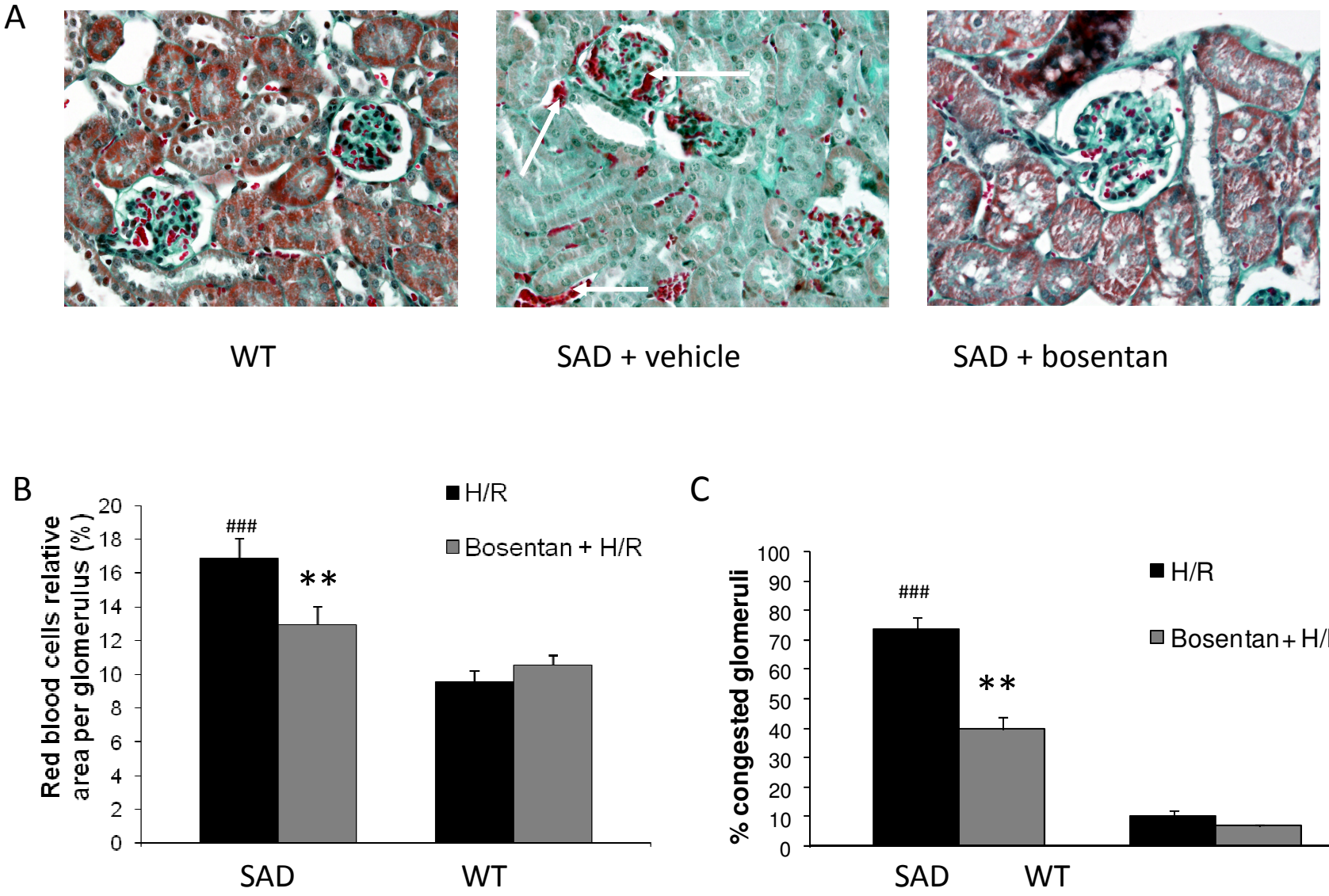
Doppler recording of renal blood flow waveforms
in SAD mouse: see supplemental video

Supplemental video 1

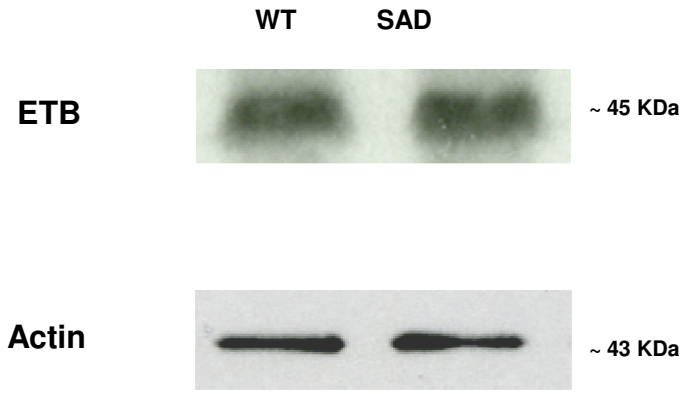


Supplemental Figure 1

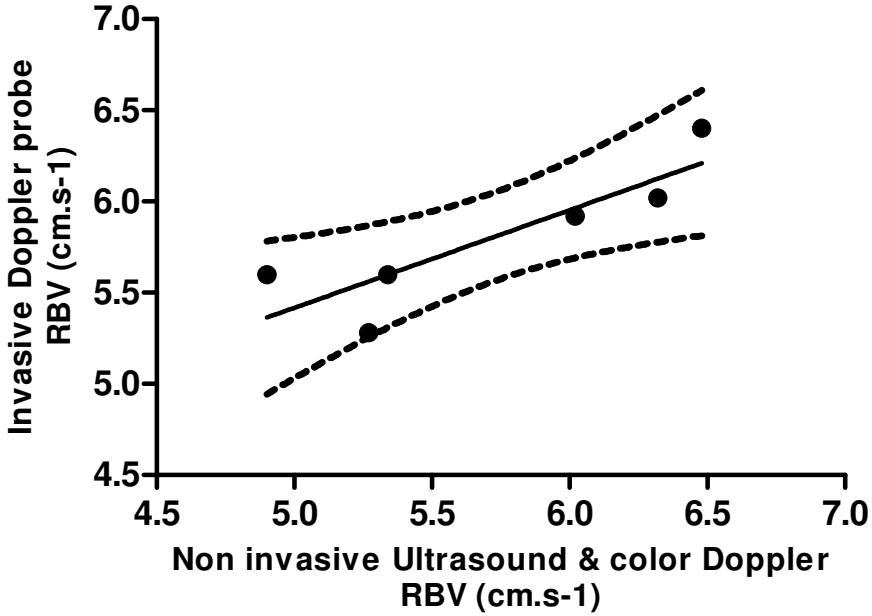
Table 1

	WT H/R					WT H/R + Bosentan					SAD H/R					SAD H/R + Bosentan					
	1	**2**	**3**	**4**	**5**	**1**	**2**	**3**	**4**	**5**	**1**	**2**	**3**	**4**	**5**	**1**	**2**	**3**	**4**	**5**	**6**
Vessels																					
Congestion	+	+	0	+	+	0	0	+	0	0	+++	++	++	++	+++	+	0	+	0	+	+
Constriction (μm)	0.02±0.004 (5)					0.06±0.005 (5)					0.09±0.002* (5)					0.04±0.009° (6)					
Thrombi	0	0	0	0	0	0	0	0	0	0	+	+	0	+	0	0	0	0	0	+	0
Bronchus																					
Mucus	0	0	0	0	0	0	0	0	0	0	++	++	+	++	++	0	+	0	+	+	+
Inflammatory cells	18.3±3.2 (5)					26.5±2.1 (5)					40.4±6 (5)*					29.6±1.4 (6)°					
Neutrophils	1.8±0.7 (5)					3.9±0.7(5)					8.7±0.9 (5)*					4.1±1.3(6)°					

Supplemental Table 1



Supplemental Figure 2



Supplemental Figure 3

Legends to supplemental material

Supplemental Video 1: Echo-Doppler recording of renal blood flow waveforms in a SAD mouse after H/R and before and after infusion of bosentan. Note the decrease in end-diastolic and time-averaged mean velocities in the SAD mouse after H/R and, following bosentan treatment, the reversal toward higher values indicating a decrease in vascular resistance.

Supplemental Figure 1: Endothelin-dependent trapping of red blood cells in glomeruli during experimental H/R-induced vaso-occlusive events in SAD mice. (**A**) Masson trichrome-stained kidney sections show congestion and dilation of glomerular and interstitial capillary loops (arrows) of a vehicle-treated SAD mouse whereas permeable capillary lumens are present in normal (WT) and bosentan-treated mice. (**B**) Image analysis of the relative area covered by RBCs in glomeruli of SAD and wild-type (WT) mice. The percentage of the glomerular area covered by RBCs in SAD mice was 165% of that in WT mice. Administration of bosentan for two weeks partially inhibited entrapment of RBCs (such that the percentage of surface area per glomerular section covered by RBCs was 126% of that in wild type mice. ### $P < 0.001$ versus untreated wild type; ** $P < 0.01$ versus vehicle-treated SAD mice. (n = 7 per group).(**C**) Assessment of the proportion of congested glomeruli in H/R-exposed animals. The percentage of congested glomeruli was lower in SAD mice treated with bosentan than in vehicle-treated SAD controls. ### $P < 0.001$ versus untreated wild type; ** $P < 0.01$ versus vehicle-treated SAD mice. (n = 7-9 per group).

Supplemental Table 1: Effect of bosentan on the histopathological scoring of acute lung injury in SAD mice after H/R. WT: wild-type; H/R: 46 hours hypoxia and 2 hours reoxygenation. *Congestion:* This was evaluated as the percentage of vessel area filled with red blood cells (RBCs): 0, no RBCs; +, RBCs filling less than 30% of the vessel section; ++, RBCs filling between 30 and 50% of the vessel; +++, more than 50% of the vessel filled with RBCs. *Constriction:* Data are expressed as mean numbers of cells ± SD per field at 250x magnification (*n* of experiments); **P* <0.05 compared to wild-type; ° *P* < 0.05 compared to SAD mice exposed to hypoxia. *Thrombi:* 0, no thrombi; +, presence of thrombi in small vessels. *Mucus:* 0, no mucus; +, mucus filling less than 50% of the area of the bronchus section; ++, mucus filling more than 50% of the area of the bronchus section; *Inflammatory cells* and *Neutrophils:* Data are expressed as mean numbers of cells ± SD per field at 250x magnification (*n* of experiments); **P* < 0.05 compared to wild type; ° *P* < 0.05 compared to SAD mice exposed to H/R.

Supplemental Figure 2: Semiquantitative representative immunoblot of ETB receptor using erythrocyte proteins prepared from SAD and normal mice. No significant difference in ETB abundance was observed between conditions after normalization for actin content.

Supplemental Figure 3: Plot of renal blood flow velocity (RBV) in the left renal artery in six C57BL/6J male mice after laparotomy with a pulsed-Doppler probe (Y axis) and a transcutaneous ultrasound and color Doppler method (X axis). Dotted lines delineate the 95% CI. Spearman r= 0.89, *P*= 0.033.

Supplemental methods

Comparison of the transcutaneous ultrasound and Doppler method with the in situ pulsed Doppler probe for the measurement of renal blood flow.

At the time of sacrifice, mice were anesthetized as indicated above. After the mouse was placed on its right side, the left kidney was exposed through a subcostal incision and the left renal artery was gently dissected and isolated from the renal vein using a method adapted from Castier Y et al. (Circ Res. 2005;97(6):533-40). The arteries were photographed *in situ*, alongside a 460 µm-wide scale placed parallel to the vessel, using a camera (Logitech) attached to the surgical microscope (Nikon). Precise arterial diameters were measured with the help of image analysis software (Histolab). Blood flow velocity was measured by using a 20 MHz pulsed Doppler system. The output of the recorder was fed to a Hewlett-Packard high speed digital voltmeter No. 3437A which is interfaced with a desk-top computer which is programmed to call upon the voltmeter to sample the wave form of the inputs separately and sequentially in periods which can be as short as 0.2 milliseconds. The pen probe (Millar Instruments) position was adjusted to obtain a beam angle of 30 degrees with the vessel axis. Blood flow was measured 2 mm from the ostium of the left renal artery. Velocities (*V*, cm/sec) were obtained from the measured Doppler frequency shifts and volume flow (Q) was calculated by multiplying the mean velocity by the cross-sectional area of the vessel lumen, using the formula Q (cm3/s) $=\pi r2\ V$, where *r* is the radius in cm. Two consecutive invasive measurements were performed on each mouse then the transcutaneous measurement of renal blood flow velocity with ultrasound and pulsed Doppler was done after localization of the renal artery with color-Doppler ultrasound as described above following the transcutaneous ultrasound and Doppler method published by Bonnin P et al. (Ultrasound Med Biol. 2008 Feb 5;34). Measurements were performed in six mice. Non parametric Spearman correlation test was performed (supplemental Figure 3).