IRF4 is a Novel Mediator for Neuronal Survival in Ischemic Stroke

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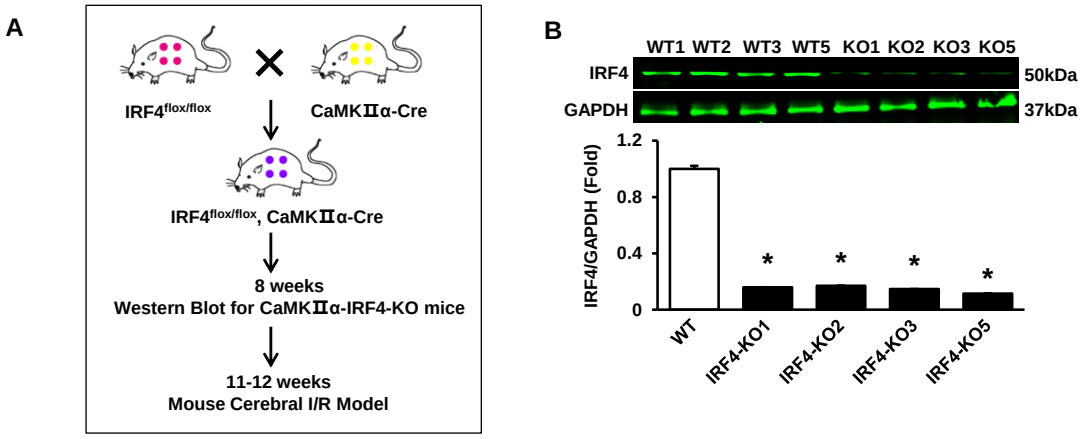
Supplementary Figure S1-S6

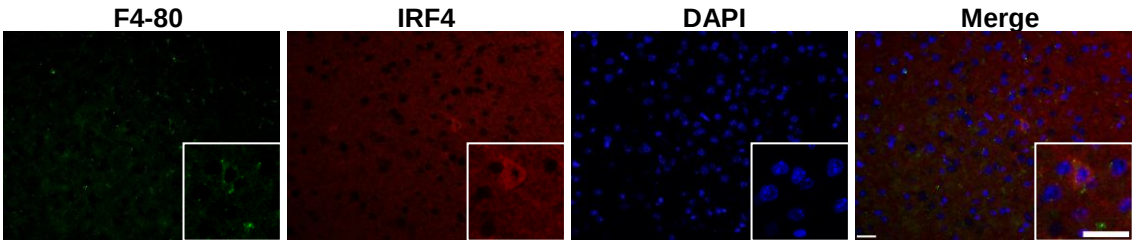
Supplementary Table S1-S4

Supplementary Figure S1



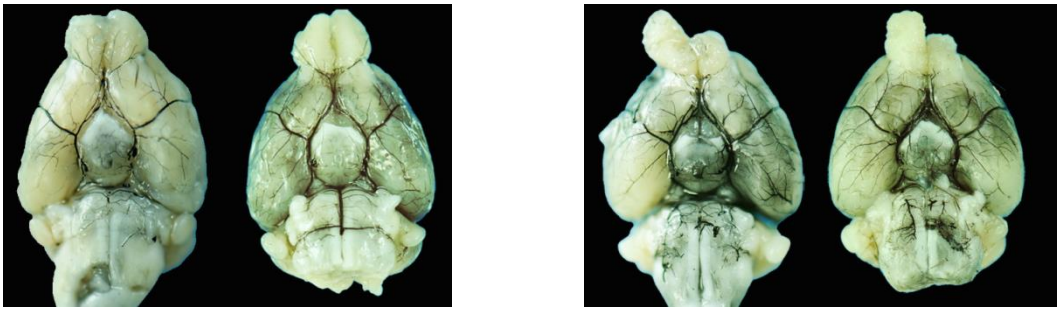
Supplementary Figure S1. Generation of neuron-specific IRF4-knockout mice. (a) Schematic representation of generation of neuron-specific IRF4-knockout (IRF4-KO) mice. **(b)** Immunoblotting of IRF4 in the brain lysates from IRF4-KO mice (left) and the relative IRF4 levels (right). *P<0.0001, <0.0001, <0.0001, <0.0001 versus wild type (WT), respectively, unpaired Student's t-test. The values are the means ± SE of three independent experiments in triplicate.

Supplementary Figure S2



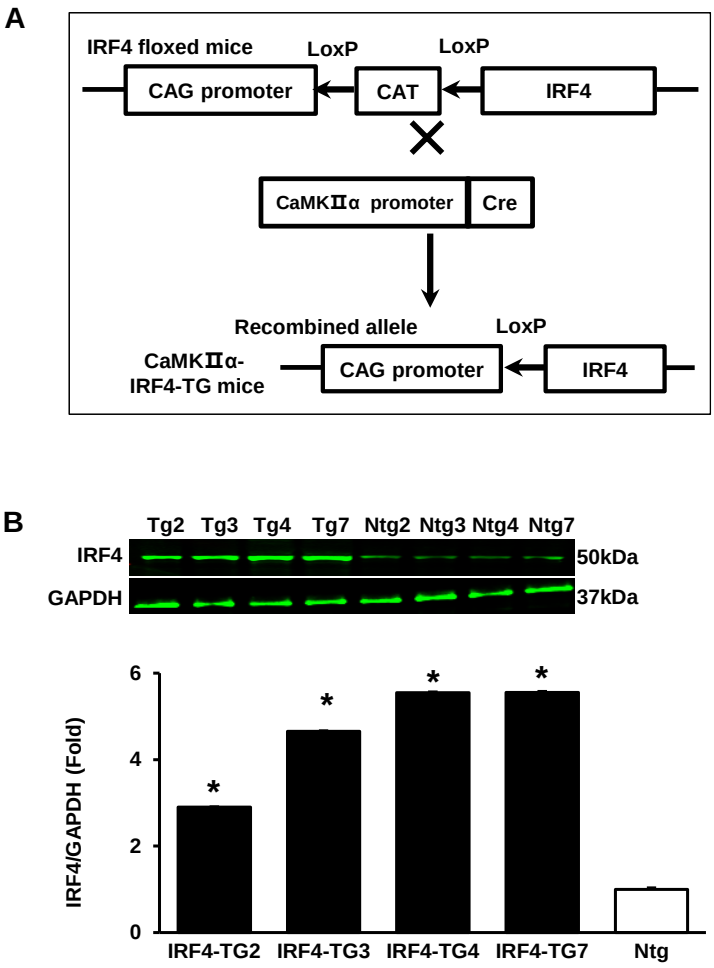
Supplementary Figure S2. IRF4 expresses at a relatively low level in microglia. Representative immunofluorescence images of IRF4 (red), F4-80 (green) and DAPI (blue) in the brains of wild-type mice out of three independent experiments. Scale bar: 20 μm.

Supplementary Figure S3



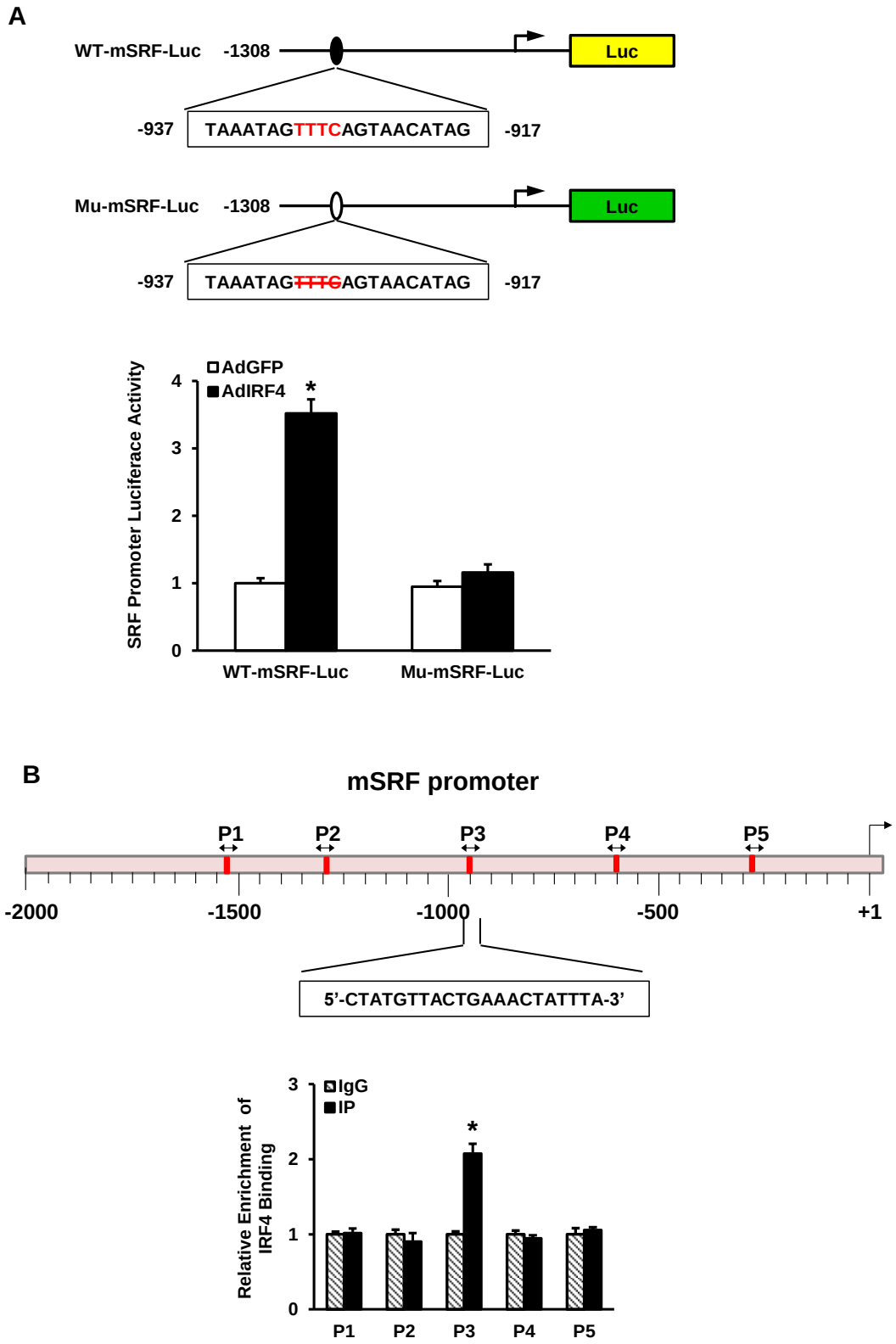
Supplementary Figure S3. Genetic manipulations of IRF4 do not impact the intergrity of cerebral vasculature. Representative images showing the integrity of the cerebral vasculature stained with India ink in WT, IRF4-KO, NTG, and IRF4-TG mice out of three independent experiments.

Supplementary Figure S4



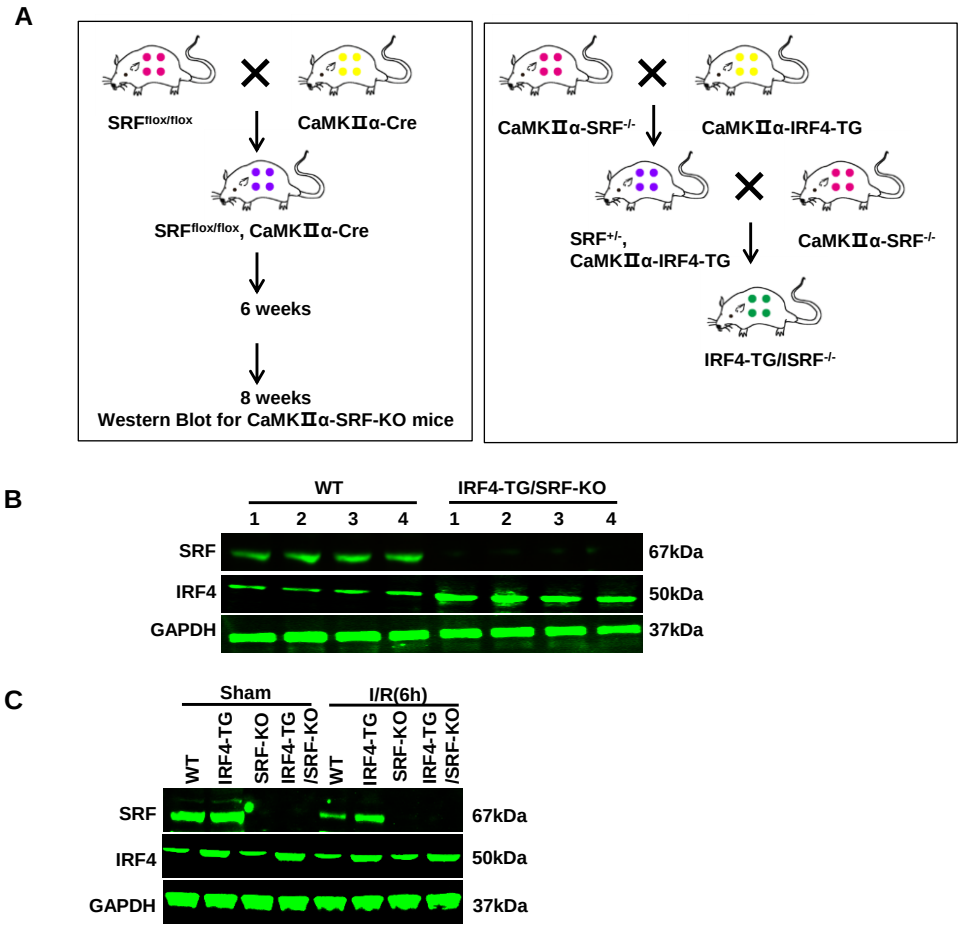
Supplementary Figure S4. Generation of neuron-specific IRF4-transgenic mice. **(a)** Schematic representation of generation of neuron-specific IRF4-transgenic (IRF4-TG) mice. **(b)** Immunoblotting of IRF4 in the brain lysates from IRF4-TG mice (left) and the relative IRF4 levels (right). *P<0.0001, <0.0001, <0.0001, <0.0001 compared with non-transgenic mice (NTG), respectively, unpaired Student's t-test. The values are the means ± SE of three independent experiments in triplicate.

Supplementary Figure S5



Supplementary Figure S5. IRF4 binds to the promoter region of SRF. (a) The primary mouse cortical neurons were transfected with the wild-type (WT-mSRF-Luc) or mutant SRF promoter (Mu-mSRF-Luc) and infected with AdGFP or AdIRF4 prior to the OGD treatment. The luciferase activity was assessed and quantified (bottom panel). The values are the means ± SE of three independent experiments. *P<0.0001 versus AdGFP. NS: not significant, unpaired Student's t-test. **(b)** The putative IRF4 binding sites (P3) were identified in the promoter region of the murine SRF gene (upper panel). P1, P2, P4, and P5 served as negative controls. Lower panel: IRF4 was enriched in the P3 region as shown by chromatin immunoprecipitation (ChIP). *P=0.0015 versus IgG. The values are the means ± SE of three to five independent experiments in triplicate, unpaired Student's t-test.

Supplementary Figure S6



Supplementary Figure S6. Generation of neuron-specific SRF knockout (SRF-KO) mice and IRF4-TG/SRF-KO mice. (a) Schematic representation of generation of SRF-KO (left panel) and IRF4-TG/SRF-KO mice (right panel). **(b)** Immunoblotting of SRF and IRF4 in WT and IRF4-TG/SRF-KO mice. GAPDH served as a loading control. **(c)** IRF4-TG, SRF-KO and IRF4nTG/SRF-KO mice were subjected to a sham operation or 24-h I/R. Using immunoblotting, the levels of SRF and IRF4 were detected in brains prior to and after I/R. GAPDH served as a loading control.

Supplementary Table S1

	WT	IRF4-KO	NTG	IRF4-TG
pH	7.333 ±0.032	7.263 ±0.056	7.348 ±0.015	7.323 ±0.027
pO_2 (mmHg)	125.50 ±2.02	126.00 ±5.73	116.75 ±2.63	125.25 ±4.66
pCO_2 (mmHg)	32.25 ±2.92	33.25 ±3.45	35.50 ±3.43	31.50 ±2.25
DBP (mmHg)	105.98 ±1.78	106.59 ±2.15	104.40 ±2.15	105.41 ±2.68
SBP (mmHg)	132.73 ±1.90	137.64 ±3.17	134.72 ±1.57	136.24 ±0.96
Heart rate (b.p.m.)	616.08 ±23.46	610.92 ±9.03	640.69 ±13.06	609.64 ±13.31

Supplementary Table S1. Physiological variables of experimental groups before MCAO. Blood gas analysis, blood pressure, and heart rate were comparable between groups. Data represent mean ± SE from 8 mice of each genotype, post hoc Tukey's test with Bonferroni correction. WT: wild-type; KO: neuron-specific IRF4 knockout; NTG: non-transgenic; TG: neuron-specific IRF4 transgenic. SBP: systolic blood pressure; DBP: diastolic blood pressure.

Supplementary Table 2

Primers used for Real-time PCR.

Genes	Sequence 5'-3'
SRF-FWD-A	TTCAGCAAGAGGAAGACGGGCA
SRF-REV-A	AGTTTGCGGGTGGCAAAGGT
BDNF-FWD-B	AACGTCCACGGACAAGGCAACTT
BDNF-REV-B	CCAAAGGCACTTGACTGCTGAGCA
c-fos-FWD-A	CGGGTTTCAACGCCGACTA
c-fos-REV-A	TGGCACTAGAGACGGACAGAT
fos-B-FWD-B	AGGAACGCCTGGAGTTTGTCCT
fos-B-REV-B	AGCCGTCTTCCTTAGCGGATGT
Junb-FWD-A	CTATCGGGGTCTCAAGGGTC
Junb-REV-A	CTGTTGGGGACGATCAAGC
Egr1-FWD-A	TATGAGCACCTGACCACAGAG
Egr1-REV-A	GCTGGGATAACTCGTCTCCA
Egr2-FWD-A	TCAGTGGTTTTATGCACCAGC
Egr2-REV-A	GAAGCTACTCGGATACGGGAG
Egr3-FWD-B	TTGCCTGACAATCTGTACCCC
Egr3-REV-B	TAATGGGCTACCGAGTCGCT
Cyr61-FWD-B	CTCCAGAATCTACCAAAACGGG
Cyr61-REV-B	CGTCCAGGGAGTCCTTAATGC
Ctgf -FWD-B	AGACCTGTGGGATGGGCAT
Ctgf -REV-B	GCTTGGCGATTTTAGGTGTCC
Gelsolin-FWD-A	ACCAGCTCTGCTGCATCAGGTA
Gelsolin-REV-A	GCACAGGCACCAGGTCAAACTT
Psd95-FWD-A	ACCAGAAGAGTATAGCCGATTCG
Psd95-REV-A	GGTCTTGTCGTAGTCAAACAGG

Sema3a-FWD-A	TGGCAAAGCCTGTGCAGAATGC
Sema3a-REV-A	TGGTGCTGCAAGTCAGAGCAGT
Ngf-FWD-B	AGACTCCACTCACCCCGTG
Ngf-REV-B	GGCTGTGGTCTTATCTCCAAC
Cdk5-FWD-A	GGGAAGGCACCTATGGAACTG
Cdk5-REV-A	CAGCCTGACACGCTTCAGAG
Actb-FWD-A	GTGACGTTGACATCCGTAAAGA
Actb-REV-A	GCCGGACTCATCGTACTCC
Actg2-FWD-A	TGAGATGGCCACAGCAGCTTCA
Actg2-REV-A	CCATGCCAATGAAGGAAGGCTGG
Npas4-FWD-A	TGCTCTGGATGCTGATCGCCTT
Npas4-REV-A	AGCGGTGAACACAGGGTTTCCT
Nur77-FWD-B	CTCCCCGAGCCAGACTTATG
Nur77-REV-B	TGTCACGGTTCGGAGAGGT
BCL2-FWD-B	CGTCGTGACTTCGCAGAGAT
BCL2-REV-B	TGACATCTCCCTGTTGACGC
GAPDH-FWD-A	ACTCCACTCACGGCAAATTC
GAPDH-REV-A	TCTCCATGGTGGTGAAGACA

Supplementary Table S3

Primers used for ChIP assays

Genes	Sequence 5'-3'
m-SRF-F1	TCCACATTGCTGTTCATCAC
m-SRF-R1	GCTGGTAGTCCTGGGTTCTA
m-SRF-F2	GCTCCTTCCTCTCTGATGACT
m-SRF-R2	CACACAAACAAGTGACCTGGT
m-SRF-F3	CAATCCTCCTGTCTCAACCTC
m-SRF-R3	GAGTCTGAATCTTTGCTCCAAG
m-SRF-F4	GGATGATGAACGATGTGACC
m-SRF-R4	AGGGCAGGGATAGATTGGA
m-SRF-F5	CAACACTCCACTACTCTCCAAC
m-SRF-R5	CATAGACATACCGAACTCGCT

Supplementary Table S4

Primers used for vector construction in luciferase reporter gene assays.

Genes	Sequence 5'-3'
SRF-IRF4-F	GTTGGTACCTCAAGTTGATGCCAAACCAG
SRF-IRF4-R	GACAAGCTTCTCACTTTCCTGCCCCTATG
SRF-IRF-MF	GCCAGATTTAAATAGAGTAACATAGAAGTGCAAGA
SRF-IRF-MR	ACTTCTATGTTACTCTATTTAAATCTGGCCGGACG