Supplementary Material

Supplementary Tables

Table 1: Absolute measurements (mean ± SEM; in mm) of the abdominal aortic diameter using B-mode ultrasound from the porcine-pancreatic-elastase (PPE) induction model from baseline until day 28. Absolute measurements correspond to Figure 1A (presented as AAD *vs*. baseline in %) in the manuscript. **P<0.05 vs. sham*.

	baseline	day 3	day 7	day 14	day 21	day 28
ELAST	0.63 ± 0.003	0.76 ± 0.04	*0.89 ± 0.04**	*1.06 ± 0.03**	*1.11 ± 0.05**	*1.12 ± 0.12**
sham	0.62 ± 0.004	0.72 ± 0.03	0.78 ± 0.02	0.78 ± 0.02	0.79 ± 0.04	0.77 ± 0.08

Table 2: Absolute measurements (mean ± SEM; in mm) of the abdominal aortic diameter using B-mode ultrasound in the angiotensinII-AAA induction model from baseline until day 28. Absolute measurements correspond to Figure 2A (presented as AAD *vs*. baseline in %) in the manuscript. **P<0.05 vs. sham*.

	baseline	day 7	day 14	day 28
ANGII	1.11 ± 0.12	*1.32 ± 0.14**	*1.74 ± 0.16**	*1.99 ± 0.17**
sham	1.1 ± 0.14	1.13 ± 0.13	1.18 ± 0.15	1.12 ± 0.13

Table 3: Absolute measurements (mean ± SEM; in mm) of the abdominal aortic diameter using B-mode ultrasound from the porcine-pancreatic-elastase (PPE) induction model from baseline until day 28 in anti-/pre-29b and scr-miR mice with AAA. Absolute measurements correspond to Figure 4B (presented as AAD *vs*. baseline in %) in the manuscript. *#P<0.05 vs. scr-miR and anti- or pre-29b*.

	baseline	day 3	day 7	day 14	day 21	day 28
pre-29b	0.62 ± 0.005	0.79 ± 0.06	*0.92 ± 0.07#*	*1.2 ± 0.08#*	*1.35 ± 0.11#*	*1.39 ± 0.13#*
scr-miR	0.63 ± 0.004	0.77 ± 0.01	0.88 ± 0.05	1.02 ± 0.02	1.12 ± 0.05	1.13 ± 0.07
anti-29b	0.62 ± 0.005	0.73 ± 0.03	*0.78 ± 0.03#*	*0.81 ± 0.06#*	*0.86 ± 0.11#*	*0.86 ± 0.06#*

Supplementary Methods

Aortic diameter measurements by ultrasound imaging

At baseline, and 3, 7, 14, 21 and 28 days after aneurysm induction, B-mode ultrasound (US) imaging was performed to assess the abdominal aortic diameter (AAD). Mice were anesthetized using 2% isoflurane (Vet One), and laid supine on a heated 37°C plate. Two-dimensional B-mode imaging was performed using a real-time microvisualization scan head (RMV 704) with a central frequency of 40 MHz, frame rate of 30 Hz, a focal length of 6 mm, and a 20×20 mm field of view (Visualsonics). Transverse image scans were performed and cine loops of 300 frames were acquired throughout the infra- and suprarenal region of the mouse aorta. The acquired images were stored digitally on a built-in hard drive for offline analysis to determine maximal AAD. All aortic diameters were measured in anterior-posterior direction during the diastolic phase. US image analysis was performed using the accompanying Vevo770 software (Visualsonics). Measurements were accomplished using random selection of each dataset and operator blinding to prevent recall bias. All measurements were collected by one observer to limit bias, while results were analyzed by a second independent observer blinded to the treatment group.

Preparation of aortic tissue

Mice were sacrificed with an inhalation overdose of isoflurane (Vet One, Meridian). Immediately following sacrifice the abdominal aorta was transected and flushed via the left ventricle with ice cold phosphate buffered saline (PBS; pH 7.4). The aorta was then dissected from fat and connective tissue from the left renal artery to the bifurcation under a microscope (Leica). Specimens were snap frozen individually in liquid nitrogen and stored at -80°C to await further processing.

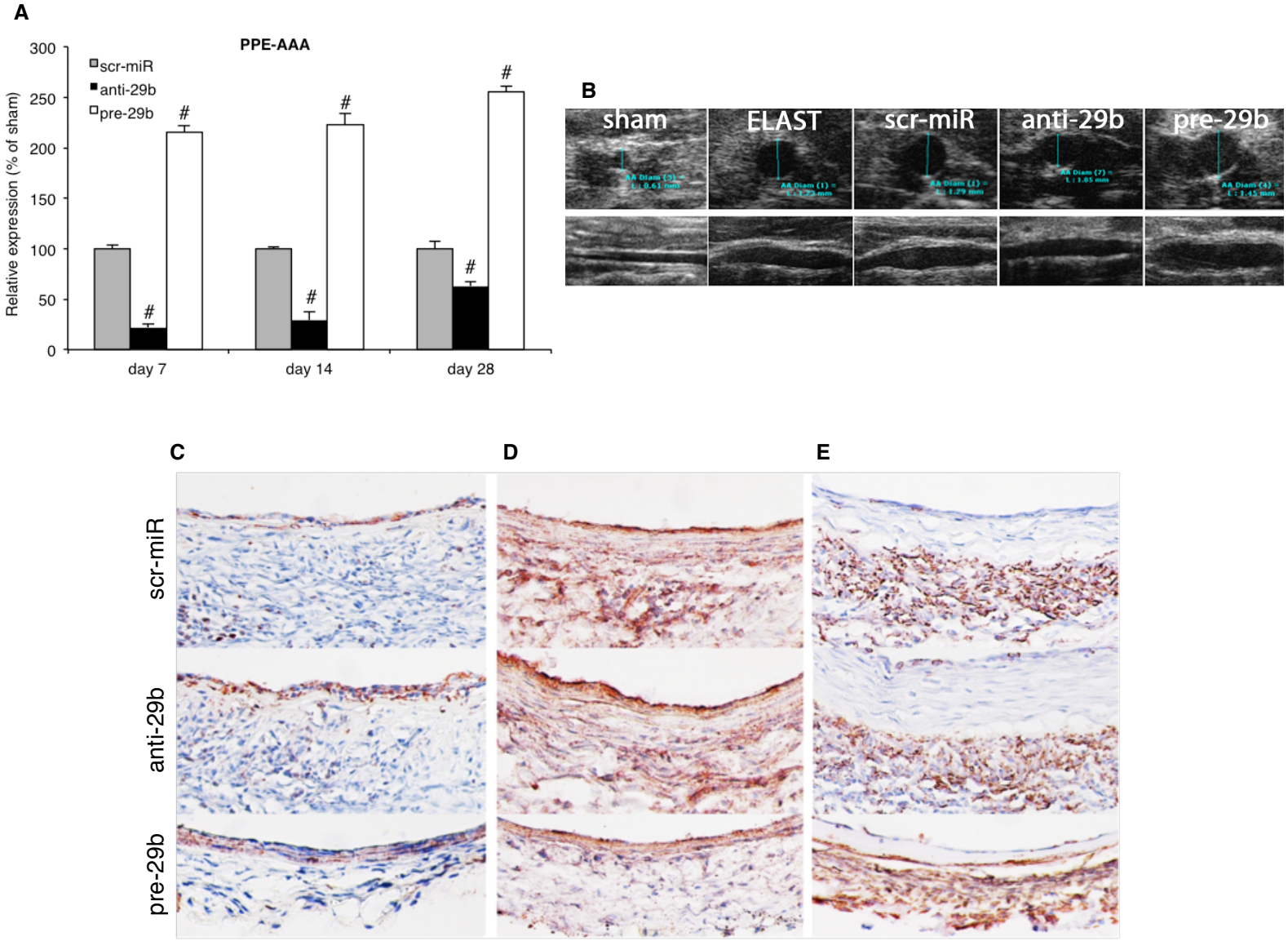
Histological and immunohistochemical analysis

Histological staining was performed in the same region of abdominal aorta that was imaged in order to obtain morphometric data to correlate with ultrasound measurements and gene expression results from qRT-PCR. Mice were euthanized and perfused at a constant pressure of 100 mmHg through the heart with saline followed by warm (37°C) agarose gel (Amresco) diluted in saline (3% w/v). After the agarose solidified, the abdominal aorta was dissected free from the surrounding connective tissue and fixed in 4% formalin. Isolated tissue was then dehydrated through a graded sucrose series and subsequently embedded in OCT blocks. Aortic tissue was segmented into 4 (0.5 mm spacing) 7 μm-thick serial sections from the left renal artery to the bifurcation and stained with Picrosirius Red (Sigma Aldrich), and in addition with a rabbit antibody against Col3a1 (Abcam) and the Vectastatin ABC kit (Vector Laboratories). Visualization was aided by AEC (DAKO) while counterstaining was performed with Mayer's Hematoxylin (Sigma Aldrich, St. Louis, MO, USA). Negative controls were performed with the omission of the primary antibody. In addition to counting the cells of specific interest as described in the manuscript, Image J (National Institute of Health) was used to perform histological analysis.

Picrosirius Red staining

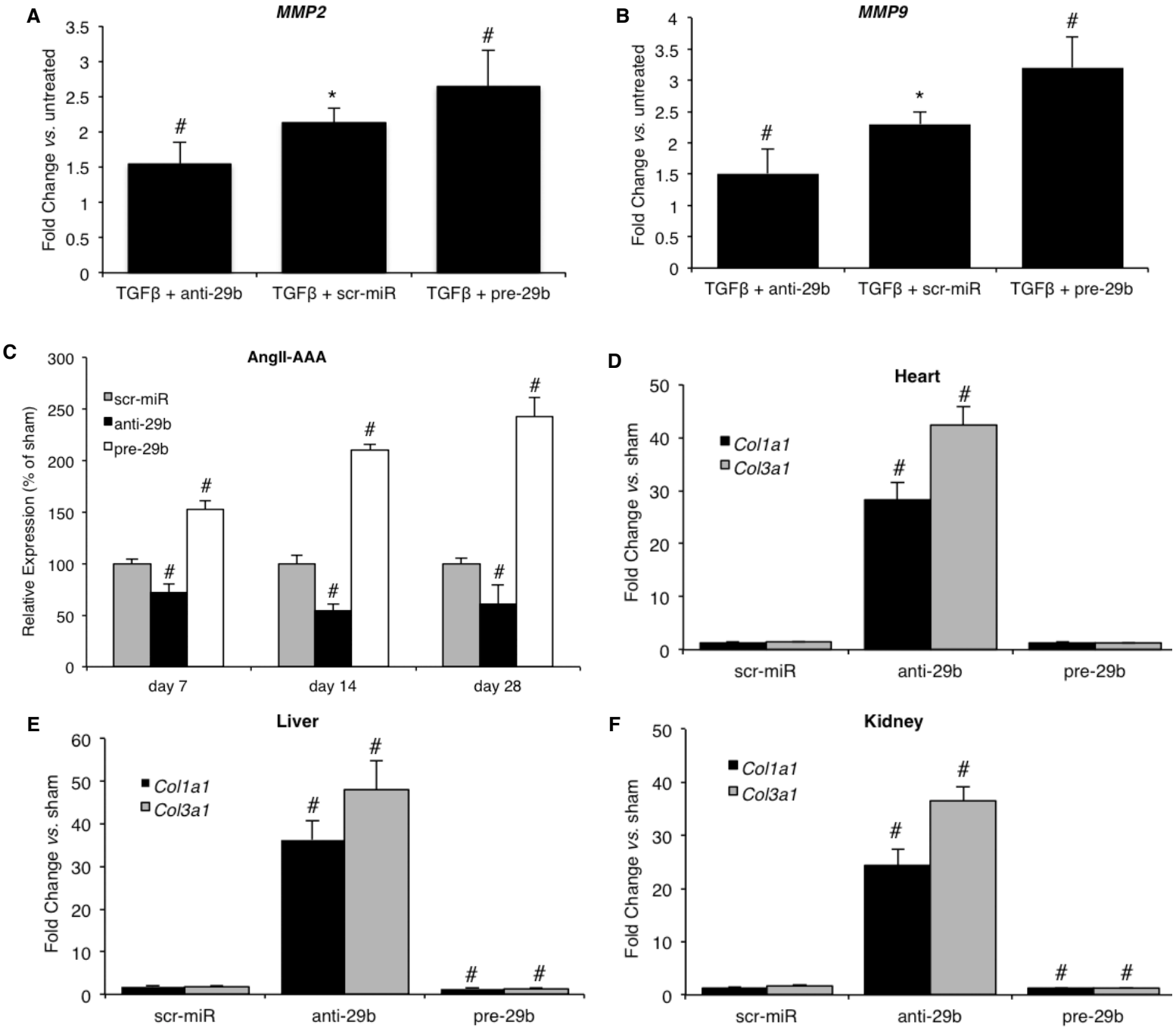
For sample preparation, please see above. Samples from sham, scr-miR-, as well as anti-/pre-29b were stained using the Picrosirius Red stain kit (Polysciences Inc) according to manufacturer's instructions. In brief, nuclei were stained using Weigert's hematoxylin. Samples were then washed in running tap water for 10 minutes, stained in Picrosirius Red for 1h, washed in 0.5% acetified water, dehydrated in 100% ethanol, cleared in xylene, and mounted using a resinous medium.

Supplemental Figure 1



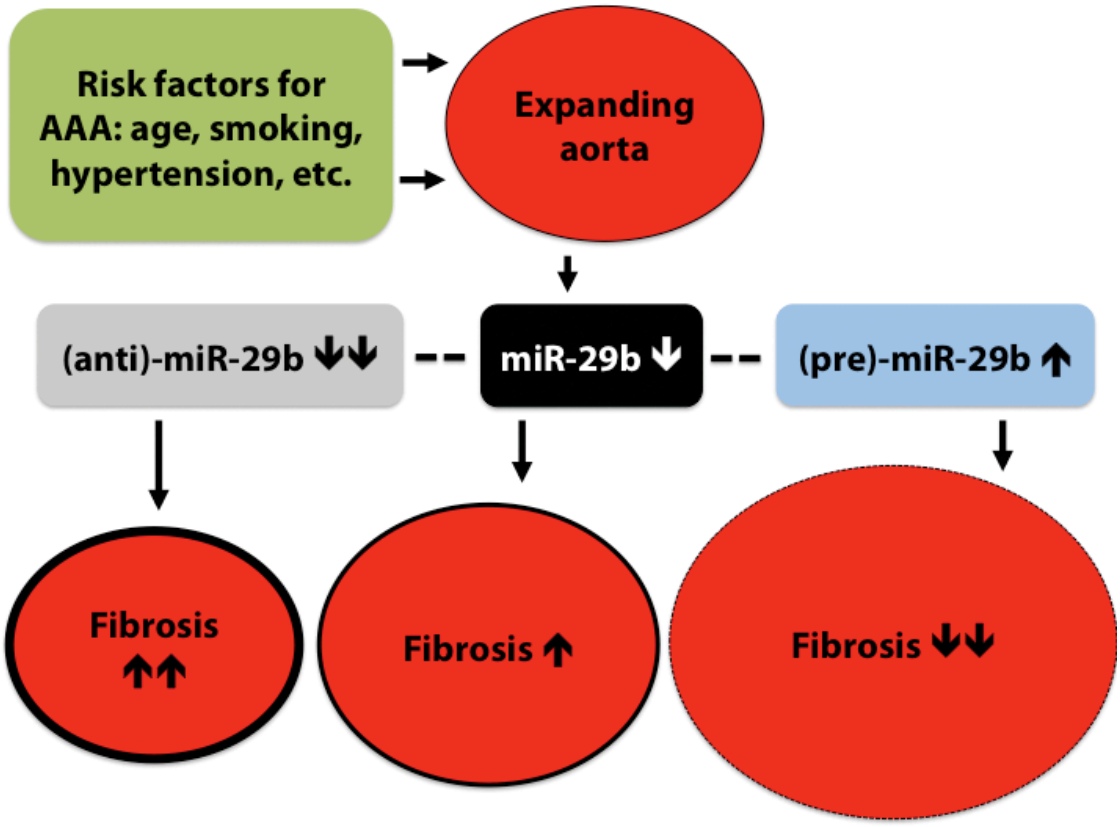
Suppl. Fig. 1. miR-29b expression in anti-/pre-29b transduced mice with PPE-AAA; representative ultrasound images and immunohistochemistry. (A) Relative miR-29b expression in anti-/pre-29b transduced mice compared to scr-miR, 14 days after injection and AAA induction using PPE. **(B)** B-mode ultrasound images with measurements in the transverse view (and corresponding longitudinal images below) of all different groups, 28 days after AAA-induction using PPE. **(C-E)** Representative immunohistochemistry images for SMA (A), Ki67 (B), and Mac-1 (C) in anti-/pre-29b and scr-miR transduced mice, 14 days after PPE-induced AAA (200x magnified). n = 4-8 mice in each group. Data are mean ± SEM. $^{\#}P<0.05$ vs. sham and scr-miR.

Supplemental Figure 2



Suppl. Fig. 2. *MMP2/9* expression in hAFB; miR-29b expression in anti-/pre-29b transduced mice with AngII-AAA and ancillary off target effects of miR-29b manipulation. (A-B) *MMP2* (A) and *MMP9* (B) expression in TGFβ +/- anti-/pre-29b transfected hAFB. **(C)** Relative miR-29b expression in anti-/pre-29b transduced mice compared to scr-miR, 14 days after injection and AAA induction using AngII. **(33)** *Col1a1* and *Col3a1* are significantly regulated in heart (D), liver (E), and kidney (F) samples of miR-29b modulated mice. *n* = 4-8 mice in each group. Data are mean ± SEM. **P*<0.05 vs. untreated. $^{\#}$*P*<0.05 vs. sham and scr-miR/pre-con.

Supplemental Figure 3



Suppl. Fig. 3. Proposed mechanism of miR-29b regulation in AAA disease.