# Supporting Information

## Ma et al. 10.1073/pnas.1113810109

### SI Text

**Measurement of Erectile Response.** Animals were anesthetized and the carotid artery was cannulated (PE-50 tubing) to measure systemic arterial pressure continuously with a transducer (BioPac Systems) connected to a computerized system for data acquisition (Windaq DI 400, DATAQ, Data Systems International, St. Paul, MN). The right jugular vein was cannulated (PE 50 tubing) to administer fluids and supplemental anesthesia. The skin overlaying the penis was incised, and the right crura exposed by removing part of the overlaying ischiocavernous muscle. A 25-gauge needle filled with 250U/mL heparin and connected to PE-50 tubing was inserted into the right crura to provide continuous measurement of intracavernosal pressures. The bladder and prostate were exposed through a midline abdominal incision. The cavernosal nerve was identified posterolateral to the prostate on one side, and an electrical stimulator with a stainless-steel bipolar hook was placed around it. Mean arterial pressure (MAP) and intracavernosal pressure (ICP) were continuously monitored and processed with the data acquisition software (Biopac Systems, Goleta, CA). The cavernous nerve stimulation (CNS) was carried out with a square pulse stimulator (Grass Instruments, Quincy, MA) using 15 Hz frequency and different voltages (2.5 V, 5.0 V, 7.5 V) for a duration of 60 s as described (1). Animals were rested for 3 min between stimulations to allow for sufficient recovery. Total erectile response was determined by measuring the area under the curve (AUC) in mmHg/s from the beginning of stimulation until ICP returned to baseline. The ratio of maximal ICP-to-MAP (mean arterial pressure) was also determined to account for variations in systemic blood pressure.

**qRT-PCR Analysis.** Total RNA was isolated from 2–3 mg of liquid nitrogen-homogenized tissue samples with RNAqueous kit (Ambion, Austin, TX). After treatment with TURBO DNase (Ambion), 1 μg of RNA was reverse-transcribed with random hexamers to obtain first-strand cDNA using iScript cDNA kit (Bio-Rad). Quantification of mRNA for the NOS family, VEGF, TGFβ1, and FGF2 was performed using CFX96 Touch real-time PCR detection system (BioRad, Hercules, CA) and SYBR Green detection kit. Primers used for reverse transcriptase-PCR are described in Table S1. Amplification conditions were as follows: 2 min of preincubation at 50 °C, 10 min at 95 °C for enzyme activation, and 40 cycles at 95 °C denaturation for 10 s, 55 °C annealing for 30 s, and 72 °C extension for 30 s. The comparative threshold cycle (Ct) method was used for the calculation of relative amplification normalized with GAPDH amplification.

**Immunofluorescence (IF).** For IF staining, the penile tissue sections were fixed in paraformaldehyde for 30 min at 4 °C and air dried and incubated overnight with various primary antibodies at 4 °C: rabbit anti-iNOS/NOS Type II polyclonal antibody (1:150 dilution; BD Biosciences); polyclonal rabbit anti-eNOS/NOS Type III (1:250; BD Biosciences); polyclonal rabbit anti-nNOS/NOS Type I (1:250; BD Biosciences); and purified mouse anti-BrdU (Clone Bu20a) antibody (1:400; Biolegend). To prevent nonspecific staining by the secondary antibody, the sections were blocked by 10% goat serum in PBS for 30 min at room temperature. Slides were washed three times and incubated for 1 h at 37 °C with these fluorescence secondary antibodies: Texas Red goat anti-rabbit IgG, Alexa Fluor 488 goat anti-rabbit IgG, goat anti-rabbit or anti-mouse IgG according to the manufacturer's instructions (Invitrogen, Carlsbad, CA). The samples processed without primary antibodies served as negative controls.

**Western Blot Analysis.** Part of the penile tissue was homogenized in a buffer containing 0.32 M sucrose, 10 mM Tris·HCl, pH 7.4, 2 mM EDTA, and 10 μl/mL protease inhibitor (Genotech). After centrifugation (900 × *g* for 10 min), the resulting supernatant was centrifuged at 100,000 × *g* for 1 h, and the final pellet was resuspended in a buffer containing 50 mM Tris·HCl, 10 mM EDTA, 100 mM NaCl, and 8 mM $MgCl_2$, pH 7.4. The proteins (40 μg protein per lane) were fractioned by 10% SDS/PAGE gel electrophoresis and transferred onto polyvinylidene fluoride (PVDF) by semi-dry electroblotting. After blocking (3% BSA for 1 h), the membranes were incubated overnight at 4 °C with primary rabbit antibodies; eNOS (1:800); nNOS (1:750); iNOS (1:1,000); VEGF (1:1,500); TGFβ1(1:1,000); FGF2 (1:1,000) (Abcam, Cambridge, MA) or loading control anti-GAPDH (1:1,000) (Cell Signaling, Danvers, MA). An anti-rabbit IgG antibody conjugated with horseradish peroxidase (Zymed Laboratories, Inc., San Francisco, CA) was used at a 1:2,500 dilution, and protein complexes were visualized using an ECL kit (GE Healthcare, Buckinghamshire, UK) and Kodak X-OMAT film (Eastman Kodak Co., Rochester, NY). Densitometry analysis was performed by Quantity One software (Bio-Rad, Hercules, CA).

1. Kendirci M, et al. (2005) Poly(Adenosine diphosphate-ribose) polymerase inhibition preserves erectile function in rats after cavernous nerve injury. *J Urol* 174:2054–2059.

**Table S1. Real-time PCR primer sequences**

| Gene | Forward primer | Reverse primer |
|---|---|---|
| *eNOS* | TATTTGATGCTCGGGACTGCAGGA | ACGAAGATTGCCTCGGTTTGTTGC |
| *nNOS* | TCTACGCCACAGAGACAGGCAAAT | CATGGACATTGCCTTGGCATCGAA |
| *iNOS* | AACCCAAGGTCTACGTTCAAG | AAAGTGGTAGCCACATCCCG |
| *VEGF* | CAGCTATTGCCGTCCAATTGA | CCAGGGCTTCATCATTGCA |
| *TGFβ1* | AGGACCTGGGTTGGAAGTGG | AGTTGGCATGGTAGCCCTTG |
| *FGF2* | TTCACAGCCTGTGCTCTAGGG | GATCGGGTCAGGTTTTGGAAA |
| *GAPDH* | ATGACTCTACCCACGGCAAG | GGAAGATGGTGATGGGTTTC |