SUPPLEMENTAL MATERIAL

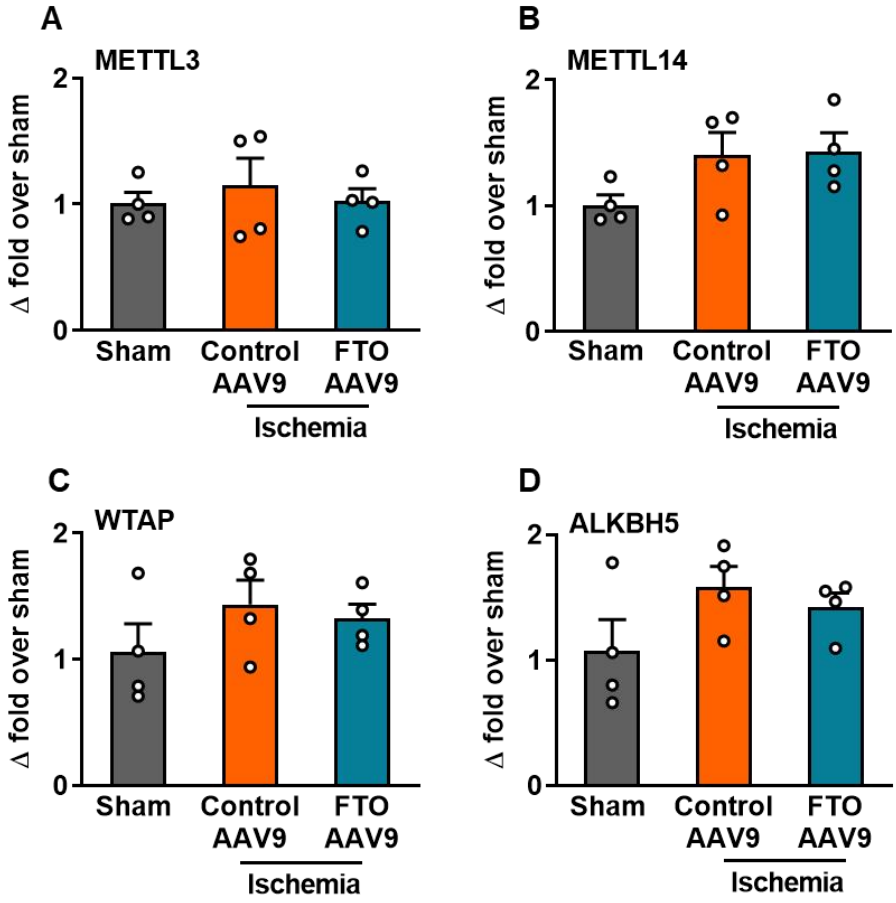
Cerebroprotective role of m^{6}A demethylase FTO after experimental stroke

Methods

***In vivo* laser speckle imaging:** Cerebral blood flow was assessed by laser speckle analysis in a subset of mice subjected to 1h of transient MCAO at 21 days after FTO AAV9 or control AAV9 injection. Readings were obtained before transient MCAO, at 40 to 55 mins of occlusion and at 2h of reperfusion. For each reading, mice were anesthetized with isoflurane and fixed into a stereotaxic frame, and the cranium was exposed via a midline incision. The laser-speckle imager (high-resolution mode) was positioned 10 cm above the skull, and light-based readings were collected at an effective rate of 2.1 images/s. PIMSoft analysis software (Perimed, USA) was used to establish an arbitrary index of cerebral blood flow (perfusion units) in the ischemic hemisphere at each time point.

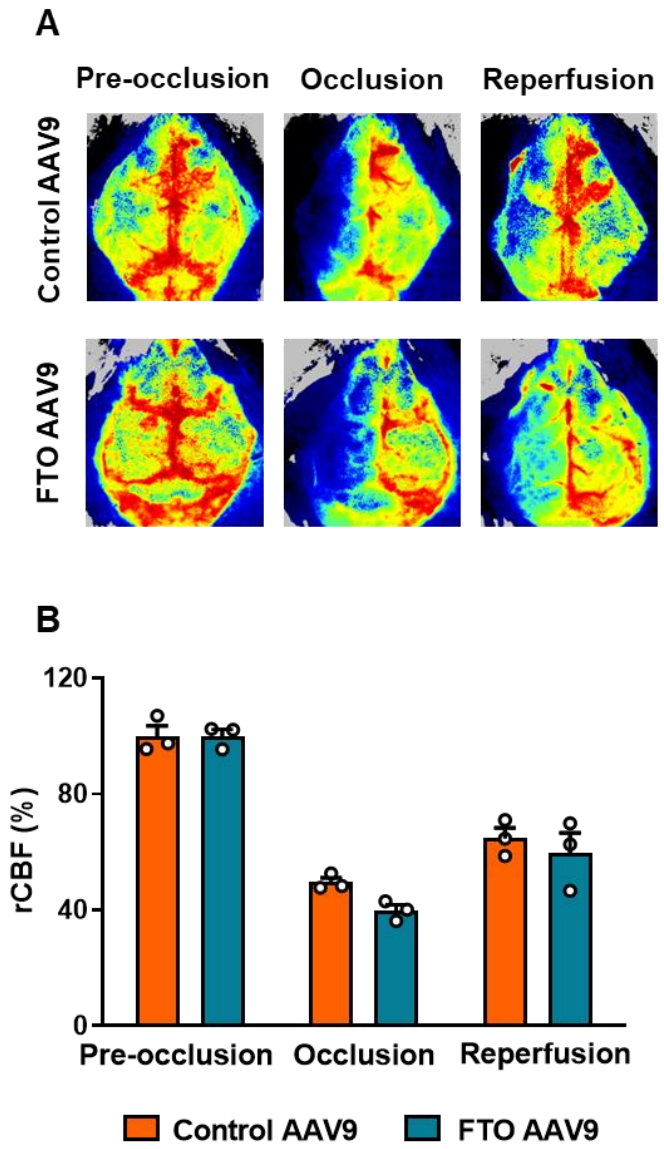
Figures and Tables

Supplementary Fig.1



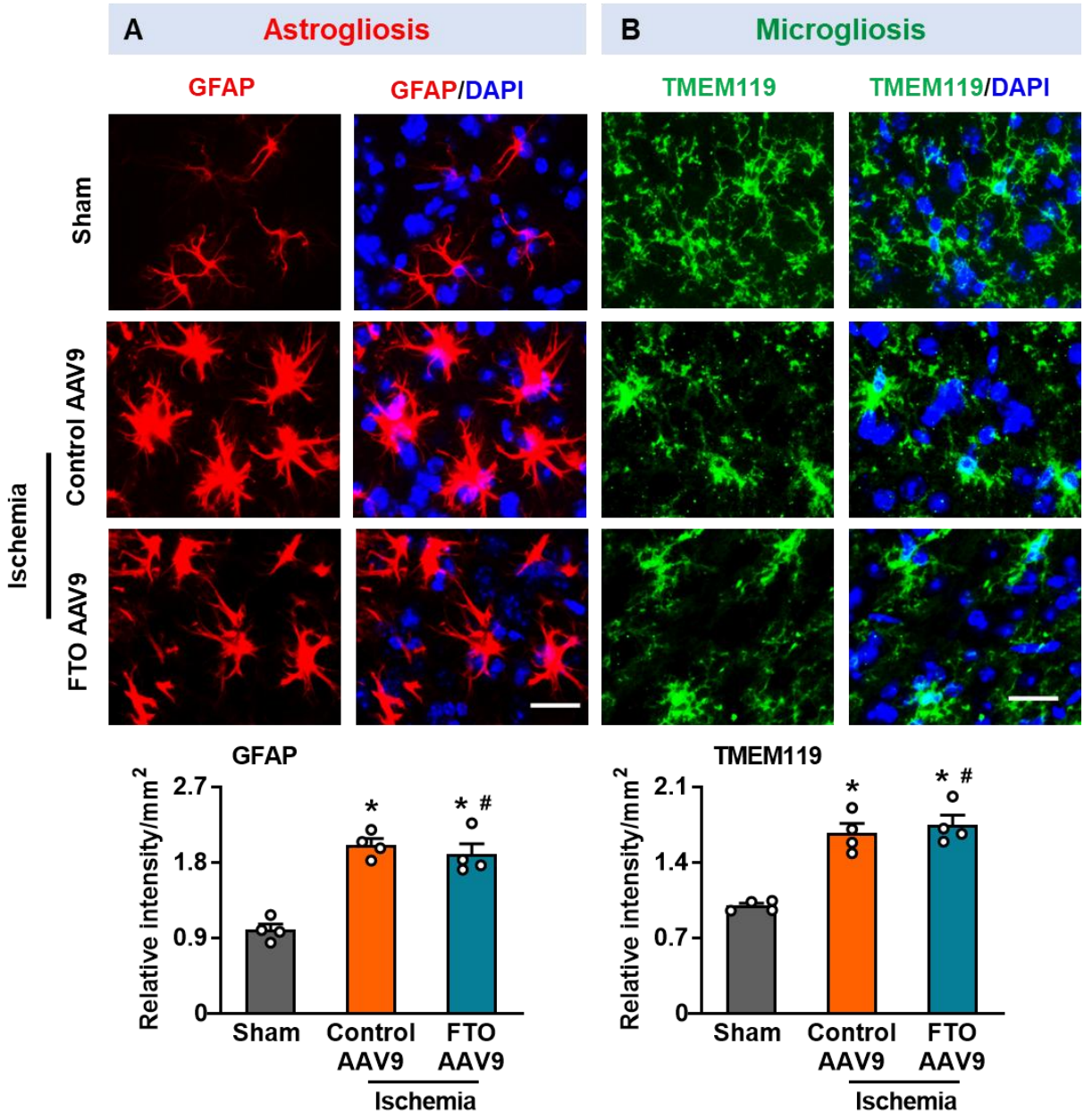
FTO overexpression did not affect the mRNA levels of m^{6}A methylase complex and m^{6}A demethylase ALKBH5. Quantification of mRNA levels of the m^{6}A methylase subunits METTL3 (A), METTL14 (B) and WTAP (C), and m^{6}A demethylase ALKBH5 (D) in the cortical peri-infarct region of control AAV9 and FTO AAV9 treated mice at 1 day of reperfusion following 1h transient MCAO compared to sham. Data are mean±SEM (n=4/group).

Supplementary Fig. 2



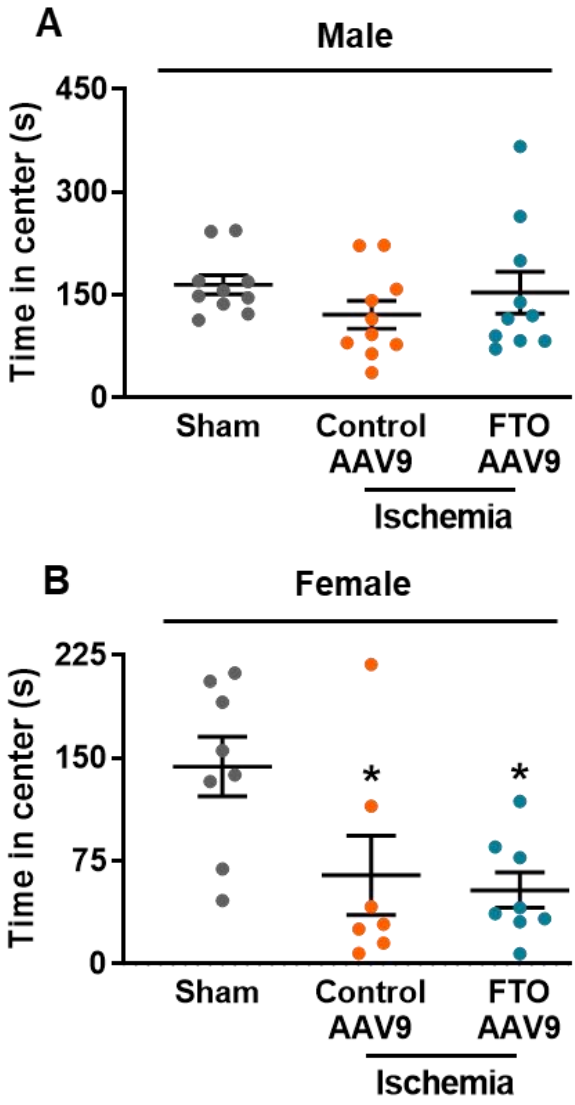
FTO overexpression did not alter the regional cerebral blood flow. Representative laser speckle images (A) showing regional cerebral blood flow (rCBF) and image quantification (B) of control AAV9 and FTO AAV9 treated male mice before, during and 2h after transient MCAO. Data are mean±SEM (n=3/group).

Supplementary Fig. 3



FTO overexpression had no gross impact on post-stroke gliosis. Representative immunostaining images and quantification of GFAP (A) and TMEM119 (B) illustrate astrogliosis and microgliosis in the cortical peri-infarct regions of control AAV9 and FTO AAV9 treated male mice at 3 days of reperfusion after 1h transient MCAO compared with sham (n=4/group). DAPI, 4',6-diamidino-2-phenylindole. Scale bars are 15 μm.

Supplementary Fig. 4



FTO overexpression did not alter anxiety-like behavior in adult male and female mice. Anxiety-like behavioral assessment in sham, control AAV9 and FTO AAV9 treated male (A) and female mice (B) by open field test at 14 days of reperfusion after 1h transient MCAO. Data are mean±SEM (n=10/group for males and n=7-8/group for females). *p<0.05 compared with sham by Mann-Whitney *U* test.

Supplementary Table 1: Physiological parameters

Parameter	Control AAV9/Sham	FTO AAV9/Sham	Control AAV9/Ischemia	FTO AAV9/Ischemia
pH	7.1±0.0	7.1±0.0	7.2±0.0	7.1±0.0
pCO_2 (mm Hg)	69.7±7.5	68.6±5.8	58.1±5.1	74.7±5.0
pO_2 (mm Hg)	117.4±29.4	188.0±5.5	113.2±13.3	119.5±12.1
Base Excess (mmol/L)	-11.2±1.7	-10.7±2.2	-8.6±1.9	-4.3±1.7
HCO_3 (mmol/L)	19.2±1.8	19.5±2.0	20.3±1.5	25.0±1.3
TCO_2 (mmol/L)	21.2±2.1	21.7±2.1	22.0±1.6	27.3±1.3
sO_2 (%)	96.8±1.2	99.0±0.0	96.0±0.9	96.5±0.6
Lactate (mmol/L)	4.5±0.3	5.1±0.3	2.2±0.3	1.7±0.2
Sodium (mmol/L)	137.6±1.0	131.3±2.5	137.2±1.2	135.8±1.5
Potassium (mmol/L)	>9	>9	>9	>9
Chloride (mmol/L)	115.0±1.3	119.3±3.5	117.6±3.4	117.3±1.3
Glucose (mg/dL)	258.4±7.0	283.3±34.5	163.8±18.4	170.5±9.8
Urea Nitrogen (mg/dL)	25.2±1.1	36.3±3.8	20.8±3.2	24.8±2.8
Creatinine (mg/dL)	<0.2	<0.2	<0.2	<0.2
Hematocrit (%PCV)	36.2±2.4	32.7±3.8	39.0±2.3	42.5±1.0
Hemoglobin (g/dL)	12.9±0.6	11.1±1.3	13.3±0.8	14.5±0.4

Physiological parameters were estimated before transient MCAO and at 1 day of reperfusion in control AAV9 and FTO AAV9 treated adult male mice. Blood (50 µL) collected from the retroorbital plexus was analyzed with the i-STAT blood analyzer in conjunction with i-STAT $CG4^+$ (blood gases) and $CG8^+$ (electrolytes) cartridges (Abbott Laboratories). None of the evaluated parameters were significantly altered between the control AAV9 and FTO AAV9 cohorts. Data are mean±SEM (n=4/group).

Supplementary Table 2: Primers used for real-time PCR

Transcript	Primer sequences (5' to 3')
FTO	TTCATGCTGGATGACCTCAATG GCCAACTGACAGCGTTCTAAG
METTL3	GGGCACTTGGATTTAAGGAACC CTTAGGGCCGCTAGAGGTAGG
METTL14	GAGCTGAGAGTGCGGATAGC GCAGATGTATCATAGGAAGCCC
WTAP	ATGGCACGGGATGAGTTAATTC TTCCCTTAAACCAGTCACATCG
ALKBH5	GCATACGGCCTCAGGACATTA TTCCAATCGCGGTGCATCTAA
18S rRNA	CGCCGCTAGAGGTGAAATTCT CGAACCTCCGACTTTCGTTCT

Primers were designed using the Primer Quest web tool (Integrated DNA Technologies). FTO, fat mass and obesity-associated; METTL3, methyltransferase like 3; METTL14, methyltransferase like 14; WTAP, Wilms tumor 1-associating protein; ALKBH5, AlkB homology 5; 18S rRNA, 18S ribosomal RNA.