# Supplement File 1. Materials and methods

## Protein immuno-blot analysis

Whole cell proteins were extracted by cell lysis buffer (NP40 buffer or RIPA buffer; Cell Signaling Technology, Danvers, MA, USA) with proteinase inhibitor cocktail (Roche, Basel, Switzerland) and phosphatase inhibitor cocktail (Millipore, Burlington, MA, USA). Cell lysates were quantified, separated by SDS-PAGE, transferred to membranes and detected by immunoblotting assay following a standard protocol for immunoblotting. Typically, the nitrocellulose membranes were blocked with 5% nonfat milk, and incubated with protein specific antibodies (Supplement Table 1). The membranes were washed and incubated with HRP-conjugated anti-IgG secondary antibodies (1:5,000; Cell Signaling Technology). Then, immunoreactive proteins were detected using an enhanced chemiluminescence detection system (Thermo Fisher Scientific, Rockford, IL, USA).

## qRT-PCR analysis

The qRT-PCR was used to determine the expression of mRNA transcripts from specific genes of interest. Total cell RNA was extracted by TRIzol reagent (Thermo Fisher Scientific). The RNAs were reverse transcribed to cDNA using a high-capacity cDNA reverse transcription kit (Thermo Fisher Scientific). Quantitative real time PCR was conducted using fast SYBR green master mix (Thermo Fisher Scientific). The $2^{-\Delta\Delta Ct}$ method was used to evaluate relative mRNA expression compared to controls. The primer sequences used for qRT-PCR detection of gene expression are listed in the Supplement Table 2. The primers used to determine the expression level of miR-92a were obtained from RiboBio (Guangzhou, China).

## Immunohistochemistry and immunofluorescence analysis

Primary antibodies (Supplement Table 1) were used for immunostaining on formalin-fixed paraffin-embedded tissue slides from the corpus cavernosum (CC) tissues. Briefly, after tissue sections were deparaffinized and rehydrated through graded alcohol, they were heated by microwave in 0.01 mol/L citrate buffer at pH 6.0 for 20 minutes to retrieve the antigens. After a 30-minutes incubation in Dako protein block, tissue sections were incubated in primary antibodies, followed by incubation in HRP polymer conjugated secondary antibody (Dako cat# K4061) or Alexa Fluor 488- or Alexa Fluor 594-conjugated secondary antibody. The specificity of immunoreactions was verified by replacing the primary antibodies with PBS.

## Measurement of the antioxidant activity

CC tissues stored at -80°C were processed and assayed for antioxidant activity. Commercial kits to detect malondialdehyde (MDA) and superoxide dismutase (SOD) were used according to the manufacturer's protocol (Beyotime Biotechnology, Shanghai, China). The penile MDA levels and SOD activities were normalized to the wet weight of the penile tissue samples. Intracellular reactive oxygen species (ROS) in the CC were detected using an oxidation-sensitive fluorescent probe (DCFH-DA; Beyotime Biotechnology).

## Analysis of NO levels and cGMP concentration

The penile tissues were homogenized, centrifuged, and extracted using $H_2O$-saturated diethylether. An enzyme-linked immunosorbent assay kit for testing cGMP (F15182; R&D Systems, Minneapolis, MN, USA) and a nitrate-nitrite assay kit for testing NO (S0024; Beyotime Biotechnology) were used according to the standard protocols. The NO levels and cGMP concentrations were normalized to the protein concentration.