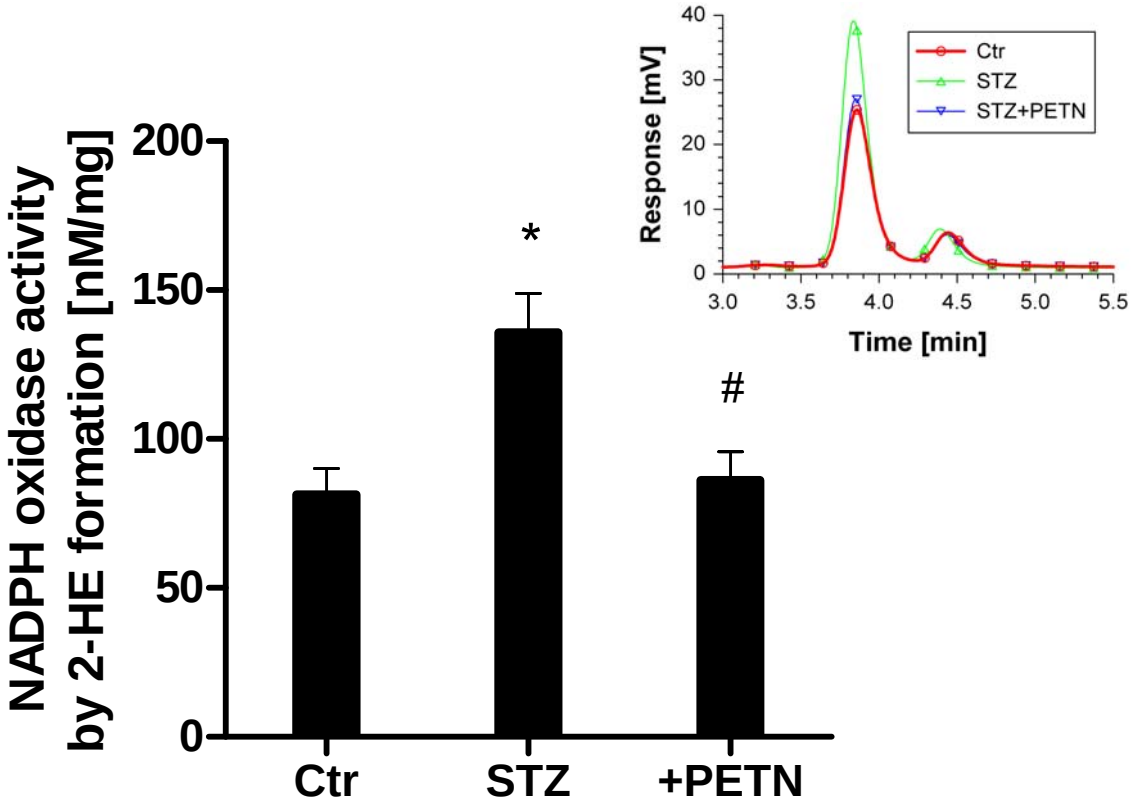
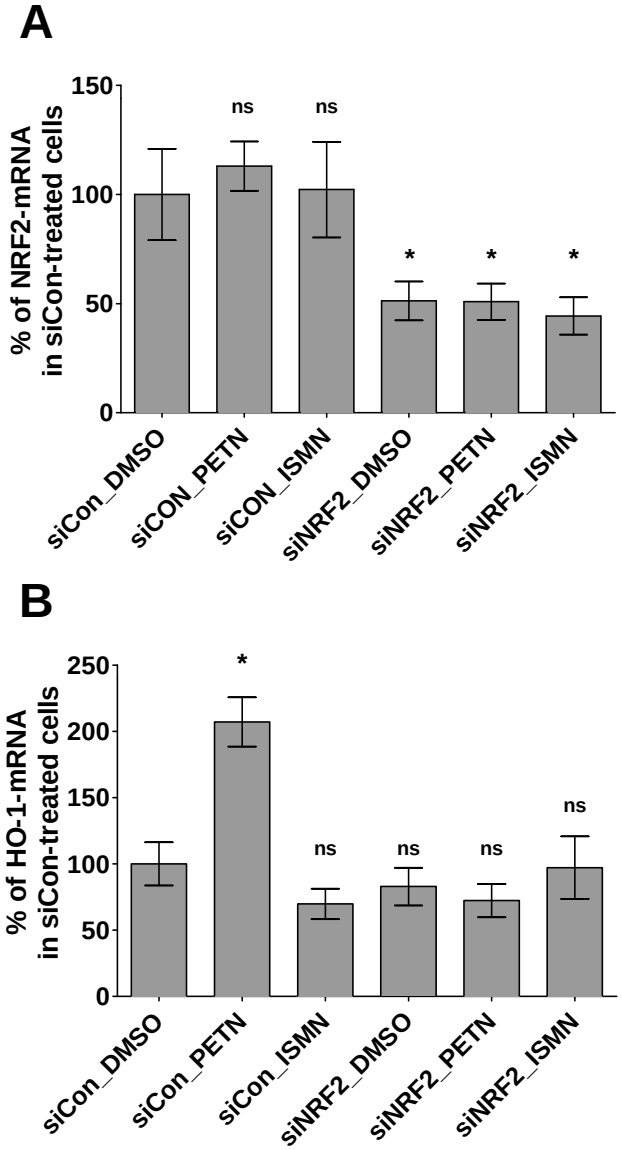
SUPPLEMENTARY DATA

**Supplementary Figure 1. NADPH oxidase activity was determined in cardiac membranous fractions by HPLC-based quantification of the superoxide-specific dihydroethidine oxidation product 2-hydroxyethidium. The insert shows representative chromatograms. The data are mean ± SEM of samples from 3 animals/group. *, p<0.05 vs. control group and $^{\#}$, p<0.05 vs. STZ-treated group.**



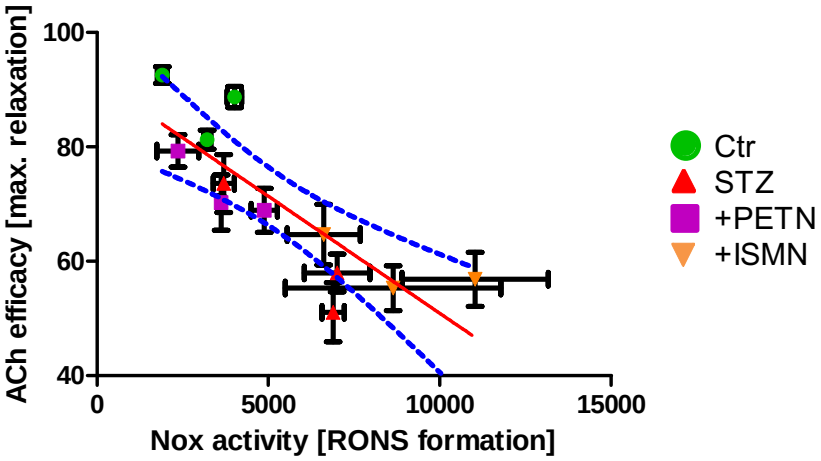
SUPPLEMENTARY DATA

**Supplementary Figure 2. Downregulation of NRF2 expression by siRNA reduces PETN-induced HO-1 mRNA and expression.** The RNA interference technique was used to downregulate NRF2 expression. DLD-1-HO-1-prom cells were transfected with a siRNA against NRF2 (siNRF2) or a negative control siRNA (siCon). After 48 h the transfected cells were preincubated for 16 h in medium without FCS and phenolred. Then the cells were incubated with 50 µM PETN, or DMSO or 50 µM ISMN for 8 h. RNA was isolated by guanidinium thiocyanate/phenol/chloroform extraction as described (1; 2) and NRF2 (**A**), HO-1 (**B**) and GAPDH mRNA expression was analyzed by two step real-time-RT–PCR as described before (3). For these qRT-PCR analyses the following oligonucleotides served as sense and antisense primers and Taqman hybridization probes: NRF2, sense 5'-AAACCAGTGGATCTGCCAAC-3', antisense 5'-GCAATGAAGACTGGGCTCTC-3', probe 5'-ACTCCCAGGTTGCCCACATTCCCA-3'; HO-1 , sense 5'-AGGCCAAGACTGCGTTCCT-3', antisense 5'-GGCTCTGGTCCTTGGTGTCAT-3', probe 5'-CTCAACATCCAGCTCTTTGAGGAGTTGCAG-3'; GAPDH, sense 5'-CCCATGTTCGTCATGGGTGT-3', antisense 5'-TGGTCATGAGTCCTTCCACGATA-3', probe 5'-CTGCACCACCAACTGCTTAGCACCC-3'. To calculate the relative expression of HO-1- or NRF2 mRNA in DLD-1 cells the $2^{(-\Delta\Delta C(T))}$ method (4) was used. A summary of four qRT-PCR analyses using RNA isolated from transiently transfected DLD-1-HO-1-prom cells is shown. Data (means + SEM) represent relative HO-1 mRNA levels (* $p < 0.05$; ns = not significant vs. siCon and DMSO treated DLD-1-HO-1-prom cells).



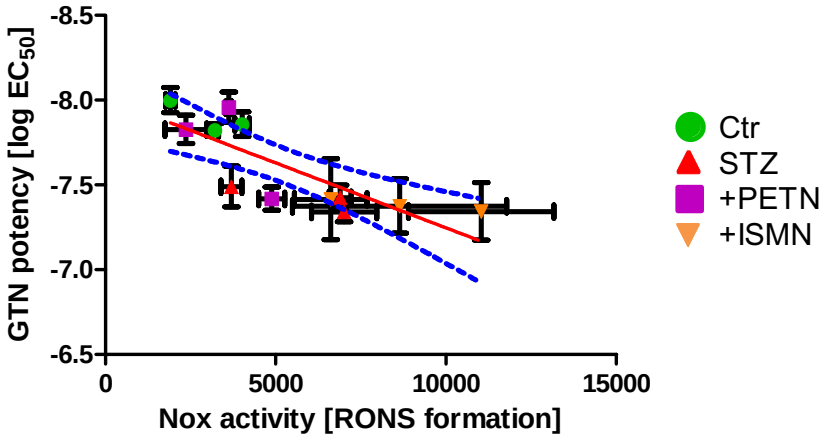
SUPPLEMENTARY DATA

**Supplementary Figure 3. Correlation between endothelium (ACh)-dependent relaxation and cardiac membranous NADPH oxidase activity.** Cardiac and aortic NADPH oxidase activity go hand in hand in the STZ-induced type 1 diabetes model (5) providing an explanation for the observed correlation between endothelial function and cardiac Nox acvtivity. Linear regression: p=0.0008, $R^2$=0.6919. The data for vascular function and oxidative stress was matched on a weekly basis. All data were divided into 3 different time intervals (three different weeks of the study) and in each time interval vascular function and oxidative stress were correlated for each treatment group. Linear regression was performed with GraphPad Prism 5 for Windows (version 5.02).



SUPPLEMENTARY DATA

**Supplementary Figure 4. Correlation between endothelium (GTN)-independent relaxation and cardiac membranous NADPH oxidase activity.** Cardiac and aortic NADPH oxidase activity go hand in hand in the STZ-induced type 1 diabetes model (5) providing an explanation for the observed correlation between GTN potency and cardiac Nox acvtivity. Linear regression: p=0.0015, $R^2$=0.6516. The data for vascular function and oxidative stress was matched on a weekly basis. All data were divided into 3 different time intervals (three different weeks of the study) and in each time interval vascular function and oxidative stress were correlated for each treatment group. Linear regression was performed with GraphPad Prism 5 for Windows (version 5.02).

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