Supplementary data

Supplementary Table 1 – List of primers

Gene	Species	Forward Sequence	Reverse Sequence
IL-6	Mouse	ACAAAGCCAGAGTCCTTCAGAGA	AGGAGAGCATTGGAAATTGGGGT
NOX 4	Mouse	CGGGATTTGCTACTGCCTCCAT	GTGACTCCTCAAATGGGCTTCC
TNF-alpha	Mouse	GATCGGTCCCCAAAGGGATG	GGCTACAGGCTTGTCAGTCG

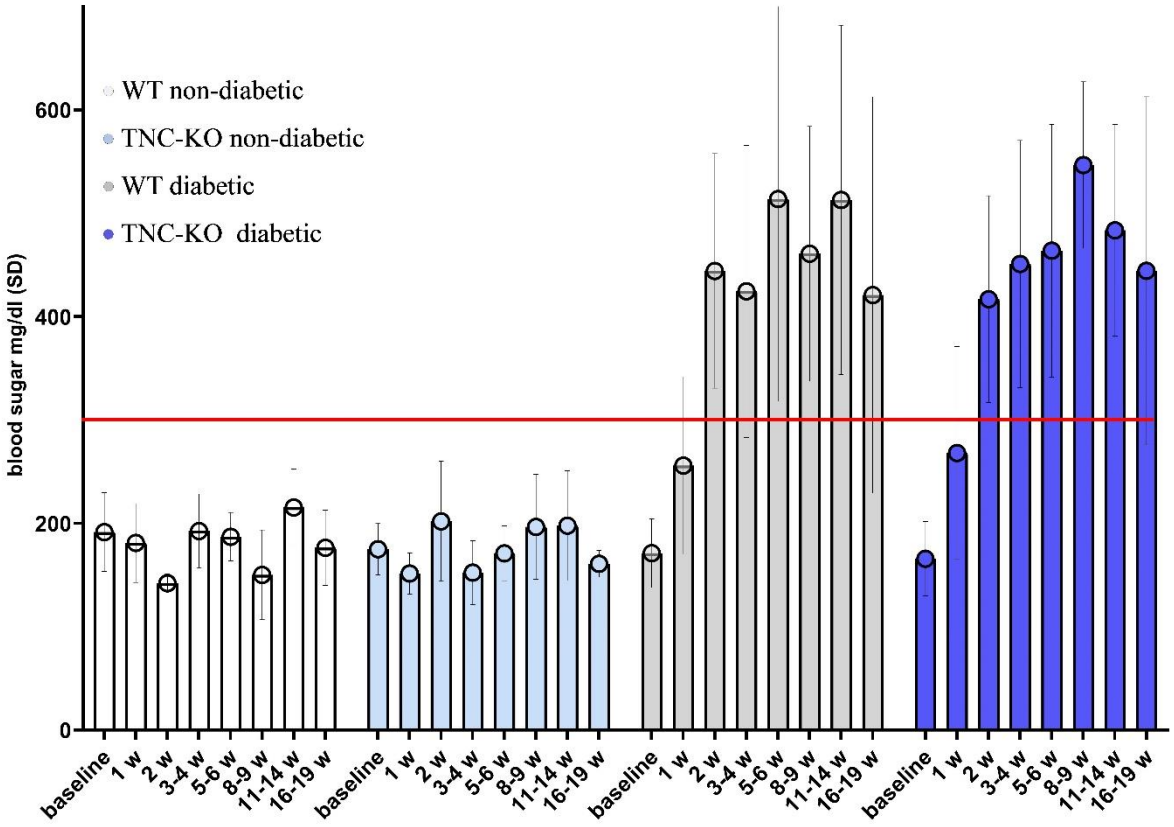
Amino acids: adenine (A), cytosine (C), guanine (G), and thymine (T); IL-6: interleukin 6; NADPH: nicotinamide adenine dinucleotide phosphate oxidase; NOX 4: NADPH oxidase 4; TNF-alpha: tumor necrosis factor alpha

Supplementary Table 2 – Baseline echocardiographic measurements

	LVEF % baseline	E/A ratio baseline	LVESD mm baseline	LVEDD mm baseline
WT non-diabetic	59.57 ± 11.05	1.76 ± 0.26	2.30 ± 0.28	3.33 ± 0.24
WT diabetic	54.75± 10.05	1.73 ± 0.64	2.50 ± 0.28	3.47 ± 0.23
TNC-KO non-diabetic	54.53 ± 9.85	2.24 ± 0.31	2.46 ± 0.41	3.40 ± 0.47
TNC-KO diabetic	56.08 ± 7.92	1.59 ± 0.28	2.17 ± 0.40	3.06 ± 0.35

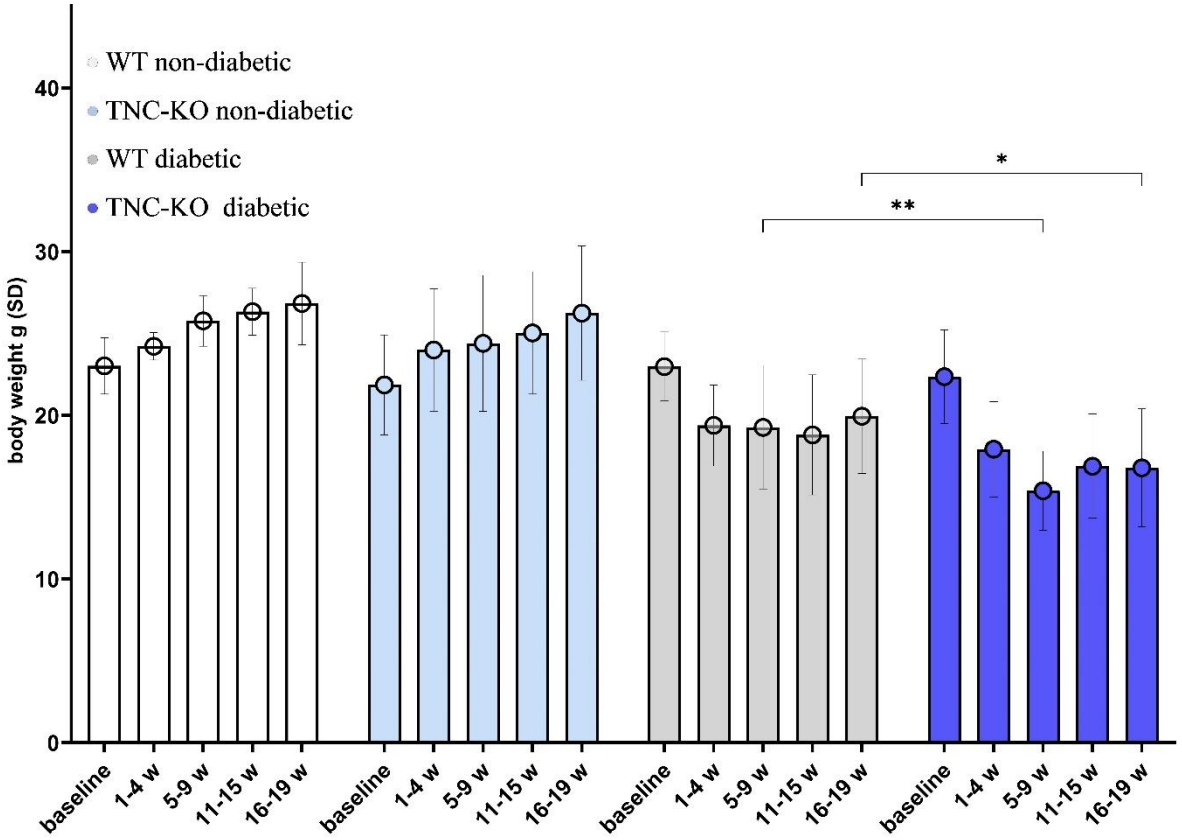
Supplementary Table 2 – Baseline echocardiographic measurements LVEDD: left ventricular enddiastolic diameter; LVEF: left ventricular ejection fraction; LVESD: left ventricular endsystolic diameter; TNC-KO: Tenascin-C knockout; WT: wild-type

Supplementary Figure 1 – Blood sugar measurements



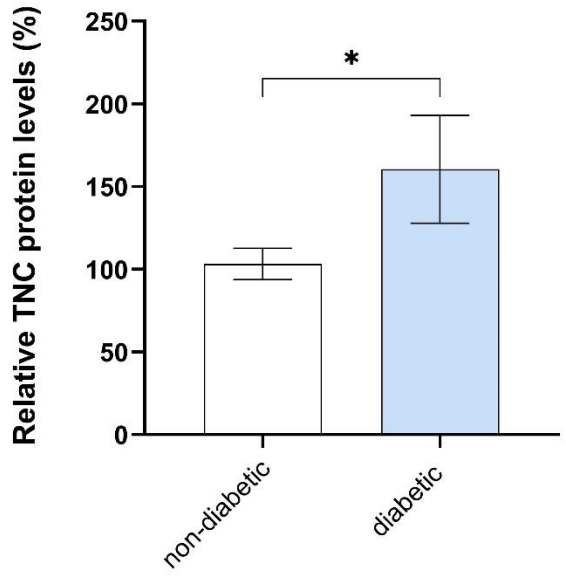
Supplementary Figure 1 Blood sugar measurements at baseline and at given intervals after streptozotocin injections. All induced animals become diabetic. No significant difference was observed between the diabetic groups: wild-type vs. Tenascin-C knockout (2way ANOVA, p = 0.5451). SD: standard deviation; TNC-KO: Tenascin-C knock-out; w: week; WT: wild-type

Supplementary Figure 2 – Body weight results after diabetes induction



*Supplementary Figure 2 Body weight measurements at baseline and after streptozotocin injections. Weight loss as a sign of untreated metabolic disturbances could be seen in all diabetic subjects. Tenascin-C knockout diabetic animals lost significantly more weight than wild-type diabetic animals. SD: standard deviation; TNC-KO: Tenascin-C knockout; w: week; WT: wild-type; **: p = 0.001-0.01; *: p = 0.01-0.05*

Supplementary Figure 3 – Cardiac expression of Tenascin-C



*Supplementary Figure 3 – Cardiac expression of Tenascin-C. Diabetic animals expressed significantly more Tenascin-C in the myocardium than non-diabetic mice. TNC: Tenascin-C; *: p = 0.01-0.05*

Supplementary Paragraph.

Angiotensin-converting enzyme activity measurement

Angiotensin-converting enzyme (ACE) activity in lung and kidney tissue samples was quantified as described previously [20]. Briefly, pieces of tissue samples were weighed, and a proportional amount of 100 mM tris(hydroxymethyl)aminomethane hydrochloride (TRIS) buffer (pH 7.0) was pipetted to them (5000X µl / X g of tissue). The samples were homogenized in this solution with TissueRuptor (Quiagen) and subsequently the same amount (5000X µl / X g of tissue) of 100 mM TRIS buffer supplemented with TritonTM X 100 (Sigma-Aldrich) was added to finish the tissue processing. This suspension was centrifuged at 13000 rpm for 5 min and the protein concentration of the supernatant was determined by PierceTM BCA Protein Assay Kit (Thermo Scientific) and TECAN (SparkControl Magellan V2.2) plate reader. Each tissue homogenate was diluted to 1 mg/ml concentration. The reaction mixtures in black 96

well microplate (Greiner Bio-One) contained 6 µl of serum or 1 mg/ml tissue homogenates in 35-fold dilution in 100 mM TRIS buffer, 50 mM NaCl, 10 µM ZnCl2 with 15 µM Abz-FRK(Dnp)P-OH substrate (synthesized by Peptide 2.0, Chantilly, VA, USA). The change of the fluorescence intensity was detected by TECAN (SparkControl Magellan V2.2) plate reader at 37 °C, λex was 340 nm and λem was 405 nm. The changes in fluorescence intensity were monitored in kinetic loops, at 1-min intervals for at least 30 min and the intensity values were plotted as a function of reaction time. The values of the fluorescence intensity were fitted by a linear regression (GraphPad Prism 6.0), and the fit was accepted only when r2 was >0.9. ACE activity was calculated by the following equation: activity=(S/k) * D/P; where S is the rate of the increase in fluorescence intensity (1/min), k is the change of fluorescence intensity during the complete cleavage of 1 pmol Abz-FRK(Dnp)P-OH substrate, D is the dilution of the sample and P is the mg/ml protein concentration. 1 unit (U) means 1 pmol substrate cleavage in 1 min by 1 mg of protein.

Plasma Malondialdehyde Analysis

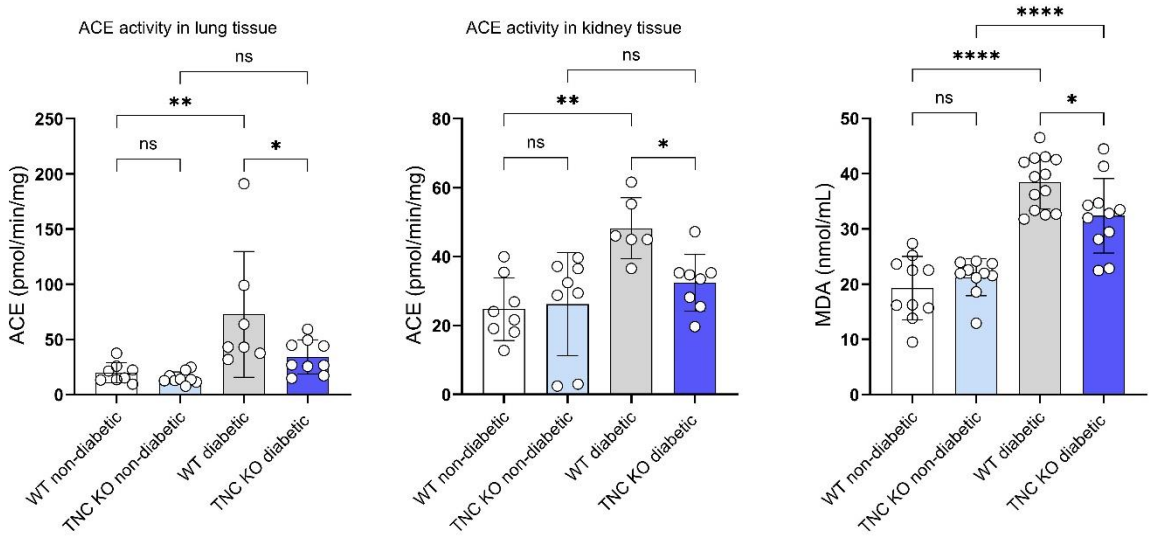
Plasma level of malondialdehyde (MDA) as a marker for oxidative stress was determined in principle according to a previously described HPLC method by Pilz *et al.* [42] after derivatization with 2,4-dinitrophenylhydrazine (DNPH). In brief, for alkaline hydrolysis of protein bound MDA 25 µL of 6 mol/L sodium hydroxide was added to 0.125 mL of EDTA plasma (1.5 mL Eppendorf tubes) and incubated at 60° (Eppendorf heater) for 30 min. The hydrolyzed sample was deproteinized with 62.5 µL 35% (v/v) perchloric acid. 125 µL supernatant obtained after centrifugation (14000 g; 2 min) was mixed with 12.5 µL DNPH solution and incubated for 10 min. This reaction mixture diluted derivatized standard solutions (0.625 nmol/mL – 10 nmol/mL) and reagent blanks were injected into the HPLC system (injection volume: 40 µL).

The MDA standard was prepared by dissolving 25 µL 1,1,3,3-tetramethoxypropane (TMP) in 100 mL bidistilled H_2O (stock solution: 1 mmol/L). The hydrolysis was performed with 200 µL TMP stock solution in 10 mL 1% sulfuric acid and incubation for 2h at room temperature [43]. The resulting MDA standard of 20 nmol/mL were further diluted with 1% sulfuric acid to the final concentrations. The DNPH derivates (hydrazones) were isocratically separated on a 5-µm ODS hypersil column (150x4.6 mm) guarded by a 5-µm ODS hypersil column (10x4.6 mm; Uniguard holder) with a mobile phase consisting of a 0.2% (v/v) acetic acid solution (bidistilled water) containing 50 % acetonitrile (v/v). The HPLC separations were performed with a SIL- 40C autosampler, a LC-40D pump and a SP–M40 diode array detector (Schimadzu, Korneuburg, Austria). Detector signals (absorbance at 310 nm) were recorded and the program Labsolution (Schimadzu, Korneuburg, Austria) was used for data requisition and analysis. Optionally EZchrom Elite (VWR) was used via a SS420x interface for recording and data analysis.

Lacking of TNC protects against increased angiotensin-converting enzyme activity in lung and kidney tissue samples as well as plasma malondialdehyde upregulation in mice

To evaluate potential factors contributing to LV dilation, myocardial fibrosis and vascular endothelial dysfunction, we assessed angiotensin-converting enzyme (ACE) activity in kidney and lung tissue samples. Diabetes induces a significant upregulation of ACE activity in both organs, and TNC-KO mice showed lower ACE activity than WT diabetic mice (Figure 5A-B). These observations were accompanied by a restoration of cardiovascular function in TNC-KO diabetic mice.

Figure 4A B C



Supplementary Figure 4 Angiotensin-converting enzyme (ACE) activity and plasma malondialdehyde (MDA) levels*. 4A-B: Diabetes increases ACE activity significantly in lung and kidney tissue, whereas TNC deficiency protects the diabetic animals from this increase. 4C: Significantly elevated MDA levels in plasma were assessed in diabetes ($p < 0.0001$) and TNC deficiency significantly attenuated lipid peroxidation by MDA plasma levels in diabetes ($p = 0.0318$). ACE: angiotensin converting enzyme; MDA: malondialdehyde; TNC-KO: Tenascin-C knock-out; WT: wild-type; ****: $p < 0.0001$; **: $p < 0.01$; *: $p < 0.05$. Data shown as mean ± SD, n = 7-14/ group.*