Supplementary materials

Supplemental Methods

Identification of smooth muscle-specific TMEM16A transgenic mice (TM^{Tg}).

Genotypes of TMEM16A transgene and Tagln-Cre offspring were examined by PCR on tail DNA using Hotstar Taq DNA Polymerase (Qiagen, Valencia, CA), and the specific expression of TMEM16A in vascular smooth muscle were confirmed by western blotting as previously described (Ma *et al.*, 2017). The primers of PCR are as follows: Tg-TMEM16A (376bp), Fw: 5'-TCATGTCTGGA TCCCCATCAAGC-3', Rw: 5'-GAGTACTTCTCGGGGACCCTCA-3'; Tagln-Cre (~100bp), Fw: 5'-GCGGTCTGGCAGTAAAAACTATC-3', Rw: 5'-GTGAAACAGCATTGCTGTCACTT-3'.

Reverse-transcription polymerase chain reaction (RT-PCR)

RT-PCR was performed as previously described (Ma *et al.*, 2017). Briefly, total RNA was isolated using Trizol reagent (Invitrogen™, Life Technologies, Grand Island, NY). 1 pg RNA was reverse-transcribed in a volume of 50 μl on a Thermal Cycler (Thermo Scientific, Waltham, MA) according to the instructions of One-step RT-PCR Kit (Qiagen, Valencia, CA). Reactants underwent a reverse transcription (50°C, 30 min), an initial denaturation (95°C, 15 min), 35 amplification cycles (94°C, 45 s; 55°C, 45 s; 72°C, 60 s), and a final extension (72°C, 10 min). PCR products were isolated in 1.5% agarose gel containing 0.1% ethidium bromide. The primers of 4 different splicing variants of TMEM16A were performed in Table S1.

Supplemental References

Ma MM, Gao M, Guo KM, Wang M, Li XY, Zeng XL, *et al.* (2017). TMEM16A Contributes to Endothelial Dysfunction by Facilitating Nox2 NADPH Oxidase-Derived Reactive Oxygen Species Generation in Hypertension. Hypertension **69:** 892-901.

Supplemental Table

Table S1. Primers of TMEM16A splicing variants

Splicing variant	Primer pairs (5'-3')
a	Fw: CACAAGAGAGCCTCGGGTAG
	Rw: ATCTTCACAAACCCGACACC
b	Fw: CAAAACCCGGAGCACAATAG
	Rw: CAGGAGTTTCCTGTCGTTGA
c	Fw: CTCTGGGCTGCCACCTTC
	Rw: TGGCTTCATACTCTGCTCTGG
d	Fw: TCCCAGAGCAGAGTATGAAGC
	Rw: AGATGGGGAGGAGTTCATGG
GAPDH	Fw: AGTGCCAGCCTCGTCTCATA
	Rw: TTGTCACAAGAGAAGGCAGC

Fw, forward primer; Rw, reverse primer.

Supplemental Figures

Figure S1

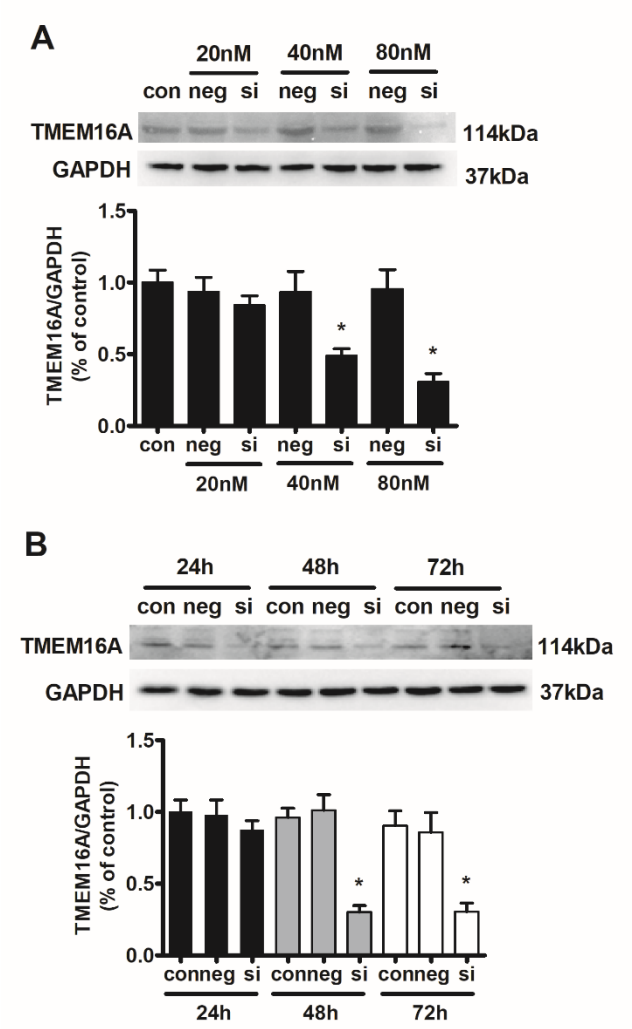

Figure S1. Effect of TMEM16A siRNA transfection on the expression of TMEM16A in basilar artery smooth muscle cells (BASMCs). **A.** Cells were transfected with TMEM16A siRNA in different concentration for 48 h. Western blot showed that the dose of 80 nmol·L^{-1} was suitable for silencing TMEM16A (n=5, *P<0.05 *vs.* con, one-way ANOVA). **B.** Cells were transfected with TMEM16A siRNA in dose of 80 nmol·L^{-1} for different time course. Western blot showed that the time course of 48 h was suitable for silencing of TMEM16A (n=5, *P<0.05 *vs.* con, one-way ANOVA).

Figure S2

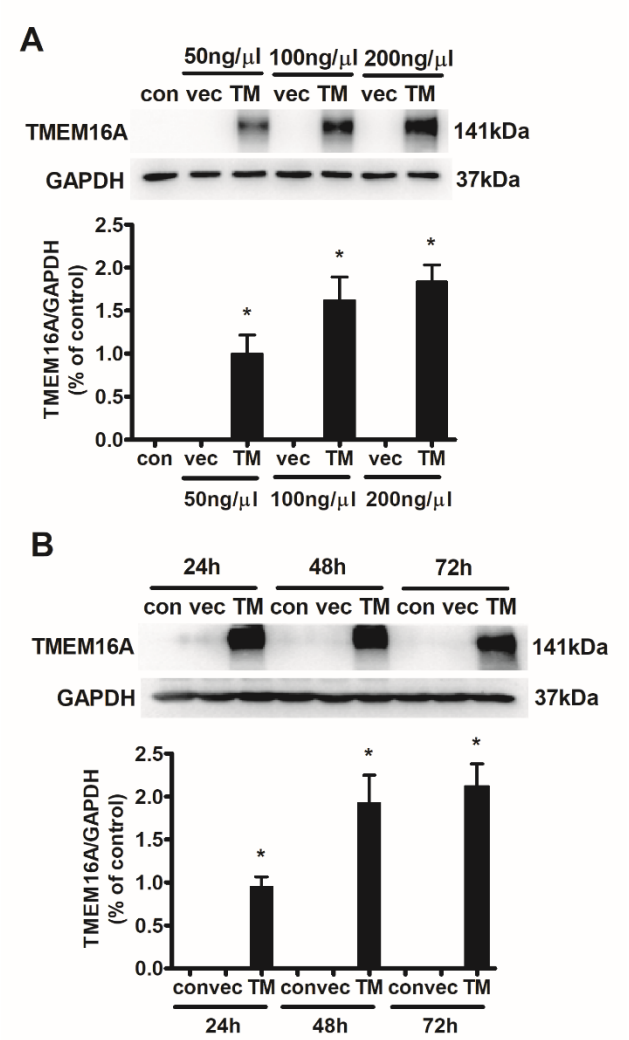

Figure S2. Effect of TMEM16A plasmid transfection on the expression of TMEM16A in BASMCs. **A.** Cells were transfected with TMEM16A plasmid tagged with RFP in different concentration for 48 h. Western blot with anti-RFP primary antibody showed that the dose of 100 ng·μL^{-1} was suitable for overexpressing TMEM16A (n=5, *P<0.05 *vs.* con, one-way ANOVA). **B.** Cells were transfected with TMEM16A plasmid in the dose of 100 ng·μL^{-1} for different time course. Western blot showed that the time course of 48 h was suitable for overexpressing TMEM16A (n=5, *P<0.05 *vs.* con, one-way ANOVA).

Figure S3

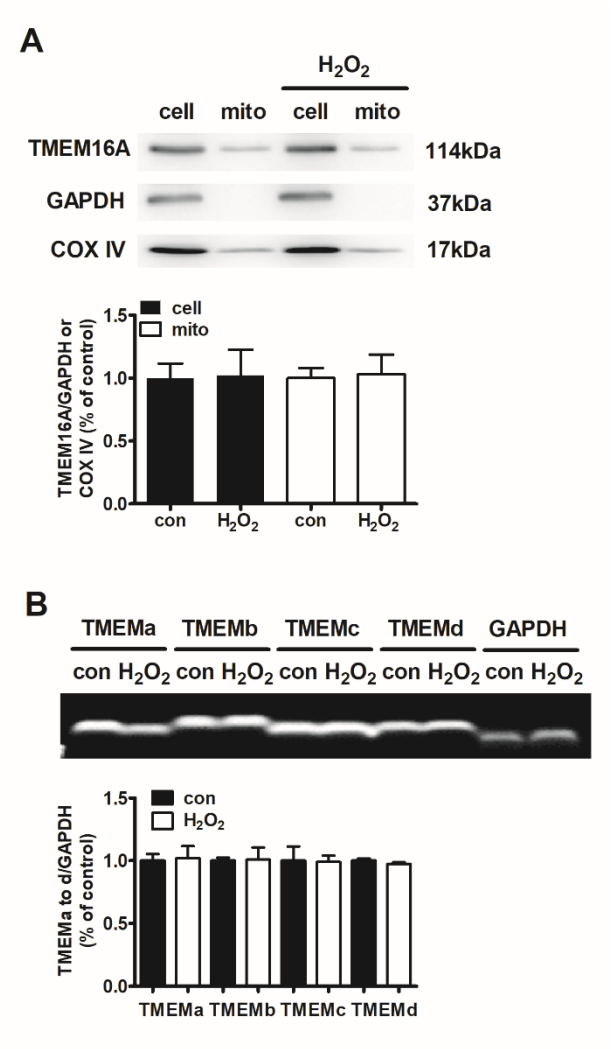

Figure S3. The effect of H_2O_2 (200 μmol·L^{-1}, 24h) on TMEM16A protein and splicing variants mRNA expression in BASMCs. **A.** The protein expression of TMEM16A was not changed in either whole-cell or in the mitochondria under the stimulation of H_2O_2. Cell and mito are abbreviations of the whole-cell and the mitochondria, respectively (n=4, 2-tailed Student's t test). **B**. The mRNA expression of TMEM16A splicing variants were not changed under the stimulation of H_2O_2 (n=4, 2-tailed Student's t test).

Figure S4

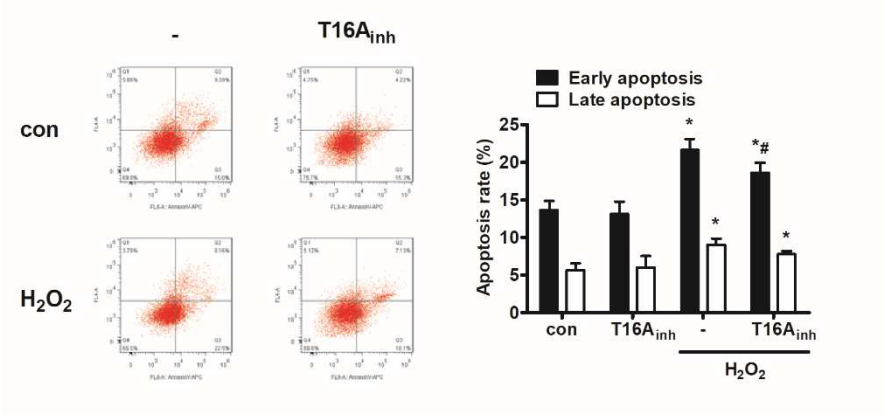

Figure S4. Effect of T16A$_{inh}$-A01 (T16A$_{inh}$) on H_2O_2 (200 μmol·L^{-1}, 24h)-induced apoptosis in BASMCs. T16A$_{inh}$-A01 (10 μmol·L^{-1}) was added 30 min before H_2O_2-treatment. Apoptosis was measured by flow cytometry. (n=5, *P<0.05 *vs.* con, # P<0.05 *vs.* H_2O_2, two-way ANOVA)

Figure S5

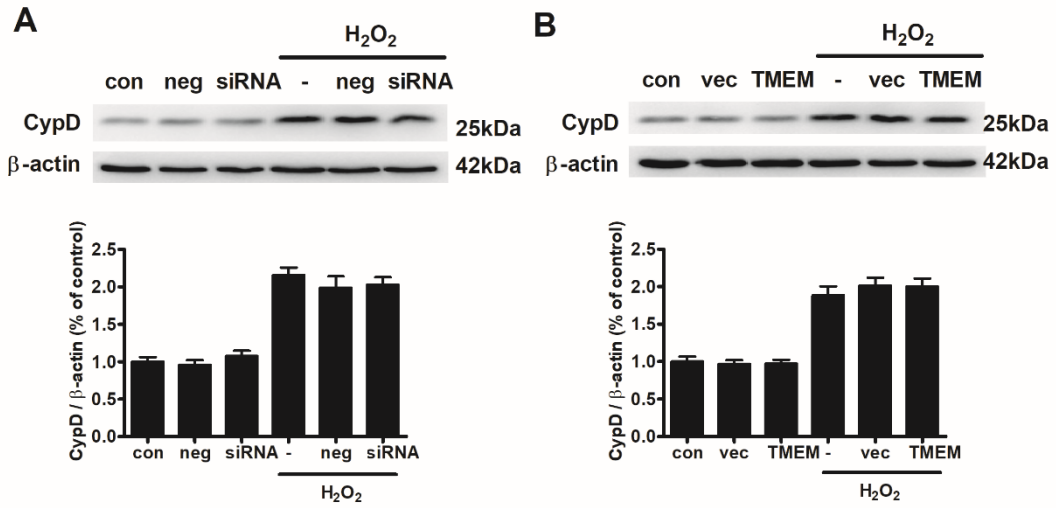

Figure S5. The effect of TMEM16A on cyclophilin D (CypD) expression in BASMCs. **A and B.** The expression of CypD was not affected by TMEM16A silencing (**A**) or overexpressing (**B**). Cells were transfected with TMEM16A siRNA or TMEM16A plasmid for 24 h and added H_2O_2 for additional 24 h. The expression of CypD was measured by western blot (n=6, two-way ANOVA).

Figure S6

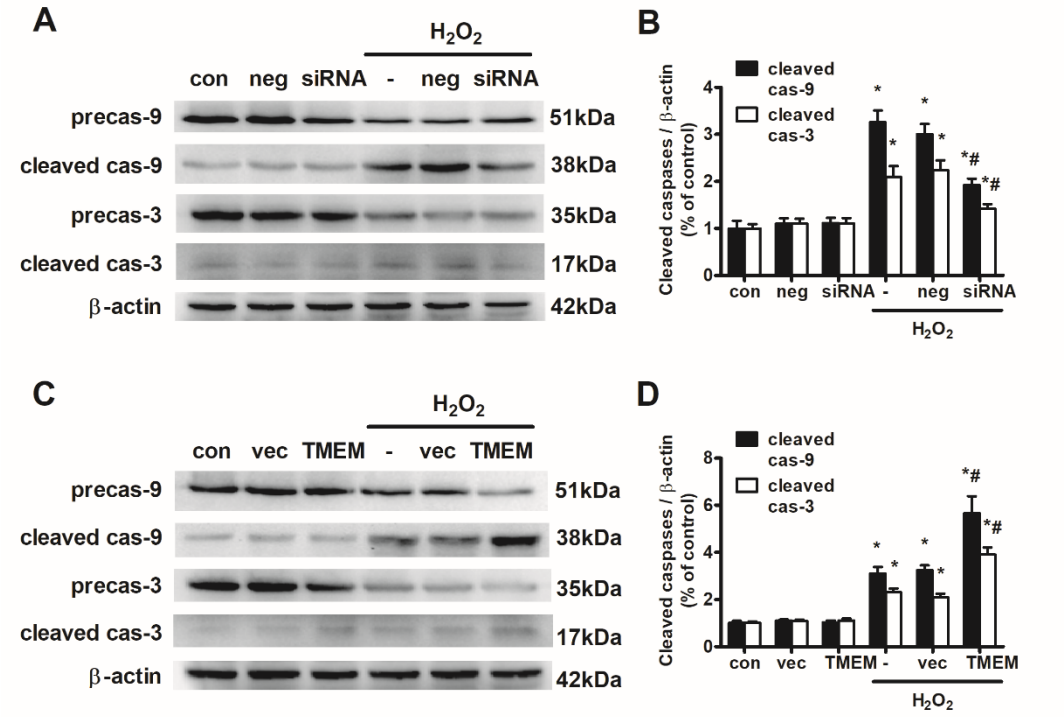

Figure S6. Effects of TMEM16A on H_2O_2-induced caspase-9 (cas-9) and caspase-3 (cas-3) activation. **A and B.** TMEM16A knockdown reversed H_2O_2-induced caspase-9 and caspase-3 activation in BASMCs. Representative western blots are shown in **A**, and the densitometric analysis is shown in **B** (n=6, *P<0.05 *vs.* con, #P<0.05 *vs.* H_2O_2, two-way ANOVA). **C and D.** TMEM16A overexpression enhanced H_2O_2-induced caspase-9 and caspase-3 activation. Representative western blots are shown in **C**, and the densitometric analysis is shown in **D** (n=6, *P<0.05 *vs.* con, #P<0.05 *vs.* H_2O_2, two-way ANOVA)

Figure S7

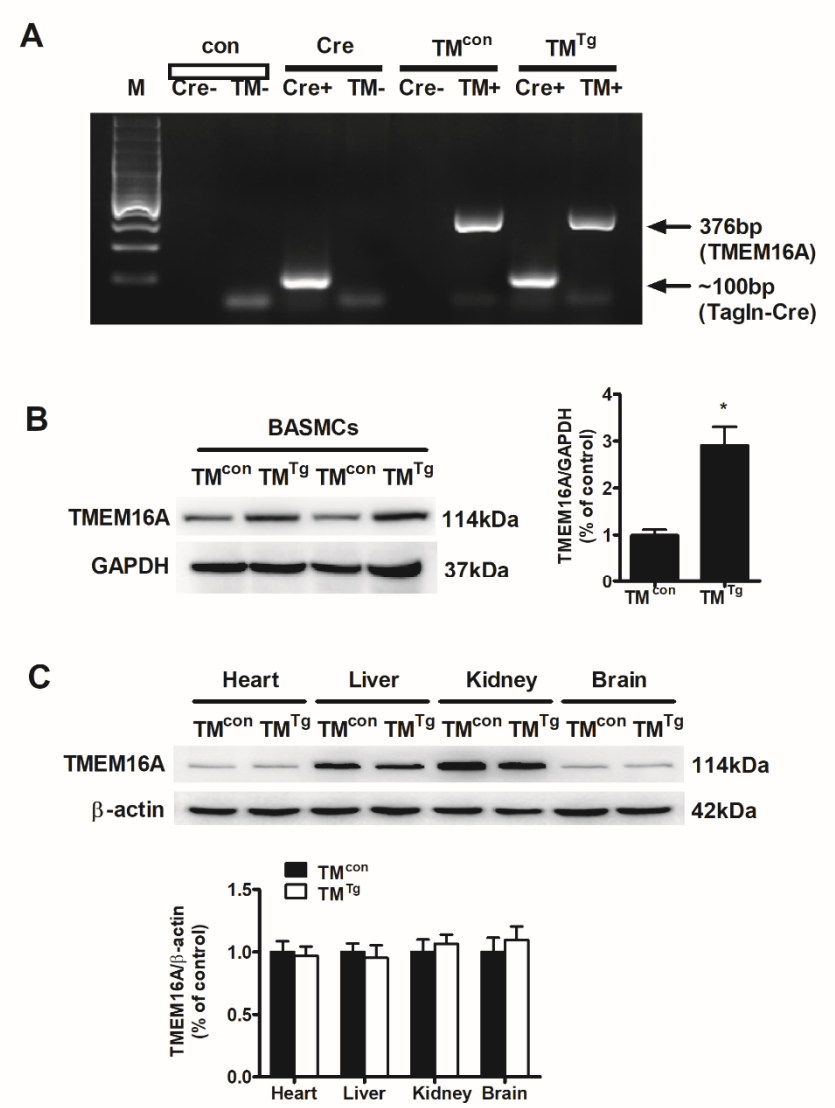

Figure S7. Identification of smooth muscle-specific TMEM16A transgenic mice (TM^{Tg}). **A.** Mice were identified by PCR amplified from genomic DNA using primers specific for transgene-TMEM16A and Tagln-Cre, respectively. Mice containing transgene-TMEM16A and Tagln -Cre were considered as TM^{Tg}. Mice containing transgene-TMEM16A, but not Tagln -Cre were considered as TM^{con}. **B.** The protein expression of TMEM16A was successfully increased in the primary cultured BASMCs isolated from TM^{Tg} compared with that from TM^{con} (n=5, *P<0.05 *vs.* con, 2-tailed Student's t test). **C.** The protein expression of TMEM16A was comparable in other tissues, including heart, liver, kidney, and brain from TM^{con} and TM^{Tg} (n=5, 2-tailed Student's t test).