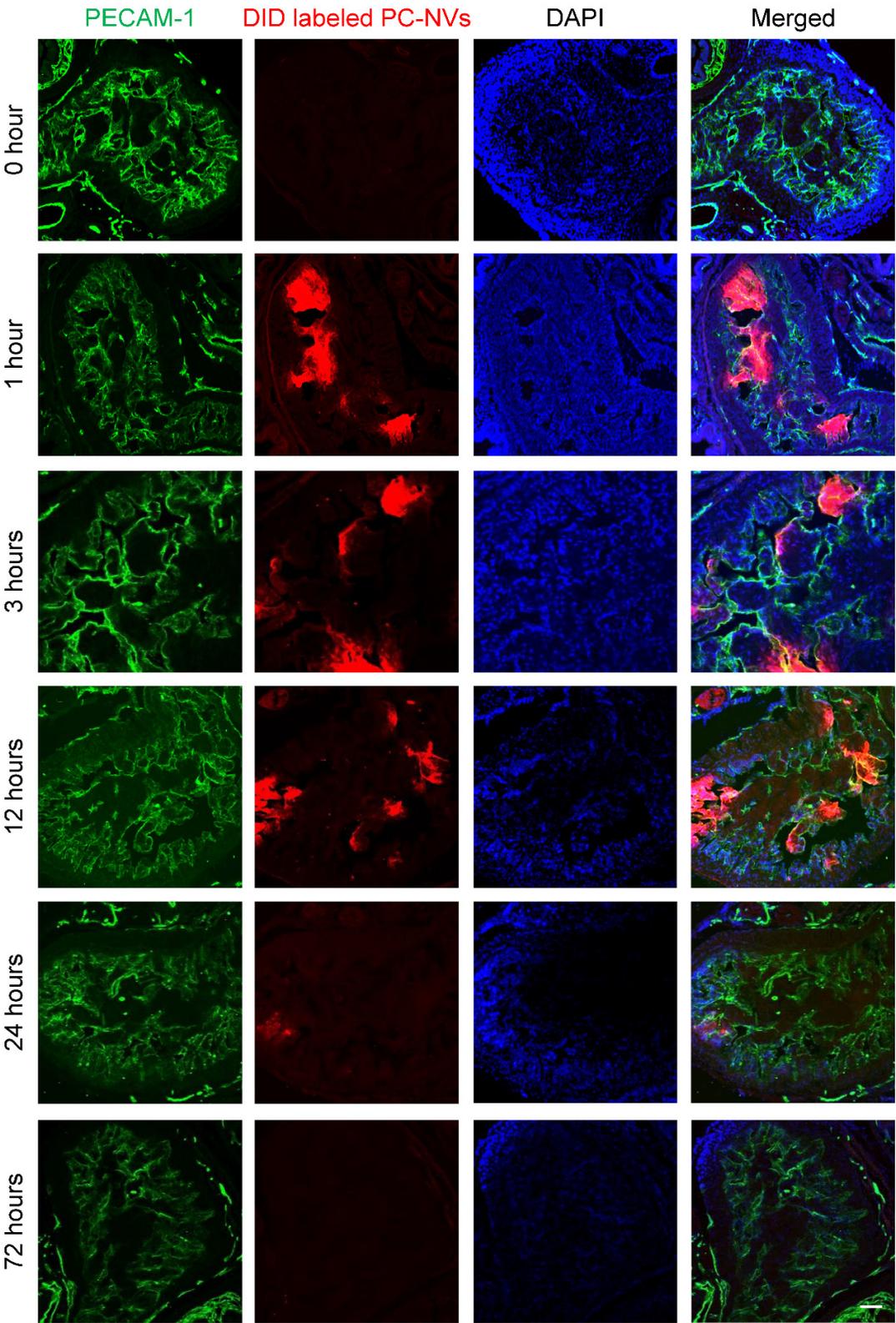
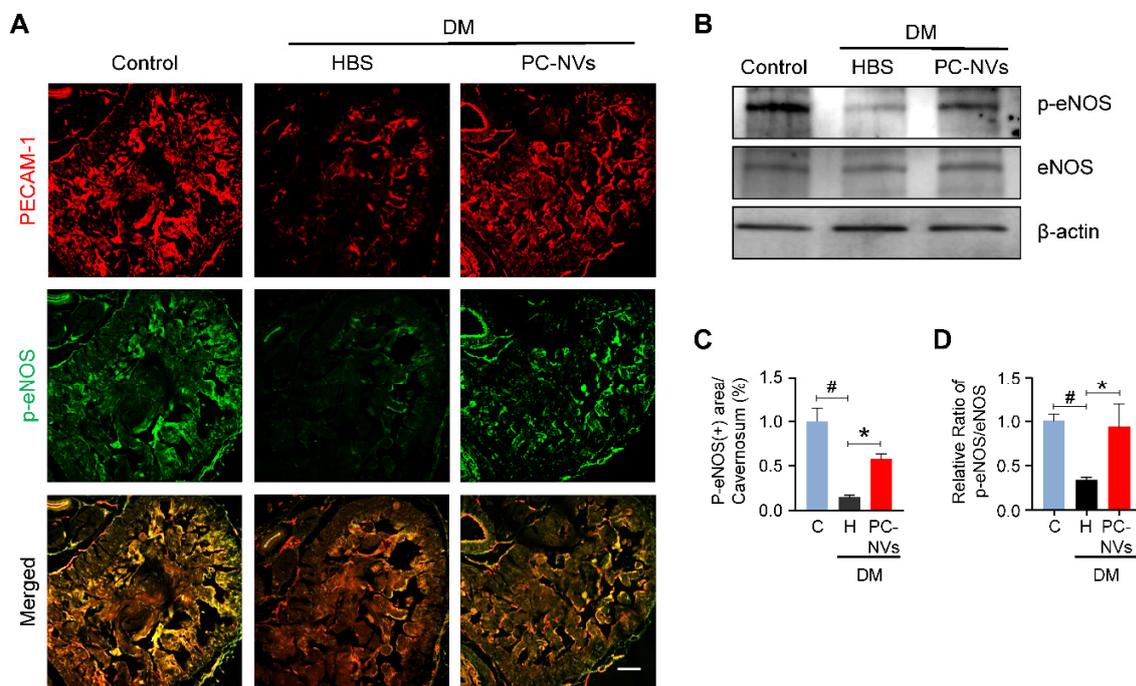


Supplemental Information

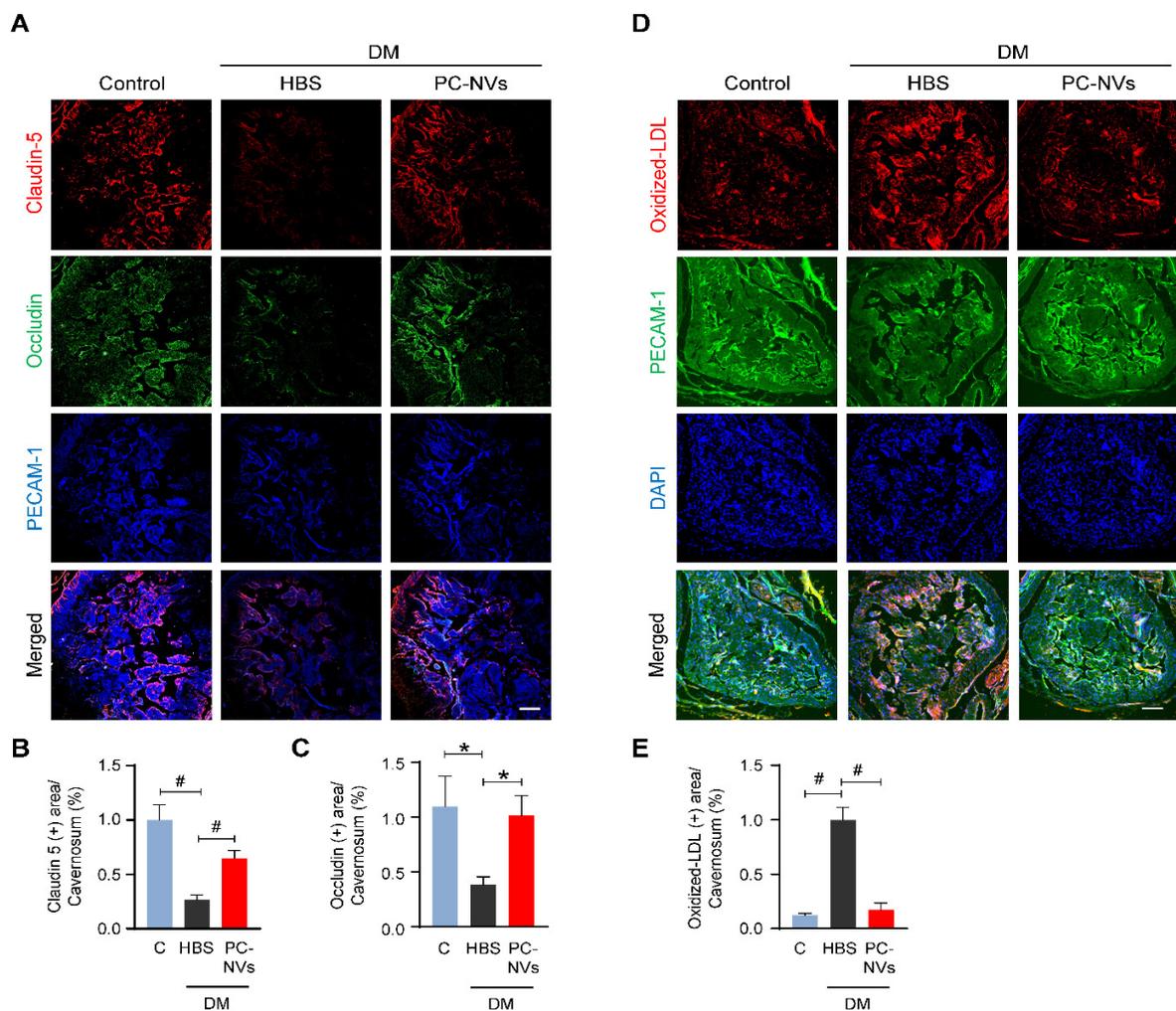


**Figure S1:** In vivo detection of DiD-red fluorescently labeled pericyte (PC)-derived extracellular vesicle-mimetic nanovesicles (PC-NVs) in the penis of normal mice. The penis tissue was harvested 0, 1, 3, 12, 24, and 72 hours after intracavernous injection of DiD-red labeled PC-NVs into the normal mice. Representative immunohistochemical staining with PECAM-1 (green) in cavernous tissue from normal mice. Nuclei were labeled with the DNA dye DAPI. Scale bar = 200  $\mu$ m. DAPI = 4,6-diamidino-2-phenylindole.



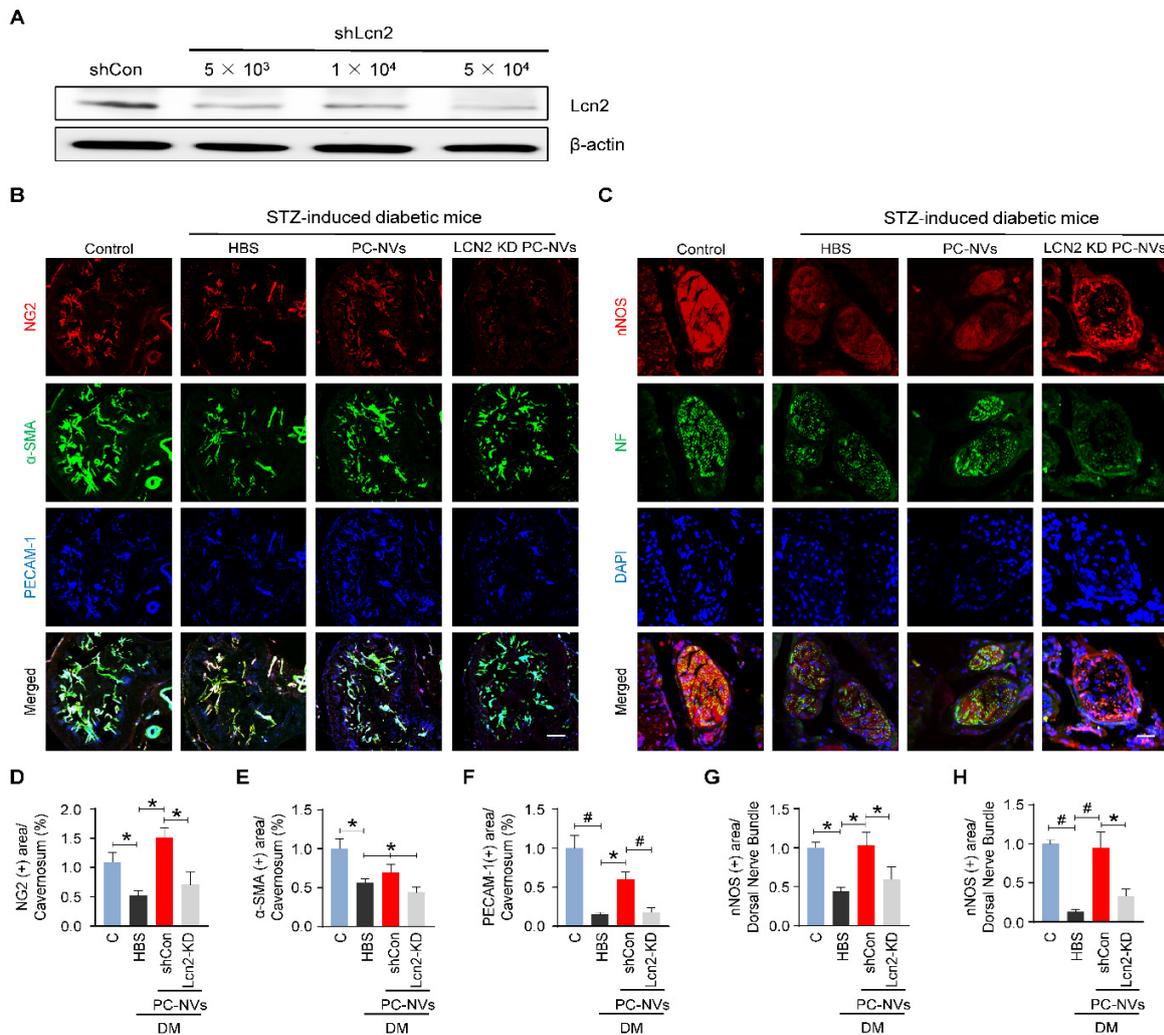
**Figure S2:** PC-NVs induce cavernous eNOS phosphorylation (p-eNOS) in diabetic mice. (A) PECAM-1 (red) and phosphor-eNOS (green) staining of cavernous tissue from age-matched control (C) and diabetic mice obtained 2 weeks after intracavernous injection of HBS (H) or PC-NVs (5  $\mu$ g/20  $\mu$ l) on days -3 and 0. Scale bar = 100  $\mu$ m. (B) Representative western blots for phosphor-eNOS and eNOS in age-matched control (C) and diabetic mice obtained 2 weeks after intracavernous injection of HBS (H) or PC-NVs (5  $\mu$ g/20  $\mu$ l) on days -3 and 0.

(C) Quantification of the phosphor-eNOS immunopositive area in cavernous tissue by Image J (n = 8). (D) Band intensity values of indicated proteins was quantified by assessing the density of the corresponding protein bands using Image J (n = 4). Results are presented as means  $\pm$  SEM (\* $P < 0.05$ ; # $P < 0.001$ ). The relative ratio in the control group was set to 1. DM, diabetes mellitus; HBS, HEPES-buffered saline.

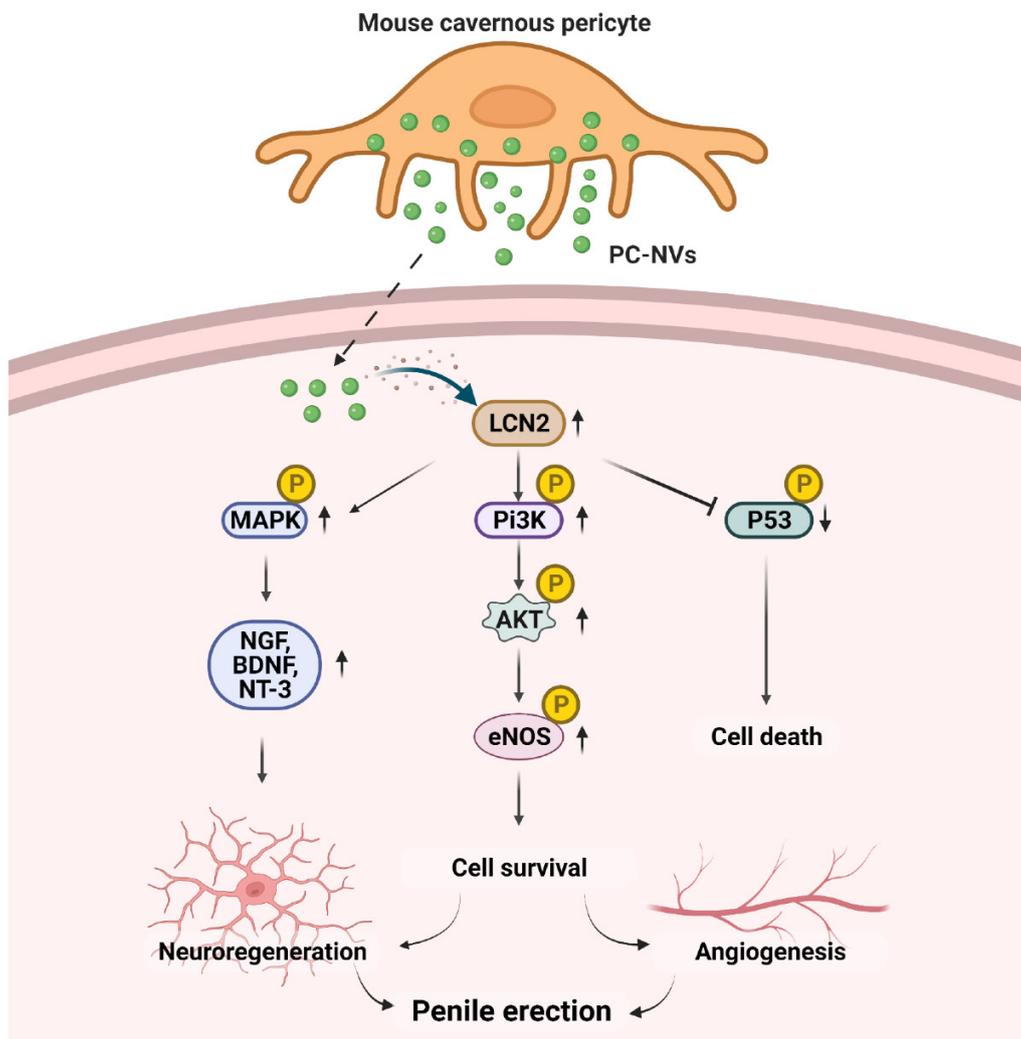


**Figure S3:** PC-NVs preserve endothelial cell-cell junctions and decrease the extravasation of oxidized-LDL under diabetic conditions. (A-C) Triple-immunostaining for Claudin-5 (red), Occludin (green), and PECAM-1 (blue) in age-matched control (C) and diabetic mice obtained 2

weeks after intracavernous injection of HBS (H) or PC-NVs (5  $\mu$ g/20  $\mu$ l) on days -3 and 0. Scale bar = 100  $\mu$ m. Quantification of claudin-5- and occluding-immunopositive area in the cavernosum using Image J (n = 10). Results are presented as means  $\pm$  SEM (\**P* < 0.05; #*P* < 0.001). (D and E) PECAM-1 (green) and oxidized-LDL (red) staining of cavernous tissue from age-matched control (C) and diabetic mice obtained 2 weeks after intracavernous injection of HBS (H) or PC-NVs (5  $\mu$ g/20  $\mu$ l) on days -3 and 0. Scale bar = 100  $\mu$ m. Quantification of oxidized-LDL-immunopositive area in the cavernosum using Image J (n = 7). Results are presented as means  $\pm$  SEM (#*P* < 0.001). DM, diabetes mellitus; HBS, HEPES-buffered saline.



**Figure S4:** PC-NVs induce neurovascular regeneration with Lcn2-dependent manner under diabetic conditions. (A) Representative Western blots for Lcn2 in mouse cavernous pericyte infected with shCon or shLcn2 lentivirus at three doses ( $5 \times 10^3$  TU,  $1 \times 10^4$  TU, and  $5 \times 10^4$  TU/ml culture medium) for at least 3 days. (B) Triple-immunostaining for NG2 (red),  $\alpha$ -SMA (green), and PECAM-1 (blue) in age-matched control (C) and diabetic mice obtained 2 weeks after intracavernous injection of HBS (H), PC-NVs (shCon) ( $5 \mu\text{g}/20 \mu\text{l}$ ), or PC-NVs (Lcn2-KD) ( $5 \mu\text{g}/20 \mu\text{l}$ ) on days  $-3$  and  $0$ . Scale bar =  $100 \mu\text{m}$ . (C) nNOS (red) and neurofilament (NF, green) staining of cavernous tissue in age-matched control (C) and diabetic mice obtained 2 weeks after intracavernous injection of HBS (H), PC-NVs (shCon) ( $5 \mu\text{g}/20 \mu\text{l}$ ), or PC-NVs (Lcn2-KD) ( $5 \mu\text{g}/20 \mu\text{l}$ ) on days  $-3$  and  $0$ . Scale bar =  $25 \mu\text{m}$ . (D- H) Intensity quantification of each protein-immunopositive area in the cavernosum using Image J ( $n = 5$ ). Results are presented as means  $\pm$  SEM ( $*P < 0.05$ ;  $\#P < 0.001$ ). DM, diabetes mellitus; HBS, HEPES-buffered saline.



**Figure S5:** Schematic depiction of the proposed mechanism for Lcn2-dependent restoration of diabetic ED by PC-NVs. PC-NVs, Pericyte-derived extracellular vesicle-mimetic nanovesicles; Lcn2, lipocalin 2; PI3K, phosphoinositide 3; Akt, protein kinase B; eNOS, endothelial nitric oxide synthase; MAPK, mitogen-activated protein kinase; NGF, nerve growth factor; BDNF, brain-derived neurotrophic factor; NT-3, neurotrophin-3; P53, tumor protein 53.

**Table S1.** The summary of Pericyte derived PC-NVs characteristics

Comparator	Cell or materials	Preparation strategy	Particle Characterization	Comparison to natural exosome	Potential application
<b>EV-mimetic NVs</b>	Mouse Cavernous Pericytes	serial extrusion (10 $\mu$ m, 5 $\mu$ m, 1 $\mu$ m) + OptiPrep DGUC	Size by TEM and Zeta potential: 134.5 $\pm$ 16.92 nm	Similar cup like morphology, size distribution, protein markers	siRNA delivery, cell proliferation enhancement, pro-angio-neurogenesis, nanocarrier alternative to exosome,

DGUC; density gradient ultra-centrifugation, TEM; transmission electron microscopy.

**Table S2.** Physiologic and metabolic parameters: 2 weeks after treatment with different doses of PC-NVs

	Control	STZ-induced diabetic mice			
		HBS	PC-NVs 0.5 $\mu$ g	PC-NVs 1.0 $\mu$ g	PC-NVs 5.0 $\mu$ g
Body weight (g)	29.1 $\pm$ 0.3	22.8 $\pm$ 1.1 <sup>#</sup>	23.0 $\pm$ 0.8 <sup>#</sup>	22.6 $\pm$ 0.4 <sup>#</sup>	22.4 $\pm$ 0.2 <sup>#</sup>
Postprandial glucose (mg/dL)	148.3 $\pm$ 7.2	577.4 $\pm$ 9 <sup>#</sup>	575.3 $\pm$ 12.6 <sup>#</sup>	572.7 $\pm$ 9 <sup>#</sup>	571.3 $\pm$ 13.5 <sup>#</sup>
Fasting glucose (mg/dL)	113.9 $\pm$ 5.7	416.4 $\pm$ 8.8 <sup>#</sup>	411.0 $\pm$ 8.5 <sup>#</sup>	408 $\pm$ 19.5 <sup>#</sup>	393.4 $\pm$ 19.7 <sup>#</sup>
MSBP (cm H <sub>2</sub> O)	83 $\pm$ 3.1	89.9 $\pm$ 1.1	87.8 $\pm$ 5.1	71.33 $\pm$ 0.7	73.89 $\pm$ 1.6

Values are the mean  $\pm$  SEM for n = 5 animals per group. <sup>#</sup>P < 0.001 vs. control group. MSBP, mean systolic blood pressure; STZ, streptozotocin.

**Table S3.** Physiologic and metabolic parameters: 2 weeks after treatment with PC-NVs (shCon) and PC-NVs (Lcn2-KD) under diabetic conditions.

	Control	STZ-induced diabetic mice		
		HBS	PC-NVs (shCon)	PC-NVs (Lcn2-KD)
Body weight (g)	28.5 $\pm$ 0.6	24.9 $\pm$ 1.4 <sup>#</sup>	24.3 $\pm$ 1.6 <sup>#</sup>	22.2 $\pm$ 1.2 <sup>#</sup>
Postprandial glucose (mg/dL)	123.6 $\pm$ 2.6	550.0 $\pm$ 21.7 <sup>#</sup>	489.4 $\pm$ 19.5 <sup>#</sup>	517.4 $\pm$ 22.15 <sup>#</sup>
Fasting glucose (mg/dL)	100 $\pm$ 3.8	376.1 $\pm$ 22.3 <sup>#</sup>	347.6 $\pm$ 18.4 <sup>#</sup>	422.0 $\pm$ 23.9 <sup>#</sup>
MSBP (cm H <sub>2</sub> O)	90.1 $\pm$ 1.1	94.3 $\pm$ 2.2	98.9 $\pm$ 4.1	104.4 $\pm$ 2.1

Values are the mean  $\pm$  SEM for n = 5 animals per group. <sup>#</sup>P < 0.001 vs. control group. MSBP, mean systolic blood pressure; STZ, streptozotocin.