SUPPLEMENTARY DATA

Immunohistochemistry.
For immunohistochemistry, frozen tissue sections (8-μm thick) were incubated with an antibody to FLAG M1 (Sigma-Aldrich, St. Louis, MO; 1:200), then treated with secondary antibodies and developed according to the instructions in the Histostain-SP kit (Invitrogen Co., Carlsbad, CA). Subsequently, sections were stained with hematoxylin. For fluorescence microscopy, the penis tissue was fixed in 4% paraformaldehyde for 24 h at 4°C, and frozen tissue sections (8-μm thick) were incubated with antibodies to platelet/endothelial cell adhesion molecule (PECAM)-1 (an endothelial cell marker, Chemicon, Temecula, CA; 1:50), phosphohistone H3 (Upstate, Temecula, CA; 1:50), fluorescein isothiocyanate (FITC)-conjugated antibody to smooth muscle α-actin (a smooth muscle cell marker; Sigma-Aldrich; 1:200), tetramethyl rhodamine isothiocyanate (TRITC)-conjugated antibody to smooth muscle α-actin (Sigma-Aldrich; 1:200), phospho-eNOS (Ser1177, Cell Signaling, Beverly, MA; 1:25), or nitrotyrosine (a marker of peroxynitrite formation, Upstate Biotechnology, Waltham, MA; 1:50) at 4° C overnight. Control sections were incubated without the primary antibody at this step. After several washes with PBS, the sections were incubated with TRITC- or FITC-conjugated secondary antibodies for 2 h at room temperature. Signals were visualized and digital images were obtained with an Apotome microscope (Zeiss, Göttingen, Germany). We also performed β-gal histochemistry as previously described (1).

The nitrotyrosine-immunopositive area in cavernous endothelial cells or smooth muscle cells was quantitatively analyzed with an image analyzer system (National Institutes of Health [NIH] Image J 1.34, http://rsb.info. nih.gov/ij/index.html). We also determined endothelium and phospho-eNOS-immunopositive area in cavernous tissue as previously described (2). The numbers of phosphohistone H3-immunopositive endothelial cells were counted at a screen magnification of ×400 in 6 or 8 different regions. Values were expressed per high-power field.

In situ detection of superoxide anion.
Hydroethidine (Molecular Probes, Eugene, OR), an oxidative fluorescent dye, was used to evaluate levels of superoxide anion in situ as previously described (2,3). The numbers of ethidium bromide fluorescence-positive endothelial cells or smooth muscle cells were counted at a screen magnification of ×400 in 6 or 8 different regions. Values were expressed per high-power field.

TUNEL assay.
The terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick end labeling (TUNEL) assay was performed by using the ApopTag® Fluorescein In Situ Apoptosis Detection Kit (S7160, Chemicon) as previously described (2). The numbers of apoptotic cells in endothelial cells or smooth muscle cells were counted at a screen magnification of ×400 in 6 or 8 different regions. Values were expressed per high-power field.

Western blot.
The collected penile tissues were immediately stored within liquid nitrogen. The tissues were then homogenized with small volume of liquid nitrogen. This experimental step was repeated several times till changing to the complete powder of tissues. And then, the Ripa buffer (200 μl, a protease inhibitor cocktail, Sigma-Aldrich) was added into the homogenates, and transferred to 1.5 ml eppendorf tubes. After incubating on ice for 20 min, the homogenates were centrifuged at 13,000 g for 10 min at 4°C and the concentrations of each protein in the supernatants were measured using the Universal Microplate Reader ELx800G (BioTek Instruments Inc., Winooski, VT). Equal amounts of protein (100 μg per lane) were electrophoresed on sodium dodecylsulfate-polyacrylamide gels (6% to 10%), transferred to nitrocellulose membranes, and probed with antibodies to FLAG M1 (Sigma-Aldrich; 1:400), eNOS (Transduction Laboratories, Inc., Lexington, KY; 1:300), phospho-eNOS (Ser1177, Cell Signaling;

1:300), $p47^{phox}$ (an active catalytic subunit of NADPH oxidase, Santa Cruz Biotechnology, Santa Cruz, CA; 1:500), inducible NOS (iNOS, Abcam, Cambridge, U.K.; 1:300), vascular endothelial (VE)-cadherin (Santa Cruz Biotechnology; 1:300), zonula occludens-1 (ZO-1, Zymed Laboratories, South San Francisco, CA; 1:300), occludin (Zymed Laboratories; 1:300), claudin-5 (Zymed Laboratories; 1:300), or β-actin (Abcam). The results were quantified by densitometry (n = 4 or 6 per group).

cGMP determinations.
At 2 weeks after intracavernous COMP-Ang1 protein injection (n = 4 per group), the corpus cavernosum tissue was removed and rinsed with PBS, quick frozen in liquid nitrogen, and stored at -70°C until cGMP determination. The samples were then processed according to the instructions provided with the kit (Cayman Chemical, Ann Arbor, MI). Data are expressed as pmol/mg of wet weight tissue.

Measurement of plasma nitrite/nitrate (NO_x) levels.
Production of nitric oxide (NO) in the plasma was evaluated by measuring nitrite/nitrate (NO_x) levels using a commercially available colorimetric assay kit (Cayman Chemical). Blood was extracted by direct cardiac puncture and was collected in chilled EDTA containers and centrifuged, and plasma was stored at -80^{o}C until analysis. Plasma was filtered through a 10-kDa molecular weight cut-off filter (Millipore Co., Bedford, MA) at 12,000 g for 20 min, and incubated with nitrate reductase for 3 h to convert nitrate to nitrite. After incubation, Griess reagents were added to the plasma and total NO_x levels were measured spectrophotometrically at 540 nm (Universal Microplate Reader ELx800G, Bio-Tek Instruments Inc., Highland Park, VT).

SUPPLEMENTAL REFERENCES

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2. Jin HR, Kim WJ, Song JS, Choi MJ, Piao S, Shin SH, Tumurbaatar M, Tuvshintur B, Nam MS, Ryu JK, Suh JK: Functional and morphologic characterizations of the diabetic mouse corpus cavernosum: comparison of a multiple low-dose and a single high-dose streptozotocin protocols. *J Sex Med* 6:3289-3304, 2009
3. Bivalacqua TJ, Usta MF, Kendirci M, Pradhan L, Alvarez X, Champion HC, Kadowitz PJ, Hellstrom WJ: Superoxide anion production in the rat penis impairs erectile function in diabetes: influence of in vivo extracellular superoxide dismutase gene therapy. *J Sex Med* 2:187-197, 2005

SUPPLEMENTARY DATA

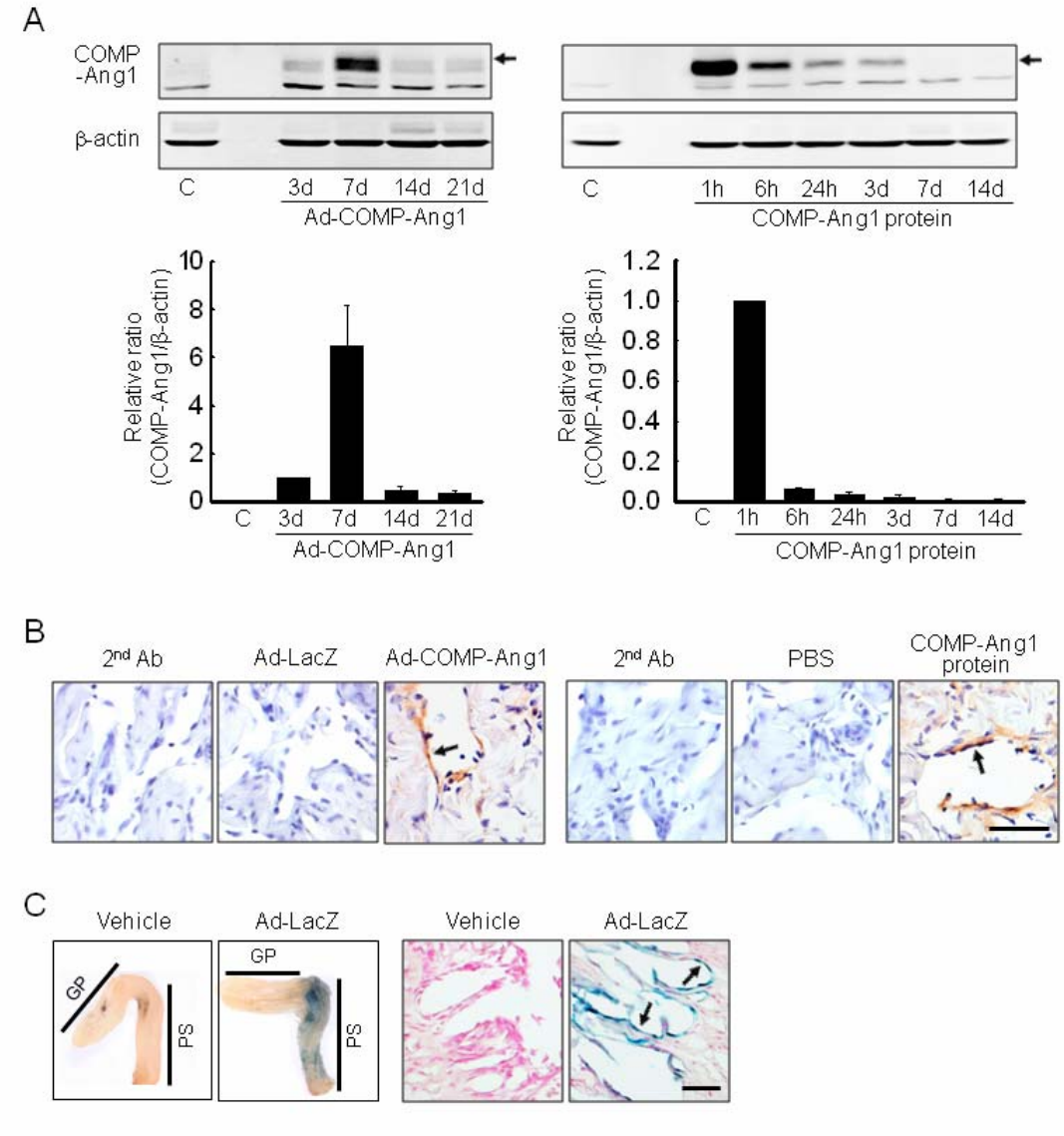
Supplementary Table 1. Physiologic and metabolic parameters: 4 weeks after treatment

		STZ-induced diabetic group				
	Control	No treatment	Ad-LacZ	PBS	Ad-COMP-Ang1	COMP-Ang1 protein
Body weight (g)	28.8±0.9	22.3±2.8*	23.7±1.9*	24.4±2.6*	24.1±1.5*	22.8±2.4*
Fasting glucose (mg/dl)	119.6±13.4	418.3±136.8*	449.1±78.2*	369.2±92.8*	415.8±73.6*	401.4±70.2*
Postprandial glucose (mg/dl)	180.1±18.3	520.3±131.4*	537.4±55.2*	519.2±137.7*	518±49.9*	506.9±44.5*
Blood pressure (mm Hg)						
SBP	99.9±9.0	102±8.0	101±8.7	99.6±3.6	96.8±5.0	97.5±7.9
MBP	69.9±15.8	67.3±11.8	67.2±16.6	69±4.7	65.7±9.3	69.3±9.8
DBP	60.9±15.8	56.1±10.8	56.1±17.3	59.2±6.0	55±9.2	58.9±9.4

Values are the mean ± SE for $n = 6$ animals per group. STZ, streptozotocin; COMP-Ang1, cartilage oligomeric matrix protein-angiopoietin-1; SBP, systolic blood pressure; MBP, mean blood pressure; DBP, diastolic blood pressure.
*$P < 0.01$ vs. control group.

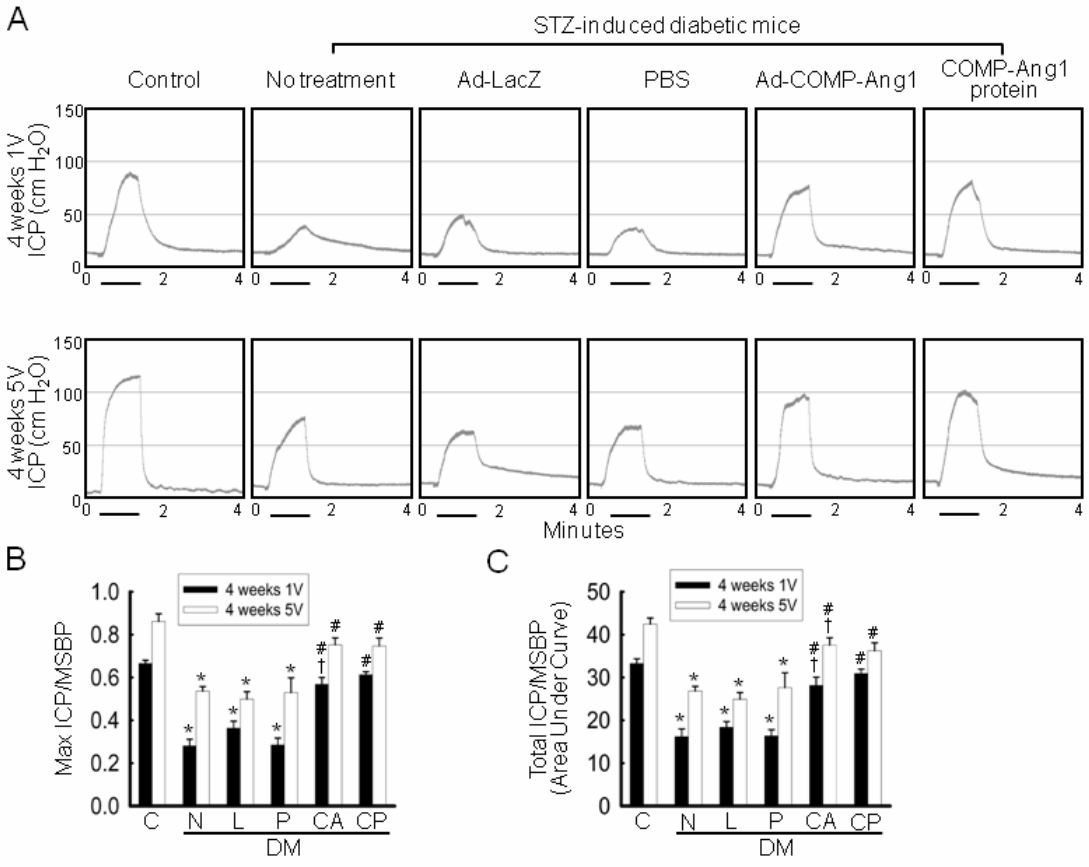
SUPPLEMENTARY DATA

Supplementary Figure 1. *In vivo* COMP-Ang1 or β-gal expression in diabetic corpus cavernosum tissue. ***A:*** Western blot analysis of the expression of COMP-Ang1 protein with anti-FLAG M1 antibody in cavernous tissues from diabetic mice 1, 6, and 24 h and 3, 7, 14, and 21 days after intracavernous injection of ad-COMP-Ang1 (left, 2×10^8 parts/20 μl) or COMP-Ang1 protein (right, 5.8 μg/20 μl). The relative ratio of COMP-Ang1 to β-actin measured 3 days after injection of ad-COMP-Ang1 or 1 h after injection of COMP-Ang1 protein was arbitrarily set equivalent to 1. Bars represent the mean ± SE of four independent experiments. ***B:*** Anti-FLAG staining of cavernous tissue from diabetic mice 7 days after intracavernous injection of ad-LacZ (2×10^8 parts/20 μl) or ad-COMP-Ang1 (2×10^8 parts/20 μl) and 1 h after intracavernous injection of PBS (20 μl) or COMP-Ang1 protein (5.8 μg/20 μl). Arrows denote positive endothelial cells. 2nd Ab, secondary antibody control. Scale bar = 50 μm. ***C:*** Localization of β-gal in the whole penis from diabetic mice 7 days after administration of virus vehicle alone or of ad-LacZ (2×10^8 parts/20 μl). Arrows denote positive endothelial cells. Tissue was counterstained nuclear fast red. Scale bar = 50 μm. GP, glans penis (corpus spongiosum); PS, penile shaft (corpus cavernosum).



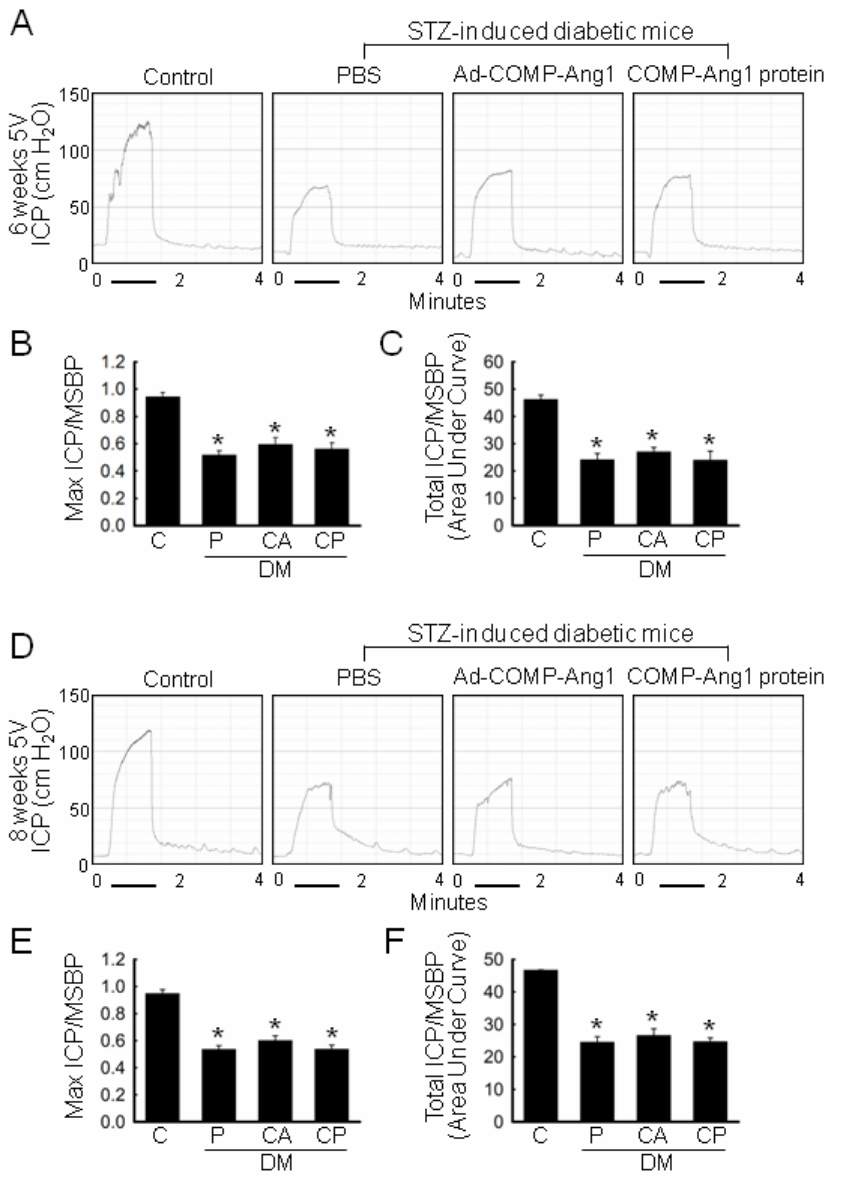
SUPPLEMENTARY DATA

Supplementary Figure 2. Adenoviral COMP-Ang1 gene or COMP-Ang1 protein transfer restores intracavernous pressure (ICP) elicited by electrical stimulation of the cavernous nerve. ***A:*** Representative ICP responses for age-matched control (**C**), untreated diabetic mice (**N**) or diabetic mice stimulated at 4 weeks after intracavernous injection of ad-LacZ (**L**, 2×10^8 parts/20 μl), PBS (**P**, 20 μl), ad-COMP-Ang1 (**CA**, 2×10^8 parts/20 μl), or COMP-Ang1 protein (**CP**, 5.8 μg/20 μl). A single injection of ad-LacZ or ad-COMP-Ang1 (day 0) and repeated injections of PBS or COMP-Ang1 protein (days -3 and 0) were done into the mid portion of the corpus cavernosum. Cavernous nerve was stimulated at 1 V and 5 V. The stimulus interval is indicated by a solid bar. ***B, C:*** Ratios of mean maximal ICP and total ICP (area under the curve) to mean systolic blood pressure (MSBP) were calculated for each group. Each bar depicts the mean ± SE from $n = 6$ animals per group. *$P < 0.01$ vs. **C**, **CA**, and **CP** groups, #$P < 0.01$ vs. **N**, **L**, and **P** groups, †$P < 0.05$ vs. **C** group. STZ, streptozotocin; DM, diabetes mellitus.



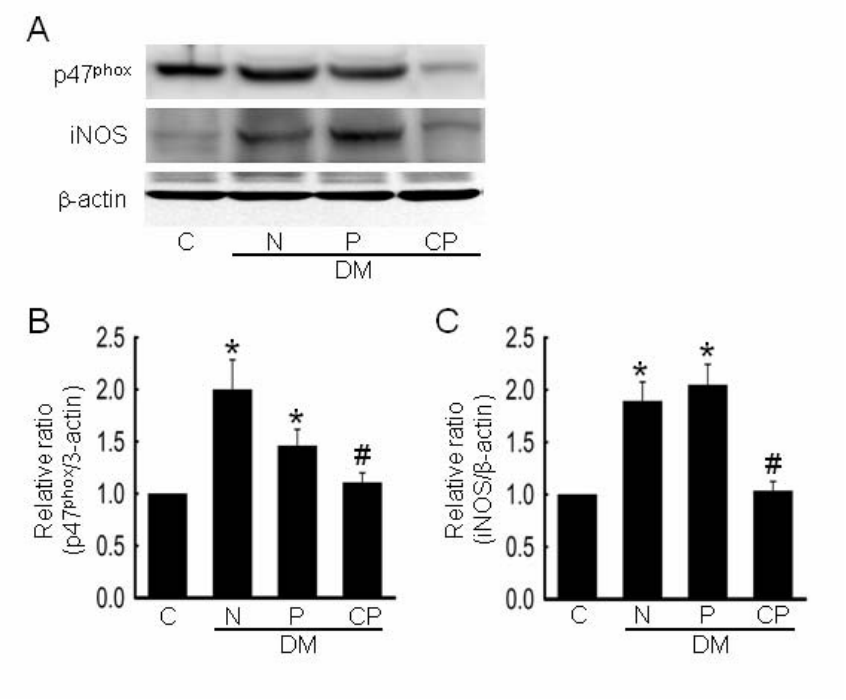
SUPPLEMENTARY DATA

Supplementary Figure 3. Intracavernous pressure (ICP) elicited by electrical stimulation of the cavernous nerve returns to baseline levels 6 and 8 weeks after administration of adenoviral COMP-Ang1 gene or COMP-Ang1 protein. ***A:*** Representative ICP responses for age-matched control (**C**) or diabetic mice stimulated at 6 weeks after intracavernous injection of PBS (**P**, 20 μl), ad-COMP-Ang1 (**CA**, 2×10^8 parts/20 μl), or COMP-Ang1 protein (**CP**, 5.8 μg/20 μl). A single injection of ad-COMP-Ang1 (day 0) and repeated injections of PBS or COMP-Ang1 protein (days -3 and 0) were done into the mid portion of the corpus cavernosum. Cavernous nerve was stimulated at 5 V. The stimulus interval is indicated by a solid bar. ***B, C:*** Ratios of mean maximal ICP and total ICP (area under the curve) to mean systolic blood pressure (MSBP) were calculated for each group. Each bar depicts the mean ± SE from $n = 4$ animals per group. $^*P < 0.01$ vs. **C** group. ***D:*** Representative ICP responses for each group of mice stimulated at 8 weeks after treatment. ***E, F:*** Ratios of mean maximal ICP and total ICP (area under the curve) to mean systolic blood pressure (MSBP) were calculated for each group. Each bar depicts the mean ± SE from $n = 4$ animals per group. $^*P < 0.01$ vs. **C** group. STZ, streptozotocin; DM, diabetes mellitus.



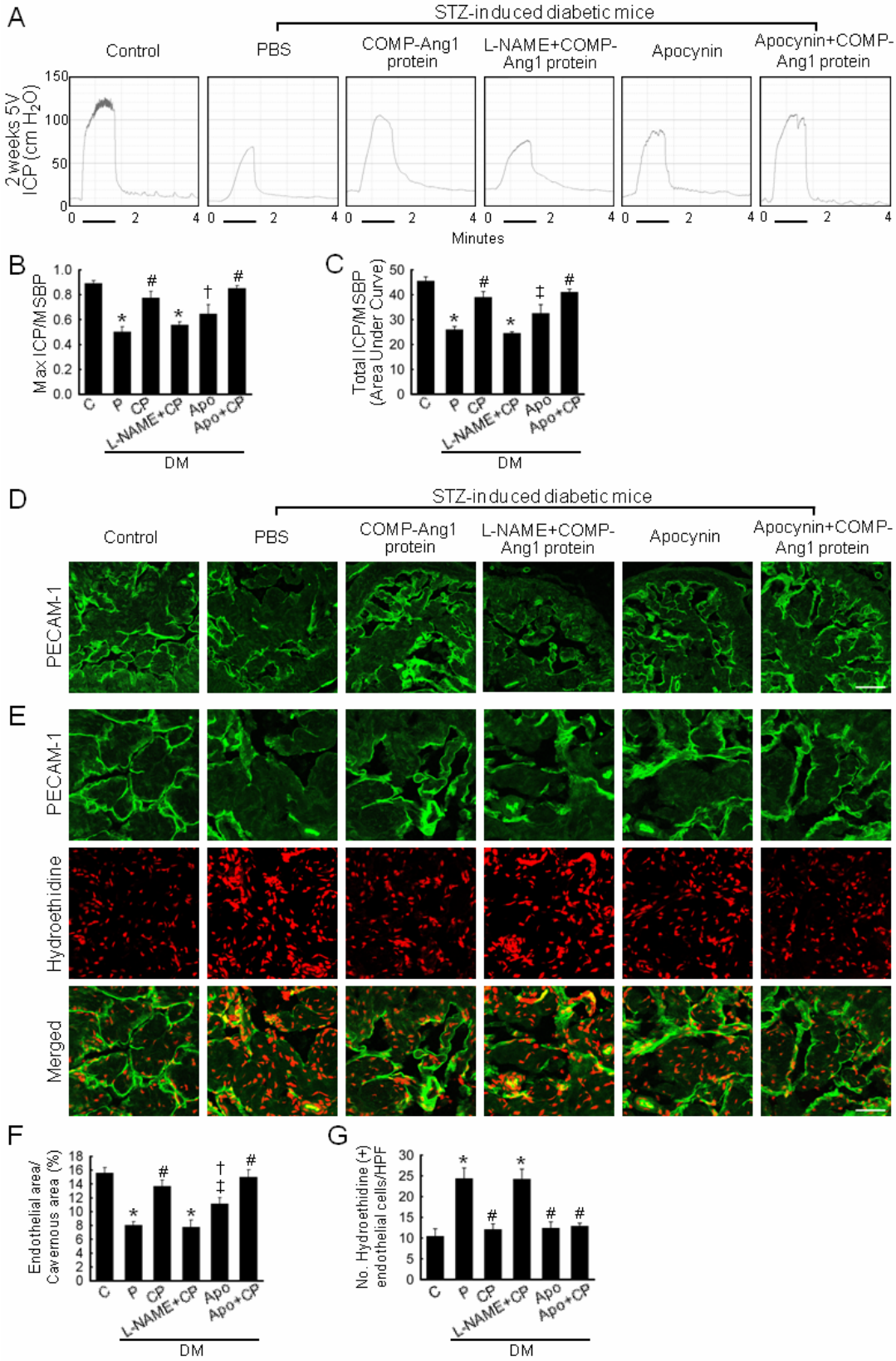
SUPPLEMENTARY DATA

Supplementary Figure 4. COMP-Ang1 protein transfer decreases $p47^{phox}$ and iNOS expression in the corpus cavernosum tissue. ***A:*** Representative Western blot for $p47^{phox}$ and iNOS in age-matched control (**C**), untreated diabetic mice (**N**), or diabetic mice 2 weeks after receiving intracavernous injection of PBS (**P**, days -3 and 0; 20 μl) or COMP-Ang1 protein (**CP**, days -3 and 0; 5.8 μg/20 μl). ***B, C:*** Data are presented as the relative density of each protein compared with that of β-actin. The relative ratio of the control group was arbitrarily set equivalent to 1. Data are representative of six independent experiments. **P* < 0.05 vs. **C** and **CP** groups, #*P* < 0.05 vs. **N** and **P** groups. iNOS, inducible nitric oxide synthase; DM, diabetes mellitus.



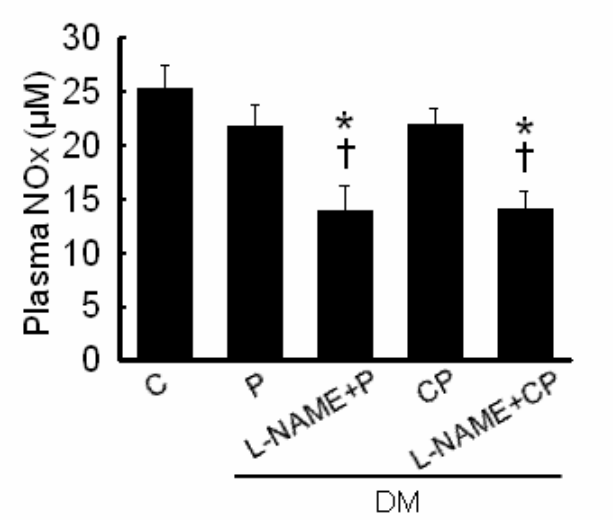
Supplementary Figure 5. COMP-Ang1 protein-induced cavernous angiogenesis and recovery of erectile function is NOS-dependent. ***A:*** Representative intracavernous pressure (ICP) responses for age-matched control (**C**), diabetic mice receiving intracavernous injection of PBS (**P**, 20 μl) or COMP-Ang1 protein (**CP**, 5.8 μg/20 μl), L-NAME-treated diabetic mice receiving COMP-Ang1 protein (**L-NAME + CP,** 5.8 μg/20 μl), apocynin-treated diabetic mice (**Apo**), or apocynin-treated diabetic mice receiving COMP-Ang1 protein (**Apo + CP,** 5.8 μg/20 μl). Cavernous nerve was stimulated at 5 V and ICP responses were measured 2 weeks after treatment. The stimulus interval is indicated by a solid bar. ***B, C:*** Ratios of mean maximal ICP and total ICP (area under the curve) to mean systolic blood pressure (MSBP) were calculated for each group. Each bar depicts the mean ± SE from *n* = 4 animals per group. **P* < 0.01 vs. **C**, **CP**, and **Apo + CP** groups, #*P* < 0.01 vs. **P** and **L-NANE + CP** groups, †*P* < 0.01 vs. **C** and **Apo + CP** groups. ‡*P* < 0.05 vs. **C**, **P**, **CP**, **L-NAME +CP,** and **Apo + CP** groups. ***D, E:*** Immunohistochemical staining of cavernous tissue using antibody to PECAM-1 and hydroethidine histochemistry in each group. Scale bar = 100 μm for **(*D*)** and 50 μm for **(*E*)**. ***F:*** Quantitative analysis of endothelial cell content in cavernous tissue was performed by using an image analyzer. Each bar depicts the mean ± SE from *n* = 4 animals per group. **P* < 0.01 vs. **C**, **CP**, and **Apo + CP** groups, #*P* < 0.01 vs. **P** and **L-NANE + CP** groups, †*P* < 0.05 vs. **P**, **CP**, **L-NAME +CP**, **and Apo + CP** groups, ‡*P* < 0.01 vs. **C** group. ***G:*** Number of ethidium bromide fluorescence-positive endothelial cells per high-power

field (screen magnification ×400). Each bar depicts the mean ± SE from $n = 4$ animals per group. *$P <$ 0.01 vs. **C, CP**, **Apo**, and **Apo + CP** groups, #$P < 0.01$ vs. **P** and **L-NAME + CP** groups. STZ, streptozotocin; DM, diabetes mellitus; HPF, high-power field.



SUPPLEMENTARY DATA

Supplementary Figure 6. Effect of L-NAME on plasma nitrite/nitrate (NO_x) concentrations. Plasma NO_x concentrations in age-matched control (**C**), diabetic mice receiving intracavernous injection of PBS (**P**, 20 μl) or COMP-Ang1 protein (**CP**, 5.8 μg/20 μl), L-NAME-treated diabetic mice receiving PBS (**L-NAME + P**, 20 μl) or COMP-Ang1 protein (**L-NAME + CP,** 5.8 μg/20 μl). Plasma NO_x levels were determined 2 weeks after treatment. Each bar depicts the mean ± SE from $n = 4$ animals per group. *$P <$ 0.01 vs. **C** group, †$P < 0.05$ vs. **P** and **CP** groups. DM, diabetes mellitus.



Supplemental FIG. 7. Schematic representation of a proposed model for how COMP-Ang1 protein restores erectile function in diabetic mice. EC, endothelial cell; O_2•-, superoxide anion; iNOS, inducible nitric oxide synthase; NO•, nitric oxide; $ONOO^-$, peroxynitrite; eNOS, endothelial nitric oxide synthase; ZO-1, zonula occludens-1; VE-cadherin; vascular endothelial-cadherin.