

Supplemental Material

Data S1.

Supplemental Methods

Cell culture

The cell experiments were performed with the myocardial cell line HL-1, which was derived from the AT-1 mouse atrial cardiomyocyte tumor line (6). The cell line was provided by Prof. W.C. Claycomb (†) (Louisiana State University, USA) and cultivated in Claycomb medium supplemented with 10 % FBS, 1 % P/S, norepinephrine (0.1 mM) and L-glutamine (2 mM).

The HL-1 cardiomyocytes were seeded in 6 well plates, which were previously coated with fibronectin and gelatin. At a confluence of 80 %, the HL-1 cardiomyocytes were starved with the described Claycomb medium, but only with a reduced FBS content of 0.5 % overnight. Cells were stimulated with 10nM of the LXR-agonist AZ876 for 24h. The cells were then harvested and stored at -80°C for further analysis.

Diet preparation and pair feeding

A dry feed for mice (V1127-000) was purchased from (ssniff, Spezialdiäten GmbH). 900g of the feed was mixed with the LXR-agonist AZ876 until complete homogeneity was achieved. 1500g of water was added to the powder in portions, while the mass was mixed continuously. The moist and homogeneous mass was applied to blotting paper about 1 cm thick and cut into squares of 2 x 2 cm. After 48 hours drying, the solid squares were stored at -20°C. To keep the differences in food intake as small as possible, mice were kept in pairs in each cage. Previous

experiments in our group resulted in a food intake of 3.5g per mouse per day. Therefore, we provided 7g food per day, either with or without LXR agonist for each cage.

Echocardiography

Echocardiography was performed with an 18-38 MHz linear array transducer using a digital ultrasound system (Vevo 2100 Imaging System, VisualSonics, Toronto, Canada). Sample size was determined based on previous experience with this model (4). The mice were placed in a supine position on a movable, heated platform at 37°C. The mice were anesthetized with 1.5% isoflurane and their pleura was removed. At each echocardiographic examination, long- and short-axis B-mode parasternal views were recorded and conventional echocardiographic parameters were determined. Trans mitral flow measurements of ventricular filling velocity were obtained from the apical four-chamber view using the pulse wave Doppler to assess diastolic function. In addition, tissue Doppler images were obtained from this view at the septal fixation of the mitral valve. Using speckle tracking based strain analysis of two-dimensional echocardiographic images obtained from the parasternal long- and short-axis views, strain parameters in the longitudinal, radial and circumferential axis were quantified (16, 17). Longitudinal strain and radial strain were obtained from the longitudinal axis parasternal views, while circumferential strain in the short axis parasternal views was determined at the medial optic disc plane. All images were acquired at a frame rate of approximately 300 frames per second. The longitudinal contraction and global longitudinal strain (GLS) represent the movement from the base to the apex. The global radial strain (GRS) represents

the myocardial thickening and thinning as the radial contraction in the short axis moves vertically to the long axis and the epicardium. Global circumferential strain (GCS) represents the radius movements in the short axis. To measure diastolic function, the mice were placed in an angled position with the head pointing slightly downward. In this position the apical four-chamber view is visual and the pulse wave doppler and tissue doppler can be determined. To determine the optimal position of the pulse wave doppler, the measurement point was placed slightly above the mitral valve with a strong color mode signal as an additional orientation aid. The incoming signals of the mitral valve show the individual phases and their contribution to filling. Since the mitral inflow represents the pressure differences between the ventricle and the atrium, all changes and anomalies in diastolic pressure affect the intensity and shape of the individual signals.

Image analyses

All image analyses were performed by one investigator and confirmed by a blinded second investigator using the software Vevo Lab (Fujifilm VisualSonics). The high-resolution images are displayed in slow motion, which allows an accurate analysis of the images despite a heart rate of about 450bpm. Official formulas of the American Society of Echocardiography were used to calculate all echocardiographic parameters. B-mode images of the parasternal longitudinal axis were used to semi-automatically analyze systolic function. Strain-based analyses were performed on B-mode images during three consecutive and electrocardiographically controlled cardiac cycles. Semi-automated tracking of the endocardium and epicardium was performed, with minor manual correction if necessary. GLS and GRS were evaluated using long-axis parasternal images

(227±1 frames/sec.). The myocardial tracings were placed starting from the middle basal region. GCS was assessed using images acquired in the short axis view (277±8 frames/sec.). The papillary muscles were used to standardize the image acquisition, but were excluded from the images. To determine the strain parameters, 48 points are placed virtually along the endocardial border and tracked over time to calculate the deformation over the cardiac cycle.

Lipid profiling and agonist detection in cardiac tissue

Lipid extraction and measurement were performed in cooperation with Dr. Carsten Jaeger (Federal Institute for Materials Research and Testing, Berlin, Germany). Dried tissue extracts were reconstituted in 100 µL isopropanol/acetonitrile/water (2:1:1). 5 µL aliquots were injected into an Agilent 1290 UHPLC system (Agilent) coupled to a TripleTOF 6600 mass spectrometer (Sciex). Chromatographic separation was achieved by gradient elution (%A: 0 min, 60%; 1.2 min, 57%; 1.26 min, 50%; 7.2 min, 46%; 7.26 min, 30%; 10.8 min, 0%; 12.96 min, 0%; 13.02 min, 60%; 14.4 min, 60%) using a solvent system of 60:40 v/v acetonitrile/water (A) and 90:10 v/v isopropanol:water (B), both containing 10mM ammonium formate and 0.1% formic acid. The column used was a 2.1 mm × 75 mm × 1.7 µm CSH-C18 UPLC column (Waters) equipped with a 0.2 µm inline filter. The flow rate was 0.5 mL/min and the column temperature 55 °C. Electrospray ionization was performed in negative mode (ESI-) with the following settings: Alternating MS and MS/MS scans were recorded using sequential window-based acquisition of all theoretical fragment ion mass spectra (SWATH) (m/z 400-1250, window width 25 Da). Mass calibration was performed at the beginning of the sequence using an ESI (-) tune

mix (Sciex). For data analysis, MS files were converted into the ABF format (Analysis Base File) and imported into MS-DIAL (18). A lipidomics project was created ("Soft Ionization", "Data independent MS/MS", "Profile data", "Negative ion mode", "Lipidomics") and parameter settings were adjusted to match chromatographic and MS settings. After completion of data processing, results were exported to a text file and further analyzed with a user defined R script. In particular, data were filtered for sparse metabolites and contradictory peak allocations were resolved. Finally, peak intensities were normalized and corrected for batch effects as described (19).

Data processing

The PLS-DA were calculated with R package "caret". Scores and loadings diagrams were generated with R package ggplot2 (20).

The Heatmaps were created with the online tool MetaboAnalyst (21). Thirty-three out of 117 detected lipid species were significantly altered by LXR treatment. The p -values obtained by two-way ANOVA were adjusted using the Benjamini-Hochberg correction ($p < 0.05$). The clustering in the lipid Heatmap was generated using average clustering and auto scaling, and shown are the color-coded intensities. Similarly, the Heatmap showing gene expression profiles of cardiac inflammatory & autoimmunity genes was generated. Average clustering and auto scaling was applied, and shown are the color-coded gene expression levels.

Histological analyses

The histological preparations and analyses were carried out in cooperation with Robert Klopfeisch from the Department of Veterinary Pathology, Free University

Berlin, Germany. At the end of the study organs of the mice were collected. The hearts were cut using a mouse heart matrix with a 0.5 mm incision. The apex, a mid-ventricular ring and the upper part of the heart were cut through and immediately formalin-fixed. The fixed midventricular ring was embedded in paraffin and the incisions were transferred to slides. The histological slides were stained with Picrosirius red to determine the collagen content. Endocardium and epicardium were defined manually using the Aperio ImageScope software. The collagen content was then automatically calculated as the percentage of collagen fibers stained with Picrosirius Red over the entire image area. To visualize the degree of macrophage infiltration, histological slides were stained for the Mac-3 marker, a macrophage surface glycoprotein, using an anti-mouse Mac3 antibody in a dilution 1:500 (#550292, BD Bioscience, CA, USA).

RNA extraction from HL-1 cells

The RNA expression was carried out with the Machery-Nagel NucleoSpin ®RNA kit. The cells were lysed in a mixture of RA1 lysis buffer and one percent β mercaptoethanol. β -Mercaptoethanol is a reducing agent that irreversibly denatures RNases by reducing disulfide bonds and destroying the native conformation required for enzyme functionality. 35 μ L of 70 % ethanol were added and the sample was resuspended. The entire sample was transferred to a NucleoSpin ®RNA column and centrifuged (30 sec at 11 000 rpm). 350 μ l membrane desalination buffer (MDB) was added. Salt removal significantly increases the effectiveness of rDNase digestion. After centrifugation (1 minute at 11 000 rpm) 95 μ l rDNase and reaction buffer were added to the column. After 15 minutes incubation at room temperature, 200 μ l wash buffer RAW2 were

added to inactivate rDNase. A second and third washing step was performed by adding 600 µl or 250 µl Wash Buffer RAW3. As a final step, 40 µl RNase-free water was added to the column and centrifuged at 11 000 rpm for 1 minute. The RNA concentration was measured immediately with a spectrophotometer (NanoDrop). The RNA samples were stored at -80°C.

RNA extraction from cardiac tissue

Tissue samples from the heart tips were frozen in liquid nitrogen. The samples were mechanically comminuted and homogenized to obtain a fine tissue powder. 5 to 10 milligrams of the sample were weighed and collected. All steps were carried out with tools and containers that were cooled in liquid nitrogen to prevent thawing of the material and thus prevent RNA degradation. The RNA was isolated with the RNeasy® Micro Kit. The samples were placed in lysis tubes containing ten ceramic beads (lysis beads) to macerate the tissue and a mixture of RLT lysis buffer and 1% β mercaptoethanol. The lysis tubes were placed in a SpeedMill for 30 seconds at 13,000 rpm and immediately placed on ice. 295 µl RNase-free water and 5 µl Proteinase K were added to the lysate and incubated for 10 min at 55°C. The samples were centrifuged for 3 minutes at 10,000 rpm. 225 µl absolute ethanol were added and the whole sample was transferred to an RNeasy-MinElute-Spin column. After 15 seconds centrifugation at 10 00 rpm 350 µl wash buffer RAW1 was added. 80 µl of a mixture of DNase and RDD buffer were added and the samples incubated at room temperature. Buffer RDD ensures efficient on-column digestion of the DNA and also ensures that the RNA remains bound to the column. After 15 minutes 350 µl RW1 buffer was added, followed by 500 µl RPE buffer and 500 µl 80% ethanol. After 2 minutes

centrifugation at 11 000 rpm and 5 minutes centrifugation at full speed, 15 μ RNase-free water was added and incubated at room temperature for 2 minutes. After a final centrifugation step for 1 minute at 11 000 rpm, the RNA concentration was measured with a spectrophotometer (NanoDrop). The RNA samples were stored at -80°C.

Single qRT-PCR analyses and qRT-PCR inflammation and autoimmunity assay

As previously described (22), RNA was reverse transcribed with reverse transcriptase, RNAsin and dNTPs (Promega) according to the manufacturer's protocol. The mRNA analysis was performed by quantitative RT-PCR analysis in presence of the fluorescent dye SYBR-Green (Life Sciences). The qRT-PCR results were normalized to 18S. Primer sequences are as follows:

<i>Gene</i>	<i>Forward Primer 5'-3'</i>	<i>Reverse Primer 3'-5'</i>
<i>18S</i>	CCTGAGAAACGGCTACCAT	TTCCAATTACAGGGCCTCGA
<i>Elovl5</i>	ATGGAACATTTTCGATGCGTCA	GTCCCAGCCATACAATGAGTAAG
<i>FADS2</i>	AAGGGAGGTAACCAGGGAGAG	CCGCTGGACCATTTGGTAA
<i>SCD2</i>	GCATTTGGGAGCCTTGTACG	AGCCGTGCCTTGTATGTTCTG

mRNA expression analyses of genes involved in inflammatory response and autoimmunity were performed using the QuantiNova LNA qPCR Assay (#249950 SBMM-077ZA, Qiagen, Hilden, Germany). Assays were performed according to the manufacturer's protocol.

Statistical methods

Data are presented as mean \pm SEM. Statistical analyses were performed using GraphPad Prism 8 (GraphPad Software, United States). For comparison of more than two groups, two-way analysis of variance (ANOVA) followed by Bonferroni post-hoc test was used. A value of $p < 0.05$ was considered statically significant. Following levels of statistical significance were used: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$. The determination of the group size for *in vivo* experiments was based on our experience with the ISO model (4). For analysis of lipidome data p-values were adjusted by Benjamini-Hochberg correction to avoid alpha inflation. Statistical significance was assumed at FDR-adjusted p-values of $p < 0.05$. Lipids were normalized to total lipid abundance in samples and either log₂-transformed or presented in measured intensities. For visualization, the log₂fold changes of the CTRL_VEH group were averaged over all samples and log₂ fold changes were calculated for the remaining groups compared to the control group.