SUPPLEMENTARY DATA

METHODS

Animals

Procedures for the maintenance and use of TREK+/+ and TREK-1−/− mice (C57BL/6J background, 20 to 30 g) were carried out in accordance with the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (NIH Publication No. 85-23, revised 1996).

Immunohistochemistry

Frozen skin sections 12 µm thick were postfixed with 4% paraformaldehyde, permeabilized in 0.3% polyoxyethylensorbitan monolaurate (TWEEN20, Sigma) and blocked with 3% goat serum/ phosphate buffered saline (PBS). The sections were stained using the following antibodies and dilutions: anti-TREK-1 (1/5000), anti-CD-31 (1/100, MAB1393 and 1/300, CBL1337, Chemicon) and secondary antibodies conjugated with Alexa Fluor 488 or 594 (1:1000; Molecular probes). Confocal microscopy observations were performed with a Laser Scanning Confocal Microscope (TCS SP, Leica) equipped with a DMIRBE inverted microscope, using a Plan Apo 63x/1.4 NA oil immersion objective.

In situ hybridization and electron microscopy

Perfused scalp sections (30 µm) were washed twice in 4X SSC/1X Denhart (30’), 4X SSC (10’) and acetylated in 0.1M triethanolamine/ acetic anhydride (100/0.25, 10 min, RT). Hybridization of DIG-dUTP labeled TREK-1 oligoprobe (CAC AAT GGT CCT CTG GGA AAT CTC CTG AGG) was performed at 42° C overnight. After several washes sections were incubated 1 hour with 1nm gold-conjugate anti-DIG antibody diluted 1/50 (Roche) in 5% sheep serum, washed 4 times in PBS and water, and treated with a set of silver enhancement reagents (20 min) (BBI international), washed in PBS (twice during 5 min), fixed with 0.1M Cacodylate/1% Glutaraldehyde (20 min in the dark). Ultrathin sections were prepared and
then transferred on 300 mesh formvar-grids. Grids were stained with 1% uranyl acetate, and observed using a Phillips CM-10 electron microscope.

**Animal preparation for in vivo experiments**

Two days prior to the *in vivo* experiments, hair was removed to present hairless areas for the cutaneous laser Doppler flow measurements. The experiments were performed in an incubator maintained at 30°C in order to better control cutaneous temperature throughout the experiment (35.5 ± 0.5°C). Animals were anesthetized with thiopental (50 mg kg⁻¹ body weight, i.p.). Blood pressure was monitored non invasively throughout the experiment using a tail cuff system (IITC INC/life science instruments). Mice were maintained in the prone position and the head was fixed on a frame. Non-invasive blood pressure was recorded before and after local pressure application and iontophoretic stimulation. Cutaneous blood flow and cutaneous temperature were continuously recorded by a data acquisition system (Biopac) and subsequently analyzed (Acknowledge Biopac).

**Role of TREK-1 in blood pressure response.**

Systolic blood pressure (SBP) was measured via a tail cuff system in anesthetized mice. SBP values were derived from an average of 6 measurements per animal at each time point (before and 1, 5, 10 and 15 minutes following stimulus). A physiological stress was induced by pinching the tail for 30 s in 9 TREK-1⁻/⁻ mice and 10 TREK-1⁺/⁻ mice. The pharmacological pressor response was induced by a bolus injection of angiotensin II (angII, 0.5 mg/kg, ip) in 7 TREK-1⁻/⁻ mice and 7 TREK-1⁺/⁻ mice. The pharmacological depressor response was induced by a bolus injection of sodium nitroprusside (SNP, 1 mg/kg, ip) in 7 TREK-1⁻/⁻ mice and 8 TREK-1⁺/⁻ mice.

**Data analysis**

The results are presented as means ± SEM. Significance of the differences between TREK-1⁻/⁻ mice and TREK-1⁺/⁻ mice was determined by ANOVA (1-factor ANOVA or ANOVA for
consecutive measurements, when appropriate). Means were compared by paired $t$ test or by the Bonferroni test for multigroup comparisons. Values of $P<0.05$ were considered to be significant.
Supplementary figure 1. TREK-1 in blood pressure response.
A: Systolic arterial blood pressure (SBP) in response to physiological stress induced by pinching the tail of TREK-1-/- mice (n=9) and TREK-1+/+ mice (n=10) for 30 s. Each point represents the systolic arterial blood pressure before (PA0) and 1 (PA1), 5 (PA5), 10 (PA10) and 15 (PA15) minutes following the stimulus.
B: Systolic arterial blood pressure (SBP) in response to bolus injection of angiotensin II in TREK-1-/- mice (n=7) and TREK-1+/+ mice (n=7). Each point represents the systolic arterial blood pressure before (PA0) and 1 (PA1), 5 (PA5), 10 (PA10) and 15 (PA15) minutes following the stimulus.
C: Systolic arterial blood pressure (SBP) in response to bolus injection of SNP in TREK-1-/- mice (n=7) and TREK-1+/+ mice (n=8). Each point represents the systolic arterial blood pressure before (PA0) and 1 (PA1), 5 (PA5), 10 (PA10) and 15 (PA15) minutes following the stimulus.