Supplemental Experimental Procedures

Hypoxia Studies with Pimonidazole *in vivo*

The Hypoxyprobe™-1(HP-1, Burlington, MA, USA, HP1-100Kit) immunofluorescent analysis can be used to evaluate the hypoxia in brain tissues with a pO_2 below10mmHg, as previously described[1]. Briefly, the Hypoxyprobe™ (pimonidazole) is resuspended at a concentration of 30 mg/ml in 0.9% sterile saline. The mice were injected with the pimonidazole solution at a recommended dose of 60 mg/kg through the tail veins after the surgery. After 90 minutes of pimonidazole circulation *in vivo*, the mice were anesthetized intraperitoneally with 40 mg/kg sodium pentobarbital and transcardially perfused with PBS. Subsequently, brains were typically harvested and placed in a cryo-embedding medium (Sakura-Finetek USA 4583). The embedded brains were cut into 7-µm thick sections that were placed on microscope slides for staining. After being fixed in cold acetone for 10 min, the sections were washed with PBS three times and blocked with 10% normal goat serum for 1 h at RT. Subsequently, the sections were incubated overnight at 4 °C with mouse monoclonal anti-pimonidazole antibody (MAb1) at a 1/50 dilution in PBS. After being washed with PBS three times, the slides were then incubated with secondary antibody conjugated with FITC (ZSGB-Bio, ZF-0311,1:100) for 1 h at RT. Finally, the slides ultimately were mounted with mounting solution DAPI (ZSGB- Bio, ZLI-9557) and a coverslip. Fluorescence images were captured using a fluorescence microscope (Ti2E, Nikon, Tokyo, Japan). The mean fluorescence intensity (MFI) of the fluorescence staining was quantified using the ImageJ software.

Viral transfection

Lentivirus harboring mouse HIF-1α expression vector JLVO-EF1a-APuro (JLV-HIF-1α) was generated and amplified as previously described[2] with the assistance of JTS scientific (Beijing, China). Lentivirus containing anti-puromycin JLVO-EF1a-APuro (JLV-Puro) was utilized as a negative control (NC). bEnd.3 cells cultured in 6-well plates to 70% confluence were transfected with lentivirus supernatant (JLV-HIF-1α, JLV-Puro) diluted with DMEM containing 10% FBS and transfection enhancer polybrene, which was replaced by fresh medium containing 1ug/ml puromycin 24 h after transfection. The transfected bEnd.3 cells were harvested after 72h for confirming the transfection efficiency by western blot analysis.

MRA experiments and methods.

MRA results were obtained by using a Biospec 94/30 preclinical system (Bruker) operating at 400 MHz (9.4 T) equipped with a gradient coil of 12 cm inner diameter, a maximum gradient strength of 660 mT/m and mice head orthogonal coil. The mice were initially anesthetized with 3–4% isoflurane in oxygen before MRA scanning and with 1.5% during scanning. The mouse head was firmly placed in a tooth bar and ear bar. The core body temperature was controlled to 37℃ via a controlled warm air system (Thermo Scientific SC100, Waltham, MA, USA) and the vital signs including body temperature and respiration rate were continuously monitored (SA Instruments, Stony Brook, NY, USA). Angiography was scanned with 3D PCA (Phase contrast) method, field of view (FOV) 20 × 20 x 16.05 mm^2, matrix 256 × 256x214, repetition time (TR) 20 ms, echo time (TE) 3.7 ms; flip angle (FA) 25°; and number of averages (NEX) 2.

Morris Water Maze Test

Cognitive function related to spatial learning and memory ability was assessed through the Morris water maze 30 days after the surgery as previously described[3]. A large circular pool with a

diameter of 150 cm and a depth of 50 cm was used in this experiment and the pool was filled with 23°C water. A white platform with a diameter of 10 cm was placed in the white pool. Mice were put into the water facing the wall of the pool and trained to escape from water to the hidden platform (1.0 cm below the surface of water). During training, visual cue placed around the pool could help the mice relate the spatial environment to the platform location. Mice were trained 4 times a day with the starting position different each trial and the training lasted for 5 consecutive days. In each trial, the mice were given 60 s to find the platform and the software would stop the trial if the mice could find the platform in 60 s. The escape latency in each trial was recorded and analyzed by ANOVA.

Supplement Reference

1. Aguilera KY and Brekken RA. Hypoxia Studies with Pimonidazole in vivo. *Bio Protoc* 2014; 4 2014/10/05. DOI: 10.21769/bioprotoc.1254.
2. Sun J, Shen H, Shao L, et al. HIF-1α overexpression in mesenchymal stem cell-derived exosomes mediates cardioprotection in myocardial infarction by enhanced angiogenesis. *Stem Cell Res Ther* 2020; 11: 373. 2020/08/30. DOI: 10.1186/s13287-020-01881-7.
3. Bromley-Brits K, Deng Y and Song W. Morris water maze test for learning and memory deficits in Alzheimer's disease model mice. *J Vis Exp* 2011 2011/08/03. DOI: 10.3791/2920.

Supplemental Legend

Figure S1. Cx43 knockdown does not affect the development of cerebral vasculature. (A) Representative MRA images from the bottom (left) and sagittal (right) view of the brains of $Cx43^{+/+}$ and $Cx43^{+/+}$ mice (n=6).

Figure S2. Cx43 regulated VEGF-induced angiogenesis through the HIF-1α-PKA signaling pathway. (A) Representative images of western blots showing HIF-1α, AKT, p-AKT and VEGF expression in the whole mouse brain 7 d after surgery. (B) Quantitative analysis of HIF-1α, AKT, p-AKT and VEGF protein level relative to GAPDH. ****P<0.0001 **P<0.01 versus sham-operated mice, ####P<0.0001 versus BCAS-operated $Cx43^{+/+}$ mice, n≥6 mice for each group.

Figure S3. Tight junctions have no significant changes at 3 days after surgery. (A) Representative images of western blots for ZO-1 and claudin-5. (B) Quantitative analysis of the band intensities relative to β-actin. n≥6 mice for each group.

Supplemental Figure

Full unedited gel/blot for Figure X

Figure 1B

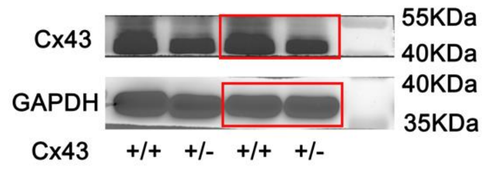


Figure 2C

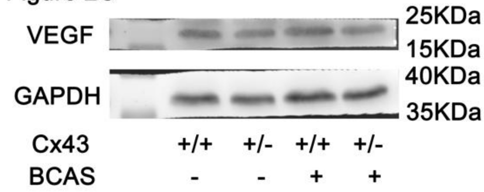


Figure 3B

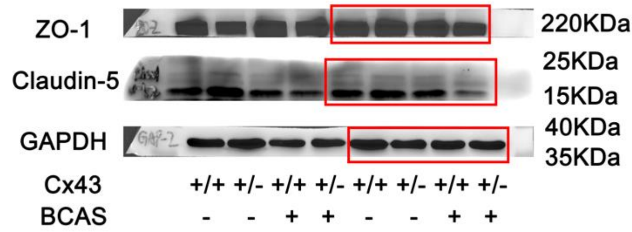


Figure 4C

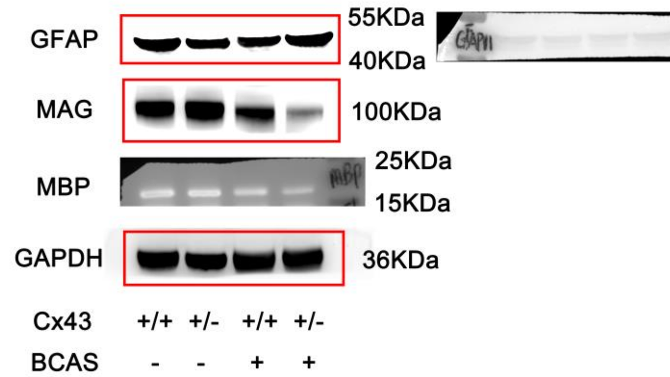


Figure 5A

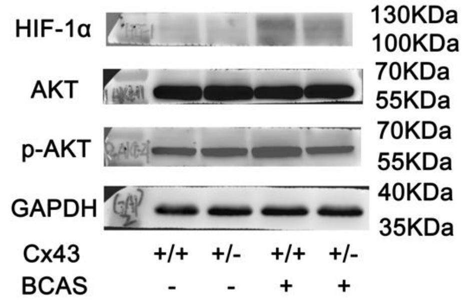
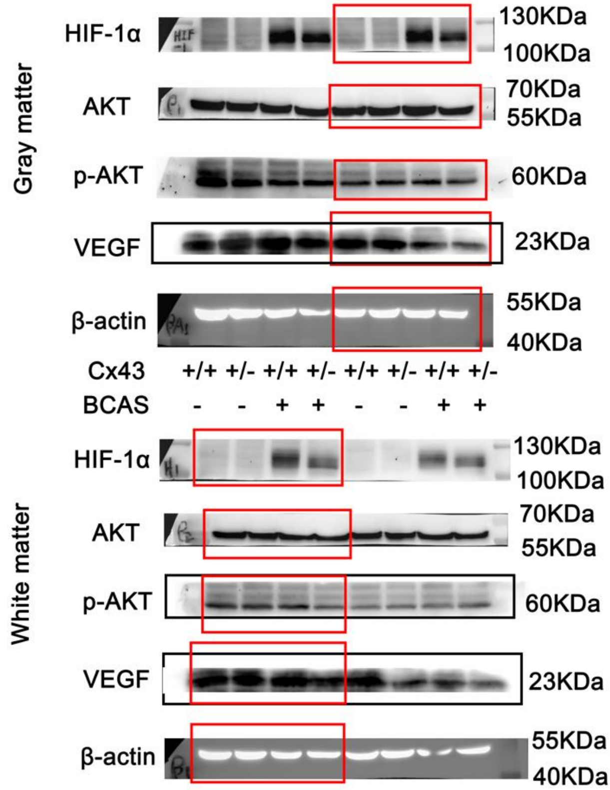


Figure 5C



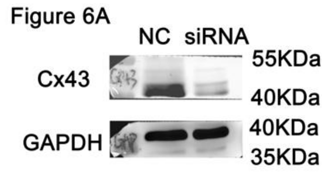


Figure 6A
NC siRNA
Cx43
GAPDH
55KDa
40KDa
40KDa
35KDa

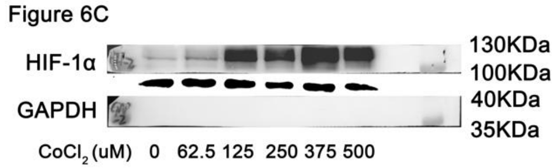


Figure 6C
HIF-1α
GAPDH
130KDa
100KDa
40KDa
35KDa
CoCl2 (uM) 0 62.5 125 250 375 500

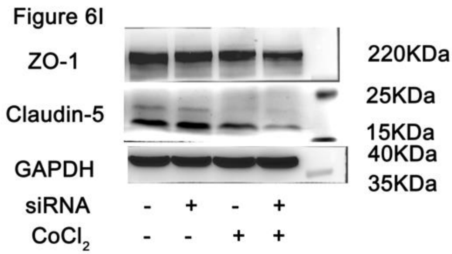


Figure 6I
ZO-1
Claudin-5
GAPDH
220KDa
25KDa
15KDa
40KDa
35KDa
siRNA - + - +
CoCl2 - - + +

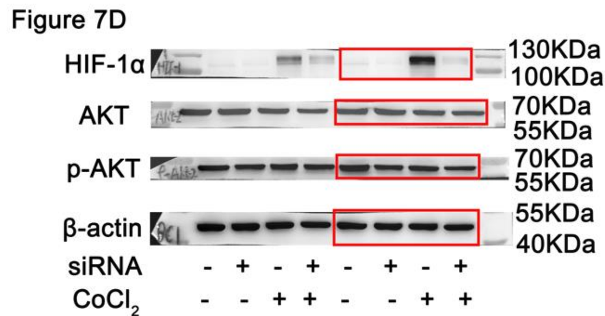


Figure 7D
HIF-1α
AKT
p-AKT
β-actin
130KDa
100KDa
70KDa
55KDa
70KDa
55KDa
55KDa
40KDa
siRNA - + - + - + - +
CoCl2 - - + + - - + +

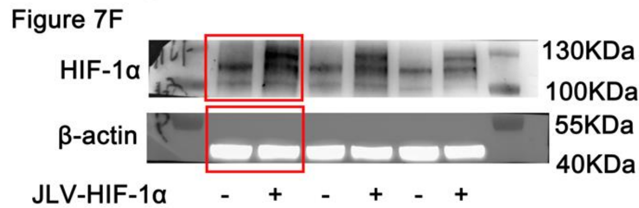


Figure 7F
HIF-1α
β-actin
130KDa
100KDa
55KDa
40KDa
JLV-HIF-1α - + - + - +

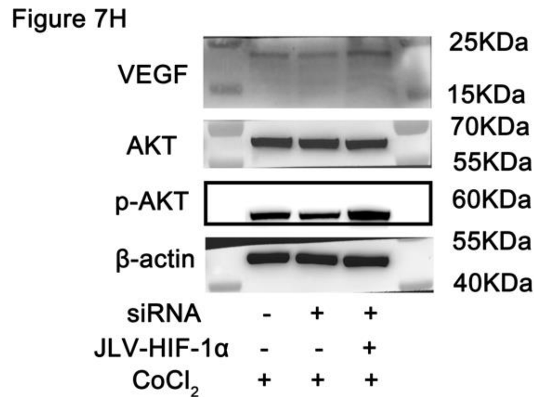


Figure 7H
VEGF
AKT
p-AKT
β-actin
25KDa
15KDa
70KDa
55KDa
60KDa
55KDa
40KDa
siRNA - + +
JLV-HIF-1α - - +
CoCl2 + + +

Figure S2A

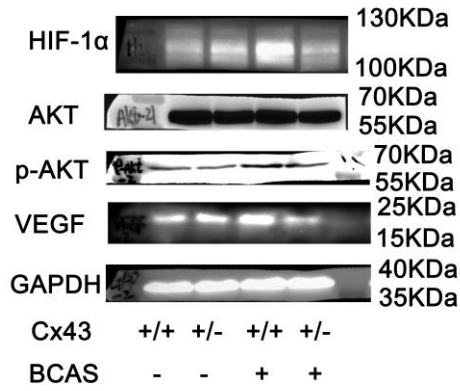


Figure S3A