Data S1.

Supplemental Materials and Methods

Mouse model of AAA
All mice were bred and housed in animal facility of University of Rochester Medical Center under a 12:12 hour light-dark cycle. Standard chow and water ad libitum were available before experiment. During the experiment, 10-week-old wild type C57BL/6J male mice were treated with 0.2% 3-aminopropionitrile fumarate salt (BAPN) (Sigma, A3134) (w/v) in drinking water. BAPN is an irreversible inhibitor of lysyl oxidase which is critical in maintaining homeostasis of the elastic lamina. BAPN treatment started from 2 days before surgery until the end of study. For surgery, mice were anesthetized with inhaled isoflurane. Periaortic application of porcine pancreas elastase (Sigma, E1250) were blindered performed as previously described with slight modifications. Briefly, the connective tissue surrounding infrarenal abdominal aorta was cleaned off from approximately 2 mm below the left renal artery to the bifurcation. A 6mm x 9mm piece of whatman paper were placed on the exposed aortic adventitia for 5 minutes and 40μl of elastase (7.6 mg protein/mL, 4 units/mg protein) were applied directly to the whatman paper. After that, the exposed area was washed gently with saline twice and the abdomen was closed routinely in layers. For sham groups, heat deactivated elastase (100°C for 30 minutes), instead of active elastase, was applied topically to the aorta. The rest of the surgical procedure was identical to the active elastase groups. Mice that received sham surgery or elastase surgery were assigned before the surgery. Extended Release Buprenorphine (0.5mg/kg subcutaneous injection) were used as analgesic prior to surgery and mice were monitored for 3 days post-surgery for recovery. Mice occasionally died shortly within the 3-day monitoring due to the surgery were excluded from following experiments. Only male mice were used because male gender is a major risk factor for AAA. Abdominal aorta size were assessed by ultrasound imaging before surgery as baseline, and assessed on the 7th day post-surgery of active elastase application. Mice were then randomly assigned into four groups on the same day right after ultrasound imaging: sham/vehicle (n=5), sham/sildenafil (n=4), elastase/vehicle (n=17), elastase/sildenafil (n=14). Treatment of drinking water with vehicle or sildenafil to mice started from the 7th day post-surgery till harvest. Animals in each group were caged together and treated in the same way until harvest, and drinking water containing vehicle or sildenafil was prepared daily from the stock solution, therefore investigators were not blinded to group allocation in terms of drug treatment. Total 3 separate experiments were included. Sildenafil was prepared in drinking water as described previously. Briefly, sildenafil citrate (100mg tablets; Viagra; Pfizer) was dissolved in drinking water (pure water acidified with citric acid to pH 5.2) to a concentration of 400 mg/L (0.6 mM), sterile filtered, and given ad libitum to mice, resulting in the ingestion of approximately 60-100 mg/kg/day (based on an average water consumption of 3–5 ml/day for an adult mouse). Sildenafil water solution was stored at 4°C for a maximum of 3 weeks. The IC50 for sildenafil inhibition of PDE5A is 4 nM. Using this approach, the average concentration of circulating sildenafil was 70 nM over a 24 hour period. Vehicle is pure water acidified with citric acid to pH 5.2 without sildenafil. All mice were euthanized at 35 days post-surgery. All animals were used in accordance with the guidelines of the National Institutes of Health and American Heart Association for the care and use of laboratory animals.

Ultrasound imaging
A Vevo 2100 ultrasound imaging platform (FUJIFILM VisualSonics) was utilized to assess aneurysm progression in periaortic elastase induced AAA model as described previously. Mice were anesthetized with isoflurane via nosecone, placed on a 37°C warming pad, in a supine position, and feet restrained. Body temperature, respiration rate and ECG were monitored. Heart rate was kept at a consistent range (500–550 beats per minute). Hair was removed from the abdomen and Aquasonic 100 ultrasound transmission gel was placed on abdomen to increase probe contact. The probe was applied on long axis to locate the aorta. Pulse wave (PW mode) doppler was used to confirm aortic flow, and M mode images were taken at the widest part of the aneurysm. Data for diastole internal diameter were shown for individual animals.

Morphometric analysis of maximal aortic width
At the end of the experiments, mice were anesthetized via intraperitoneal injection of ketamine (100 mg/kg), midazolam (5mg/kg) and heparin (1600 units/kg). Mice were euthanized via cervical dislocation when lost toe pinch response. Aortas were perfused with saline and fixed with 10% phosphate-buffered formalin (NBF) for 2 minutes. Whole aorta was dissected from the surrounding connective tissue and fixed with 10% NBF for 24 hours at 4°C. Subsequently aortas were further cleaned with a dissecting microscope. Pictures were taken with a digital camera with a ruler set aside. The adventitial circumferences at the maximal expanded portion of the aneurysm were quantified as the maximal abdominal aortic diameter. The maximum diameter of the abdominal aorta was analyzed using Image J software after adjusting the scale according to the ruler in aorta pictures. At least 3 measurements of the maximal expanded portion of the infrarenal aorta for each mouse were averaged before calculating the mean of each experimental group. Aortas were subsequently embedded in paraffin. Aortic cross sections (5 μm each) were collected serially from proximal to distal abdominal aorta in levels.

Van Gieson elastin staining
Paraffin sections were stained for elastic fibers using van Gieson Elastic Stain Kit (Thermo Scientific 87017) according to the manufacturer’s instructions. The section from the level of largest diameter of AAA for each animal was selected for staining. Briefly, deparaffinized and hydrated sections were stained in elastic stain solution for 30 min and decolorize in differentiating solution. Following that, sections were rinsed in sodium thiosulfate solution shortly and stained in Van Gieson stain solution for 15min. Sections were viewed with a BX51 upright microscope (Olympus) using Olympus CellSens Standard acquisition software. The area of elastic fibers staining was quantified using ImageJ as described previously. Eight visual fields (magnification 200) evenly distributed at media of every section were included to quantify the amount of elastin staining. All images were set to the same hue, saturation and brightness and measured for positive staining area. The elastin content is expressed as a percentage of elastic fiber area in media area.

Immunofluorescence staining
Sections were deparaffinized, followed by heat treatment with citrate buffer for antigen retrieval. Following that, sections were permeabilized by 0.2% Triton X-100/PBS for 10 minutes. Nonspecific binding sites were blocked with Dako serum-free blocking solution at room temperature for 1 hour. Sections were incubated with rabbit anti-PDE5 polyclonal primary antibody (1:500)(Cell Signaling Technology #2395), rabbit anti-phospho-myosin light chain 2 at Ser19 (pMLC) polyclonal primary antibody (1:300) (Cell Signaling Technology #3671), mouse anti-PKG1α/β monoclonal primary antibody (1:300) (Santa Cruz sc-271765), rabbit anti-calponin
polyclonal antibody (1:200)(Proteintech 13938-1-AP), rabbit anti-MYH11 polyclonal primary antibody (1:200)(Abcam ab53219), mouse anti-α-SMA monoclonal primary antibody (1:800)(Dako M0851), rabbit anti-LMOD1 polyclonal primary antibody (1:200) (Proteintech 15117-1-AP), rabbit anti-F4/80 monoclonal antibody (1:200)(Cell Signaling Technology #30325), rat anti-Mac2 monoclonal antibody (1:200)(Cedarlane CL8942AP), or rabbit anti-MMP9 polyclonal primary antibody (1:200)(Millipore AB19016) overnight at 4°C. Subsequently, the sections were incubated with Alexa Fluror-594 or 488 conjugated anti-rabbit, anti-mouse, or anti-rat secondary antibody for 1 hour at room temperature. Sections were subsequently incubated with DAPI for nuclei staining and mounted with ProLong™ Gold Antifade Mountant.

Total myosin light chain 2 (MLC) staining were performed on adjacent section of pMLC staining by immunofluorescence with HRP-tyramine signal amplification (TSA), using PerkinElmer TSA Plus fluorescence kits (NEL741001KT). Briefly, after deparaffinization, antigen retrieval and permeabilization, endogenous peroxidase was blocked in 0.3% hydrogen peroxide in PBS for 30 minutes, followed by incubation with Dako serum-free blocking solution for 1 hour. After that, sections were incubated in rabbit anti-MLC polyclonal primary antibody (1:300) (Cell Signaling Technology #3672) diluted in DAKO antibody dilution buffer overnight at 4°C. Sections were then washed with PBS for 3 times and incubated in biotinylated goat anti-rabbit (Vector BA-1000) secondary antibody for 1 hour, washed with PBS for three times and incubated in Avidin-biotinylated enzyme complex for 30 minutes. Sections were then washed with PBS for 3 times and incubated in TSA Plus fluoroscein working solution (1:50) diluted in 1X amplification diluent for 3 minutes. After that, sections were stained for DAPI and mounted with ProLong™ Gold Antifade Mountant.

One cross section from the level of largest diameter of AAA for each animal was selected for staining. Matched IgG was used in place of the primary antibody as a negative control. Sections were viewed with a confocal microscope (Olympus FV1000-IX81) using 20X objective. Differential interference contrast (DIC) image was taken at the same field to see elastic lamina structures. Media lesion of AAA was defined as the area inward of external elastic lamina (EEL). Staining was analyzed with NIH ImageJ software by calculating the integrated optical density value of positive staining per media lesion area for PDE5, MLC, pMLC, PKGI or total section area for F4/80, Mac2, MMP9. Media lesion area was drawn with the freehand selection tool. The positive staining of each group was standardized to the averaged amount of staining in sham/vehicle group expressed as fold change.

**Proximity ligation assay (PLA)**

PLA is used to detect and quantify protein-protein interactions in situ. If cGMP physically interacts with PKGI, antibodies bound to each other of the molecules are in a close proximity (maximum 40nm) that oligonucleotides conjugated to the antibodies serve as guides to ligate additional oligonucleotides and form a circular template for DNA synthesis. Amplified DNA was detected with a fluorescent dye-labeled probe (signal amplification up to 1000 times). A single PLA fluorescence dot represent a site of colocalization between cGMP and PKGI. In this study PLA were performed using Duolink™ In Situ Red Starter Kit (Sigma, DUO92102-1KT) following the manufacturer’s instructions. Briefly, aorta cross sections were deparaffinized, followed by heat treatment with citrate buffer for antigen retrieval. Then sections were permeabilized by 0.1% TritonX-100/PBS for 10min and then incubated with Duolink® Blocking Solution at 37°C for 1
hour. Following that, sections were incubated with rabbit anti-cGMP polyclonal primary antibody (Millipore Sigma 09-101)(1:200) and mouse anti-PKG1α/β monoclonal primary antibody (Santa Cruz sc-271765)(1:200) diluted in Duolink® Antibody Diluent at 4°C overnight. Sections were then incubated with anti-rabbit PLUS and anti-mouse MINUS PLA probes diluted (1:5) in the Duolink® Antibody Diluent at 37°C for 1 hour. PLUS and MINUS PLA probes are secondary antibodies coupled with oligonucleotides that target primary antibodies. Sections were then incubated with ligase diluted in 1X ligation buffer (1:40) at 37°C for 30 minutes. Ligase hybridize connector oligonucleotides when the PLA probes are in close proximity, which forms a closed circular DNA template. After that, sections were incubated with DNA polymerase diluted in 1X amplification buffer (1:80) at 37°C for 100 minutes. DNA polymerase amplify the closed circular DNA template by rolling-circle amplification using PLA probe as a primer. The generated concatemeric sequences are coupled to fluorochromes (labeled oligos) that hybridize to the complementary repeating sequences. After final washes, sections were mounted with Duolink® In Situ Mounting Media with DAPI.

One section from the level of largest diameter of AAA for each animal was used for each animal. PLA signal was visualized by confocal microscope FV1000-IX81 with 60X objective. Fluorescence images and DIC images were acquired in stack of 3.2μm intervals. PLA dots at media lesion area were counted. Total cell number in media lesion layer were counted by DAPI. 5 fields were acquired of each tissue section. Number of PLA dots per cell averaged from 5 fields were quantified for each animal. The fold change was calculated by normalizing PLA dots per cell of each animal in each group to the averaged PLA dots per cell in the sham/vehicle group.

Statistical analysis
Data of Fig.1B pass normality and constancy of variance; and was analyzed by unpaired Student’s t-test. Data of Fig.1E reject normality and accept constancy of variance; and was analyzed by nonparametric paired Wilcoxon test. Data of Fig. 1G pass normality and reject constancy of variance; and was analyzed by parametric Welch ANOVA with Dunnett's T3 post-hoc test. Data of Fig. 1I, Fig. 2C, Fig. 2D, Fig. 3E pass normality and constancy of variance; and was analyzed by parametric one-way ANOVA with Holm-Sidak's post-hoc test. For Fig. 2E, elastase/vehicle group was compared to elastase/sildenafil group by nonparametric Mann-Whitney test as data reject normality and constancy of variance. Data of Fig. 3B pass normality and constancy of variance; and was analyzed by parametric one-way ANOVA with Holm-Sidak's post-hoc test. One data point (0.422) in elastase/sildenafil group of Fig. 3B is an outlier based on the interquartile rule (1.5 times interquartile range) and was removed from statistics.
Figure S1. This is a supplementary to Fig. 1A. A, Left panels show Hematoxylin and eosin (HE) staining in sham abdominal aorta and abdominal aortic aneurysm. Middle panels show the whole cross section of PDE5 immunostaining on adjacent section. White box highlight the magnified area shown in right panels. B, Negative control of PDE5 immunostaining in sham abdominal aorta.
Figure S2. The media lesion area of abdominal aortic aneurysm (AAA) (the area inward of external elastic lamella) were dominated by smooth muscle cells (SMCs). A, Cross section of AAA from elastase/vehicle mouse. Blue box highlight the magnified area shown in panel B-F. B, Differential interference contrast (DIC) image show the external elastic lamella (EEL), dashed line. Adv, adventitia. C-F, SMC markers calponin, myosin heavy chain 11 (MYH11), leiomodin 1 (LMOD1), α-smooth muscle actin (α-SMA) were stained on AAA sections from the same level.
Figure S3. Images of all sham or abdominal aortic aneurysm samples shown in Fig. 1G. Mice #1-11 in the elastase/vehicle group and Mice #1-9 in the elastase/sildenafil group were included for histology and immunostaining analysis.
Figure S4. This is a supplementary to Fig. 2A and 2B, showing negative control of myosin light chain 2 (MLC) or myosin light chain 2 phosphorylated at Ser19 (pMLC) immunostaining in mouse AAA.
Figure S5. Negative controls of PLA (A) and cGMP-dependent protein kinase I (PKGI) immunostaining (B) that used cGMP primary antibody alone or PKGI antibody alone in mouse AAA.
Figure S6. Legend on the next page.
**Figure S6.** Immunostaining of macrophage marker F4/80, Mac2 (A) and MMP9 (B) in sham or abdominal aortic aneurysm. Scale bar, 30 µm. C-E, Staining intensity of F4/80 (C) or Mac2 (D) or MMP9 (E) in total section area. Data (C, D) analyzed by parametric Welch ANOVA with Dunnett’s T3 post-hoc test (data pass normality and reject constancy of variance). The probability of a type II error for C between elastase vehicle and elastase sildenafil is 0.795. The probability of a type II error for D between elastase vehicle and elastase sildenafil is 0.952. Data (E) analyzed by parametric one-way ANOVA with Holm-Sidak's post-hoc test (data pass normality and constancy of variance). The probability of a type II error for E between elastase vehicle and elastase sildenafil is 0.705. F-G, Negative control of F4/80 and Mac2 (F), and MMP9 (G) immunostaining in mouse AAA.