SUPPLEMENTAL MATERIAL

Long noncoding RNA FosDT is developmentally dispensable but vital for shaping the post-stroke functional outcome

Supplemental Information

Methods

Animals: Transient middle cerebral artery occlusion (MCAO) was induced in adult male and female (~3 months) FosDT$^{+/+}$ and FosDT$^{-/-}$ rats (generated on an SD background). Physiological parameters were estimated in sham and after the induction of transient MCAO and 30 min of reperfusion. Rats that showed no signs of neurological deficits during the acute phase after MCAO and or a hemorrhage after euthanasia (2 males and 1 female) and that died during the course of reperfusion (8 males and 4 females) were excluded.

RNA-seq analysis: Ribosomal RNA depleted DNA was fragmented and used for preparing the DNA library as per Illumina's Truseq-stranded-total-RNA-sample preparation protocol. Following quantitation of DNA library on a high sensitivity DNA chip (Agilent), paired-end (150 bp) sequencing was performed on. Reads containing sequencing adaptors, sequencing primers, and sequences with q quality score <20 were removed and were aligned with the reference genome using the HISAT2 package, with a maximum of two mismatches and assembled using StringTie. Transcriptomes from all samples were then merged to reconstruct a comprehensive transcriptome using a proprietary Perl script of LC Sciences USA. Following transcriptome reconstruction, FPKM reads were evaluated by StringTie, and differentially expressed genes were assessed by edgeR.

Immunostaining: Brain sections were stained with antibodies against cleaved caspase-3 (1:400; catalog no. 9661S; Cell Signaling Technology), pDrp-1 (1:400; catalog no. 3455S, Cell Signaling Technology), 3-NT (1:500; catalog no. ab61392, Abcam), and NeuN (1:300; catalog no. MAB377, Millipore) and suitable secondary antibodies as described earlier.[3] The homologous areas were used to examines the changes due to ischemic injury.[3]

Results

FosDT expression as a function of development in peripheral organs. In the peripheral organs, only muscle and spleen showed significant FosDT levels in P7 rats (Supplemental Fig.

IA). A similar pattern of FosDT was also seen in adult rats (Supplemental Fig. IB). Data presented as a fold-over the liver. FosDT levels in the heart of adult rats increased significantly, while liver levels decreased over P7 (Supplemental Fig. IC). Lung, muscle, kidney and spleen showed comparable FosDT expression in both P7 and adult rats (Supplemental Fig. IC). Fos expression mirrored FosDT expression in all peripheral organs in P7 and adult rats (Supplemental Fig. IA-C).

FosDT knockout had no effect on the peripheral organ structure. None of the peripheral organs evaluated (lung, liver, heart, spleen, muscle and kidney) showed any cytoarchitectural differences between the adult FosDT$^{+/+}$ and FosDT$^{-/-}$ rats (Supplemental Fig. IIC).

Transcripts induced by focal ischemia in FosDT$^{+/+}$ rats: Reactome, GO and KEGG pathways analysis showed that inflammation, apoptosis, angiogenesis, and glutamate signaling are the major categories of the transcripts altered after focal ischemia in the FosDT$^{+/+}$ rats (Supplemental Fig. VA and B).

Physiological parameters

		pH	pCO_2 (mmHg)	pO_2 (mmHg)	HCO_3 (mol/L)	TCO_2 (mol/L)	sO_2 (%)	Lactate (mol/L)
Sham	$FosDT^{+/+}$	7.3±0.0	51.7±2.3	53.3±19.7	29.3±0.5	30.7±0.6	93.0±5.6	1.9±0.3
	$FosDT^{-/-}$	7.4±0.0	44.8±3.2	67.0±14.5	29.1±0.9	30.7±1.2	92.0±4.6	2.0±0.4
30 min reperfusion	$FosDT^{+/+}$	7.3±0.0	48.9±4.48	64.3 ±12.5	25.9±1.5	27.3±1.5	89.3±5.5	2.8±0.7
	$FosDT^{-/-}$	7.4±0.0	46.7±3.1	71.7±25.1	27.9±1.5	29.0±1.7	91.3±6.5	2.8±0.4

Physiological parameters were estimated in sham and after the induction of transient MCAO and 30 min of reperfusion in both genotypes of rats. Blood (50 µl) collected was analyzed with the i-STAT blood analyzer (Abbott Point of Care, NJ USA). None of the parameters estimated were significantly different between the $FosDT^{+/+}$ and $FosDT^{-/-}$ cohorts.

Supplemental Figures and Figure Legends

Figure I

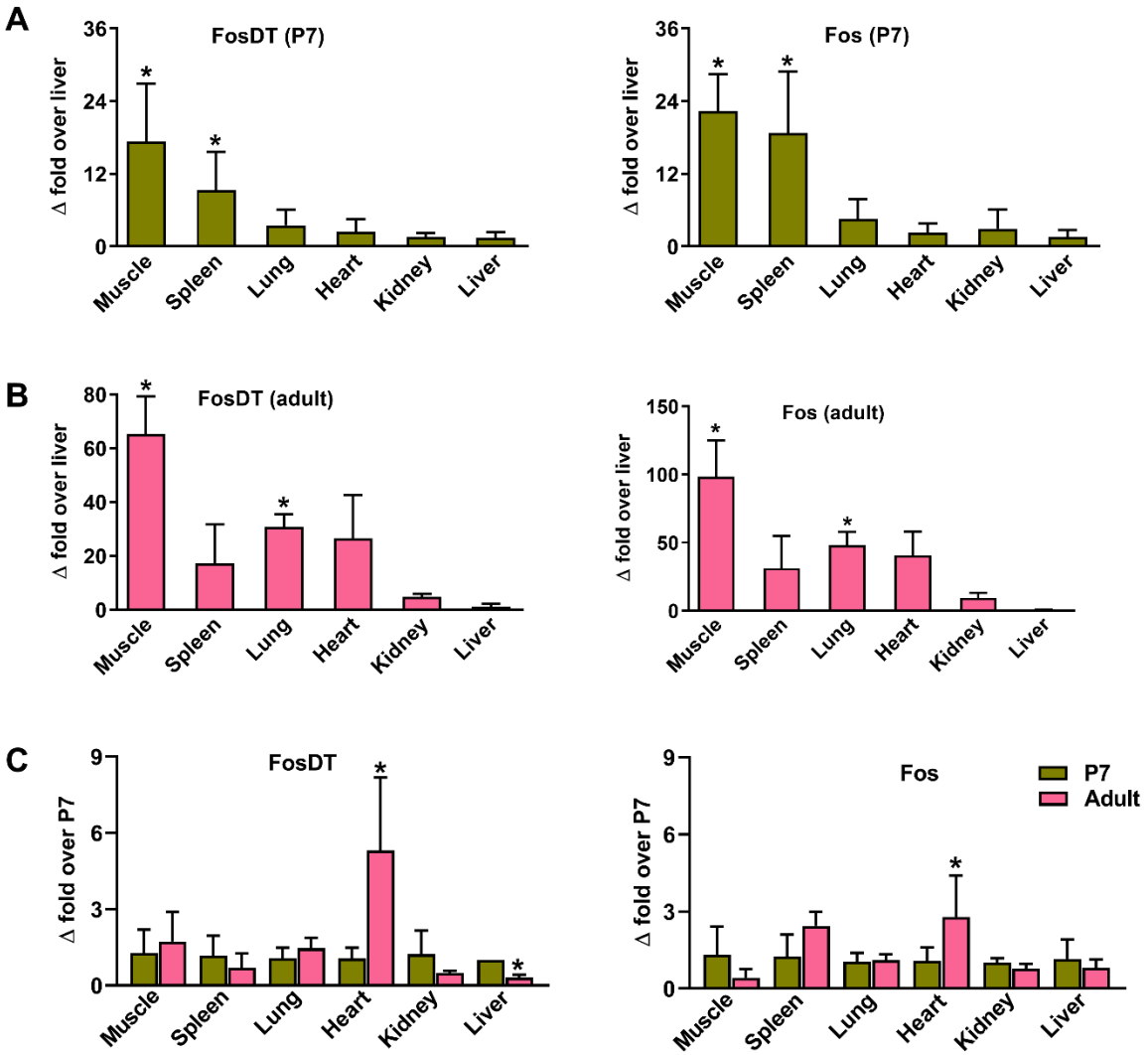


Fig. I: Developmental expression pattern of FosDT and Fos in the rat peripheral organs. Expression of both FosDT and Fos was lowest in the liver at both P7 (A) and adult (B) stages. The expression of FosDT and Fos in various organs was presented as a percent of the expression in the liver (A and B). FosDT expression is significantly higher in the adult heart compared to the P7 stage of rats (C). Fos expression mirrored FosDT expression at both P7 and adult stages (A, B and C). Values are mean ± SD (n = 3-4/group). P7, postnatal day 7. *p<0.05 compared to the liver or P7 by the Mann-Whitney U-test.

Figure II

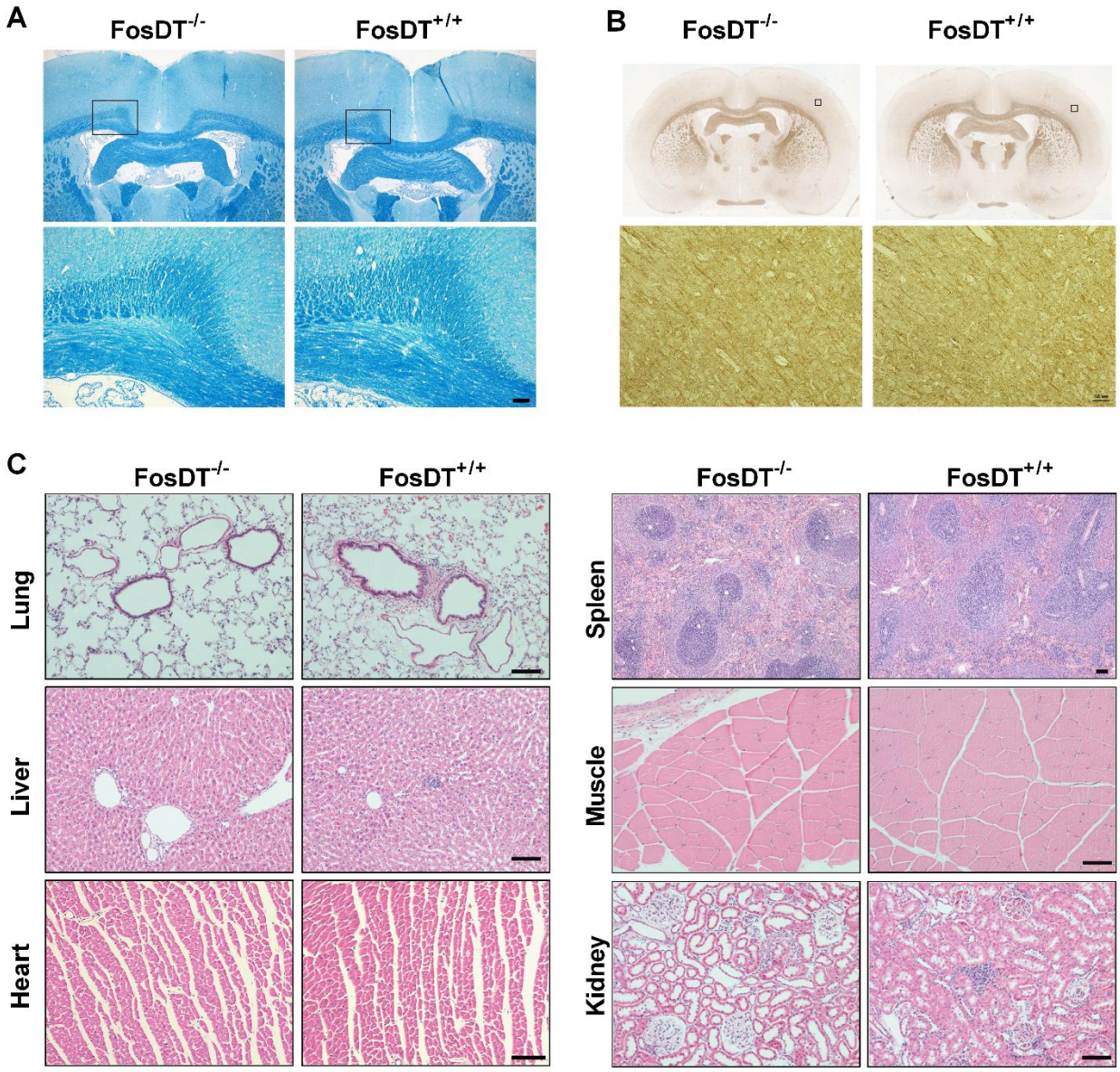


Fig. II: FosDT deletion had no effect on cytoarchitecture of central and peripheral organs of the adult rat. Luxol fast blue staining showed no significant differences in the white matter in the corpus callosum between the FosDT$^{-/-}$ and FosDT$^{+/+}$ cohorts (A). FosDT deletion did not cause neuronal degeneration in the cerebral cortex as delineated by silver staining (B). Images of H&E stained sections of lung, liver, heart, spleen, muscle, and kidney from representative adult FosDT$^{-/-}$ and FosDT$^{+/+}$ rats (C) shows comparable histopathologic changes (n = 3/group). Scale bar = 50 µm.

Figure III

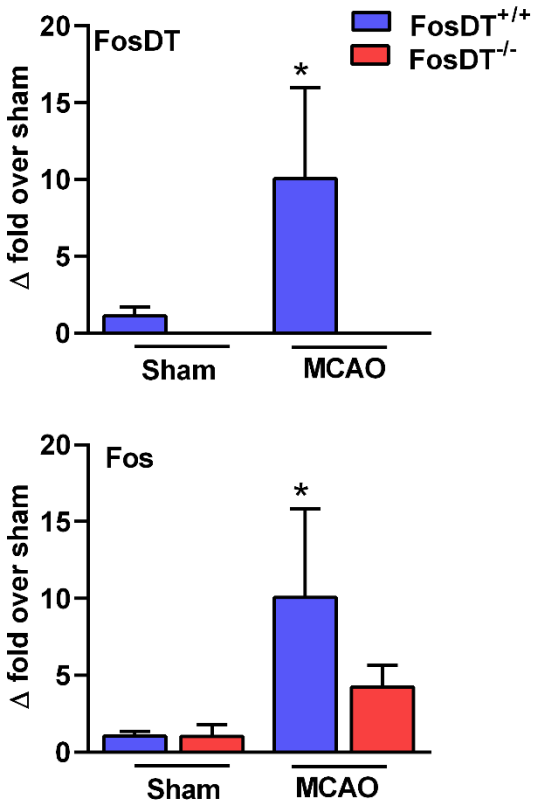


Fig. III: Post-ischemic induction of FosDT was abolished by its genetic deletion. FosDT and Fos expression in FosDT$^{-/-}$ and FosDT$^{+/+}$ rats subjected to transient MCAO/12h reperfusion or sham operation. Values are mean ± SD (n = 3/group). *p<0.05 compared to sham by Mann-Whitney U test.

Figure IV

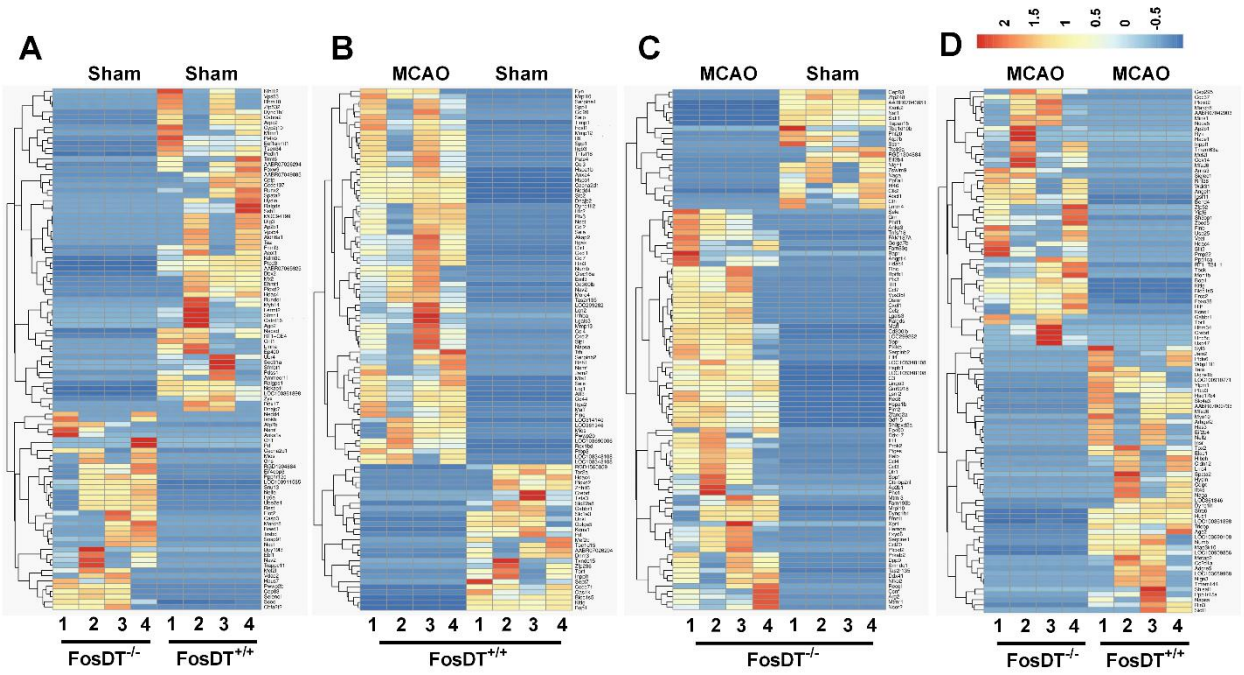
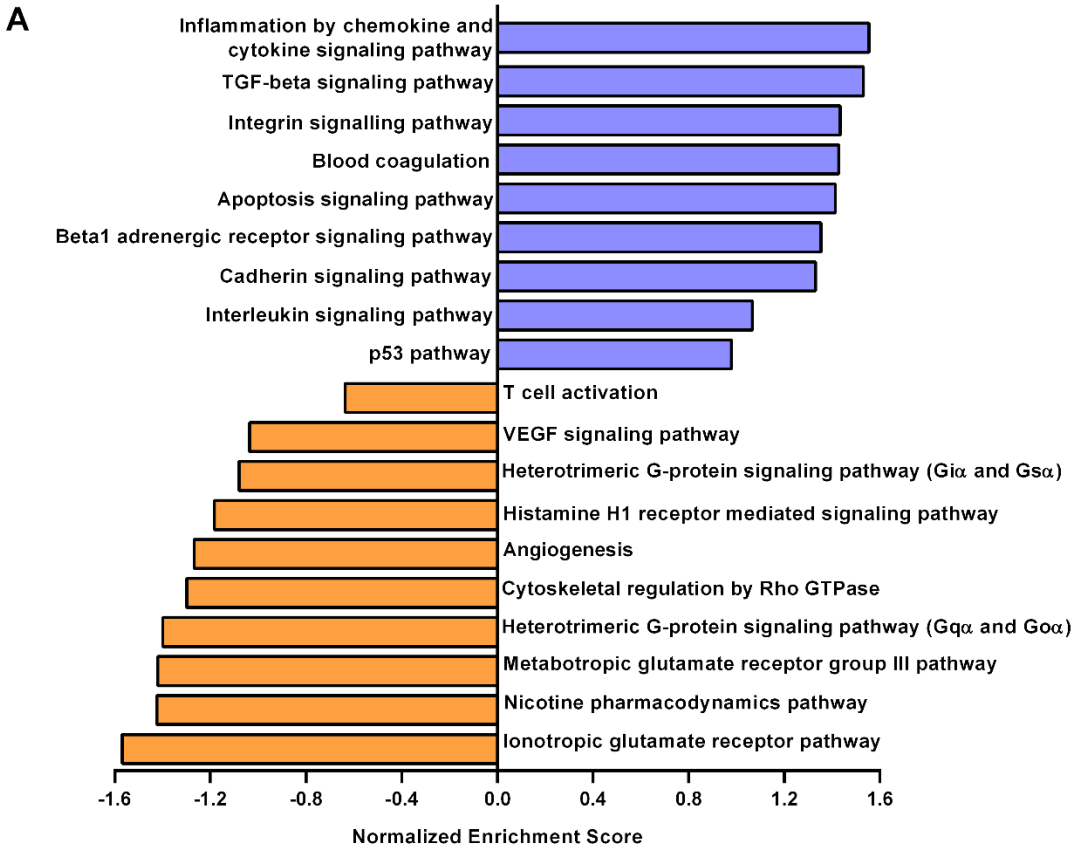


Fig. IV: FosDT deletion altered the cerebral RNA expression profiles. Heatmaps show the distinct pattern of transcripts expressed among FosDT$^{-/-}$ sham vs FosDT$^{+/+}$ sham (A), FosDT$^{+/+}$ MCAO vs FosDT$^{+/+}$ sham (B), FosDT$^{-/-}$ MCAO vs FosDT$^{-/-}$ sham (C) and FosDT$^{-/-}$ MCAO vs FosDT$^{+/+}$ MCAO (D) that showed a fold change of >3.0 (log2 fold change >1.7) and a p-value of <0.05 (n = 4/group).

Figure V


A
Inflammation by chemokine and cytokine signaling pathway
TGF-beta signaling pathway
Integrin signalling pathway
Blood coagulation
Apoptosis signaling pathway
Beta1 adrenergic receptor signaling pathway
Cadherin signaling pathway
Interleukin signaling pathway
p53 pathway
T cell activation
VEGF signaling pathway
Heterotrimeric G-protein signaling pathway (Giα and Gsα)
Histamine H1 receptor mediated signaling pathway
Angiogenesis
Cytoskeletal regulation by Rho GTPase
Heterotrimeric G-protein signaling pathway (Gqα and Goα)
Metabotropic glutamate receptor group III pathway
Nicotine pharmacodynamics pathway
Ionotropic glutamate receptor pathway
-1.6
-1.2
-0.8
-0.4
0.0
0.4
0.8
1.2
1.6
Normalized Enrichment Score

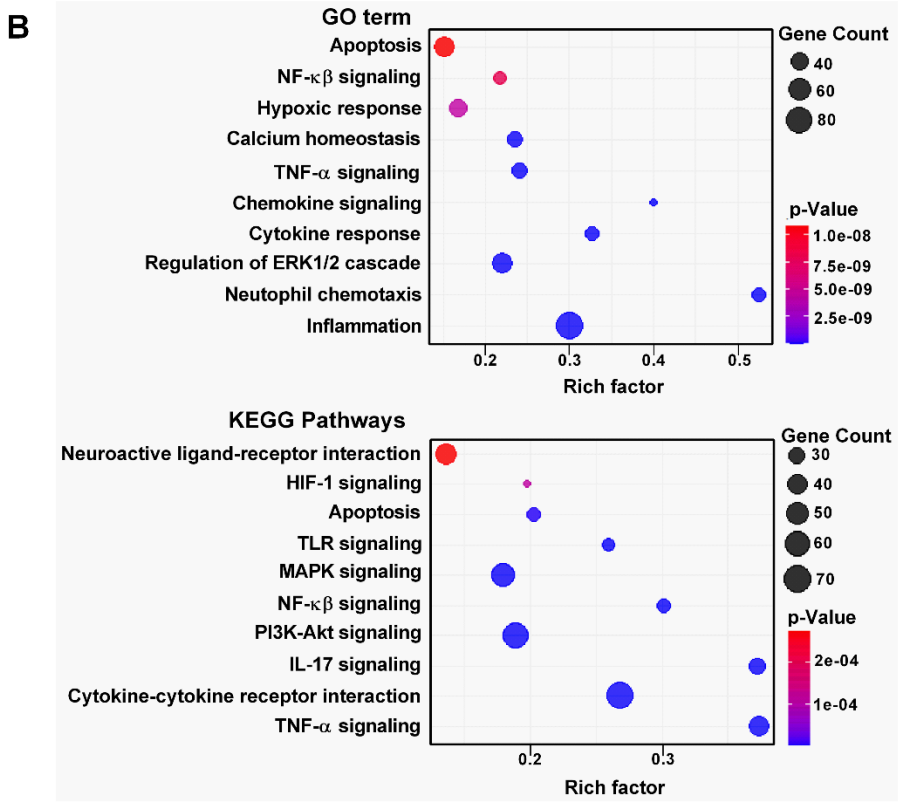

B
GO term
Apoptosis
NF-κβ signaling
Hypoxic response
Calcium homeostasis
TNF-α signaling
Chemokine signaling
Cytokine response
Regulation of ERK1/2 cascade
Neutophil chemotaxis
Inflammation
Gene Count
40
60
80
p-Value
1.0e-08
7.5e-09
5.0e-09
2.5e-09
0.2
0.3
0.4
0.5
Rich factor
KEGG Pathways
Neuroactive ligand-receptor interaction
HIF-1 signaling
Apoptosis
TLR signaling
MAPK signaling
NF-κβ signaling
PI3K-Akt signaling
IL-17 signaling
Cytokine-cytokine receptor interaction
TNF-α signaling
Gene Count
30
40
50
60
70
p-Value
2e-04
1e-04
0.2
0.3
Rich factor

Fig. V: Transient focal ischemia-induced many pathological changes. Gene Set Enrichment Analysis (GSEA) analysis of differentially expressed transcripts at 12h of reperfusion following 90 min of transient MCAO in FosDT$^{+/+}$ rats compared to the sham control (A). Various pathways, including inflammation and apoptosis, were upregulated, whereas angiogenesis and VEGF signaling were downregulated after stroke. KEGG pathways analysis revealed that inflammation, as well as metabolism, are some of the categories affected by cerebral ischemia (B).

*** Preclinical Checklist**

Preclinical Checklist: Prevention of bias is important for experimental cardiovascular research. ***This short checklist must be completed, and the answers should be clearly presented in the manuscript.*** *The checklist will be used by reviewers and editors and it will be published. See "Reporting Standard for Preclinical Studies of Stroke Therapy" and "Good Laboratory Practice: Preventing Introduction of Bias at the Bench" for more information.*

This study invovles animal models:
Yes

Experimental groups and study timeline

The experimental group(s) have been clearly defined in the article, including number of animals in each experimental arm of the study:	Yes
An account of the control group is provided, and number of animals in the control group has been reported. If no controls were used, the rationale has been stated:	Yes
An overall study timeline is provided:	Yes

Inclusion and exclusion criteria

A priori inclusion and exclusion criteria for tested animals were defined and have been reported in the article:	Yes

Randomization

Animals were randomly assigned to the experimental groups. If the work being submitted does not contain multiple experimental groups, or if random assignment was not used, adequate explanations have been provided:	Yes
Type and methods of randomization have been described:	Yes
Methods used for allocation concealment have been reported:	No

Blinding

Blinding procedures have been described with regard to masking of group/treatment assignment from the experimenter. The rationale for nonblinding of the experimenter has been provided, if such was not feasible:	Yes
Blinding procedures have been described with regard to masking of group assignment during outcome assessment:	Yes

Sample size and power calculations

Formal sample size and power calculations were conducted based on a priori determined outcome(s) and treatment effect, and the data have been reported. A formal size assessment was not conducted and a rationale has been provided:	Yes

Data reporting and statistical methods

Number of animals in each group: randomized, tested, lost to follow-up, or died have been reported. If the experimentation involves repeated measurements, the number of animals assessed at each time point is provided, for all experimental groups:	Yes
Baseline data on assessed outcome(s) for all experimental groups have been reported:	Yes
Details on important adverse events and death of animals during the course of experimentation have been provided, for all experimental arms:	Yes
Statistical methods used have been reported:	Yes
Numeric data on outcomes have been provided in text, or in a tabular format with the main article or as supplementary tables, in addition to the figures:	Yes

Experimental details, ethics, and funding statements

Details on experimentation including stroke model, formulation and dosage of therapeutic agent, site and route of administration, use of anesthesia and analgesia, temperature control during experimentation, and postprocedural monitoring have been described:	Yes
Different sex animals have been used. If not, the reason/justification is provided:	Yes

Statements on approval by ethics boards and ethical conduct of studies have been provided:	Yes
Statements on funding and conflicts of interests have been provided:	Yes

---

Date completed: 03/05/2021 17:26:02
User pid: 45430