 (3-15)	**0.009**
Previous mRS score median (IQR)	1 (0.5-2)	1 (1-2)	0.835
Glycemia, mg/dL mean ± SD	128.89 ± 34.36	140.25 ± 43.44	0.357
SBP, mm Hg mean ± SD	151.00 ± 25.26	142.44 ± 24.49	0.341
DBP, mm Hg mean ± SD	83.14 ± 16.54	72.94 ± 12.90	0.059*
Smokers % (n)	13.3 (2)	10.0 (2)	1.000
Arterial hypertension % (n)	70.0 (14)	84.0 (21)	0.301
Diabetes mellitus % (n)	25.0 (5)	28.0 (7)	0.821
Dyslipidemia % (n)	45.0 (9)	40.0 (10)	0.469
Atrial fibrillation % (n)	20.0 (3)	31.8 (7)	0.481
Cardiopathy % (n)	45.0 (9)	48.0 (12)	0.841
Previous stroke % (n)	25.0 (5)	16.0 (4)	0.482
rt-PA administration % (n)	80.0 (16)	36.0 (9)	**0.003**
Time-to-treatment, min mean ± SD	156.92 ± 62.24	147.89 ± 65.32	0.746
TOAST			**0.035**
- Atherothrombotic % (n)	40.0 (8)	8.0 (2)	
- Cardioembolic % (n)	50.0 (10)	56.0 (14)	
- Undetermined % (n)	10.0 (2)	28.0 (7)	
HT % (n)	33.3 (4)	7.7 (1)	0.160
In-hospital death % (n)	25.0 (5)	28.0 (7)	0.821
Z-SRSF1 median (IQR)	1.59 (1.59-1.59)	1.59 (1.59-2.09)	0.587
SAHH2 pg/mL median (IQR)	985.10 (910.48-1434.84)	1418.84 (1134.75-2003.27)	**0.013**
SAHH2 <993.23 pg/mL % (n)	47.1 (8)	8.7 (2)	**0.009**

NIHSS: National Institutes of Health stroke scale; mRS: modified Rankin scale; SBP: systolic blood pressure; DBP: diastolic blood pressure; SAHH2: adenosylhomocysteinase-2; Z-SRSF1: standardized values for serine/arginine-rich splicing factor-1. Statistically significant

Supplementary Figure S1. Top molecular networks of interacting proteins in (**A**) neurons and (**B**) BBB structures. The intensity of protein (geometric forms) color indicates the degree of downregulation (green) or upregulation (red) of protein presence in the infarcted (IC) region when compared to contralateral (CL). Grey proteins did not pass our stringent selection criteria ($p<0.05$, fold-change >|2|); white proteins were included from IPA database. Continuous lines indicate direct interaction and discontinuous lines indicate indirect interaction.



Supplementary Figure S2. Venn diagram of proteins altered by ischemia in neurons and BBB structures when paired-samples parametric (red) or non-parametric (yellow) tests were applied. Numbers represent proteins with a p-value <0.05 in each test but for non-parametric test in BBB, in which the limited sample size (N=4) hindered a p-value lower than 0.125. * denotes a p-value <0.125.