SUPPLEMENTARY MATERIALS AND METHODS

Immunofluorescence labeling and confocal imaging. Staining procedures were performed following standard approaches as follows. To account for possible inter-experimental variability in fluorescence signal intensity, staining during each experiment was performed on specimens in parallel. Middle cerebral arteries (MCAs) of rat were dissected out, rinsed with phosphate buffered saline (PBS) and fixed with 4% paraformaldehyde (PFA) on ice for 30 min. MCAs were permeabilized with 0.5% Triton X-100 in PBS at room temperature for 30 min. After thoroughly washing, blocking was carried out in an Antibody Dilution Buffer (ADB) supplemented with 50 µL/mL goat serum on ice for 30 min. The ADB consisted of 1% bovine serum albumin (BSA) in PBS. For immunostaining, arteries were incubated at room temperature for 2 hours in the following primary antibodies: rabbit polyclonal antibody with an epitope on the TPD2 (90-103 aa) against the BK β1 subunit (5:1,000; PA1-924, Thermo Fisher Scientific, Waltham, MA) or anti CD-31 (5:1,000, ab33858, Abcam, Cambridge, MA). After primary antibody washout, the arteries were incubated at room temperature in the dark for 2 hours in a goat anti-rabbit preabsorbed secondary antibody conjugated with Cy5 (1:1,000, ab6564, Abcam, Cambridge, MA) and goat anti-mouse preabsorbed secondary antibody conjugated with Alexa488 dye (1:1,000, ab6879, Abcam, Cambridge, MA). After washout, cellular nuclei were stained with DAPI (Life Technologies Corporation, Willow Creek Road Eugene, OR). After each stage, washing with PBS was performed at room temperature 3 times for 5 min using a 96-well plate and a benchtop orbital shaker (VWR Scientific Products, Radnor, PA). The cover slips were mounted using the ProLong AntiFade kit (P10144, Invitrogen, Carlsbad, CA). The cover slips were then dried for 24 hours at room temperature in the dark, and subsequently sealed using clear nail polish.

The specimens were imaged using 40x, imaging was performed using 405 nm (DAPI), 488 nm (Alexa488), and 635 nm (Cy5) laser lines of the Olympus FV-1000 laser scanning confocal system (Tokyo, Japan). Sequential line acquisition was used to minimize the probability of fluorescence emission crossover. The acquisition settings of the confocal

microscope system remained unchanged throughout the imaging of all immunostained specimens. Z-stacks were obtained for each artery segment, with the step of 1 μm.
The fluorescence was quantified using a built-in function in FV10-ASW 3.1 software (Olympus American Inc., Center Valley, PA). Three artery segments of equal size were imaged from each artery. For fluorescence quantification, one layer of an artery segment within a z-stack was selected based on the sharpness of the DAPI nuclear staining. For tunica media I and II, quantification was performed from the slides located at 19-29% and 71-87% of the z-stack thickness, respectively. Tunica intima was quantified from the slides located at 50-53% of the z-stack. Background fluorescence outside the artery edge was subtracted from the mean pixel intensity of each artery segment. Resulting values were used for averaging and for statistical analysis by one-way ANOVA followed by Tukey post-test.

Cerebral artery myocyte isolation and patch-clamp recordings. Middle cerebral arteries were placed into dissociation medium (DM) with the following composition (mM): 0.16 $CaCl_2$, 0.49 EDTA, 10 HEPES, 5 KCl, 0.5 KH_2PO_4, 2 $MgCl_2$, 110 NaCl, 0.5 NaH_2PO_4, 10 $NaHCO_3$, 0.02 phenol red, 10 taurine, 10 glucose, pH=7.4. DM was supplemented with 0.03% papain, 0.05% bovine serum albumin (BSA), and 0.004% dithiothreitol, and arteries were incubated in this solution at 37ºC for 4 min in a shaking water bath. Then, the supernatant was replaced with DM containing 0.06% soybean trypsin inhibitor, 0.05% BSA, and 2% collagenase (26.6 units/ml). The artery was incubated again in a shaking water bath at 37ºC for 4 min. Finally, the supernatant was replaced with DM containing 0.06% soybean trypsin inhibitor. Tissue-containing DM was pipetted using borosilicate Pasteur pipettes with fire-polished tips. The resulting cell suspension was stored in ice-cold DM containing 0.06% BSA, and the cells were used for patch-clamp recordings up to 3 h after isolation.

Patch-clamp experiments on freshly isolated mouse arterial myocytes. BK currents at single channel resolution were recorded from excised, inside-out membrane patches. Both bath and electrode solutions contained (mM) 130 KCl, 5 EGTA, 2.44 $MgCl_2$, 15 HEPES, 1.6 HEDTA, pH 7.35. Free Ca^{2+} was calculated with MaxChelator Sliders (C. Patton, Stanford University, CA), achieved by adding $CaCl_2$ stock solution to render the desired calcium concentration, and validated experimentally using Ca^{2+}-selective electrodes (Corning Incorporated Science Products Division, Corning, NY). An agar bridge with Cl^- as the main anion was used as a ground. Membrane patches were excised from myocytes at 3 μM free Ca^{2+} in bath and pipette solutions. When required, solutions with 30 μM and 1 mM free Ca^{2+} were applied onto the

patches by using an automated drug delivery system (Octaflow, ALA Scientific Instruments, Farmingdale, NY) via a micropipette tip with an internal diameter of 100 μm. To achieve BK channel block, paxilline was added to the dish with dispersed myocytes from a 22.8 mM stock solution in dimethyl sulfoxide to render final concentration of 1 μM (Zhou and Lingle, 2014).

Experiments were carried out at room temperature (21°C). Ionic currents were recorded with an EPC8 amplifier (HEKA) at 1 kHz using a low-pass, eight-pole Bessel filter. Data were digitized at 5 kHz using Digidata 1320A and pCLAMP 8.0 (Molecular Devices, San Jose, CA).

References

Zhou Y, Lingle CJ (2014) Paxilline inhibits BK channels by an almost exclusively closed-channel block mechanism. *J Gen Physiol* **144:** 415-440.