Mast cells are the trigger of small vessel disease and diastolic dysfunction in diabetic obese mice

Authors and affiliations

Sarah Guimbal, PhD[1], Lauriane Cornuault, MS[1], Paul Rouault, MS[1], Pierre-Louis Hollier, PhD[1], Candice Chapouly, PharmD, PhD[1], Marie-Lise Bats, PharmD, PhD[1], Julien Imbault, MD[1], Alain-Pierre Gadeau, PhD[1], Thierry Couffinhal, MD, PhD[1*] and Marie-Ange Renault, PhD[1*]

* These authors contributed equally to this work

[1] Univ. Bordeaux, Inserm, Biology of Cardiovascular Diseases, U1034, CHU de Bordeaux, F-33604 Pessac, France

Supplemental material

Supplemental methods

Blood tests/NFS

Blood samples were collected on heparin retroorbital bleeding. Blood cell counts were determined using an automated counter (scil Vet abc Plus+). Plasmas were separated by a 10 min centrifugation at 2500 g and stored at -80°C. Concentrations of the following biochemical markers were measured using an Architect CI8200 analyzer (Abbott Diagnostics, North Chicago, Illinois, USA): triglycerides by the lipoprotein-lipase/glycerol kinase/oxidase/peroxidase method; total cholesterol by the esterase/oxidase/peroxidase method and HDL cholesterol by the accelerator/selective detergent/esterase/oxidase/peroxidase method. LDL cholesterol was then estimated using the Friedewald formula (LDL cholesterol (mmol/L) = total cholesterol – HDL cholesterol – (triglycerides/2,2) or LDL cholesterol (mg/dL) = total cholesterol – HDL cholesterol – (triglycerides/5)).

Isolation of mouse cardiac vascular fraction

Heart were dissociated using 2 mg/mL type IV collagenase (Gibco™, ThermoFisher) for 1 hour at 37°C and the resulting dissociated cells were filtrated on a 30 µm strainer. ECs cells were labelled with rat anti-mouse CD31 microbeads (Miltenyi Biotec). Labelled ECs and attached cells were then isolated magnetically. Importantly, collagenase digestion is incomplete and the CD31+ positive fraction included about 70% CD31+ endothelial cells, 29% NG2+ pericytes and 1% CD45+ leucocytes. The CD31+ fraction was referred as cardiac vascular fraction in the entire manuscript.

RNA sequencing

RNA was isolated using Tri Reagent® (Molecular Research Center Inc) as instructed by the manufacturer from the cardiac vascular fraction of $Lepr^{db/db}$ mice and control $Lepr^{db/+}$ mice. mRNA library preparation were realized following manufacturer's recommendations (KAPA mRNA HyperPrep Kit from ROCHE). Final samples pooled library prep were sequenced on Nextseq 500 ILLUMINA, corresponding to 2x30Millions of reads per sample after demultiplexing.

Quality of raw data has been evaluated with FastQC [16]. Poor quality sequences has been trimmed or removed with Trimmomatic [17] software to retain only good quality paired reads. Star v2.5.3a [18]has been used to align reads on mm10 reference genome using standard options. Quantification of gene and isoform abundances has been done with rsem 1.2.28, prior to normalisation with edgeR bioconductor package [19]. Finally, differential analysis has been conducted with the glm framework likelihood ratio test from edgeR. Multiple hypothesis adjusted p-values were calculated with the Benjamini-Hochberg procedure to control FDR. The FPKM values of all transcripts of which expression was significantly different between both groups in Supplemental table 2.

Isolation of mouse cardiac Leucocyte/Flow cytometry

Heart were dissociated using 2 mg/mL type IV collagenase (Gibco™, ThermoFisher) for 1 hour at 37° and the resulting dissociated cells were filtrated on a 30 µm strainer. Leucocytes cells were labelled with rat anti-mouse CD45 microbeads (Miltenyi Biotec). Labelled Leucocytes were then isolated magnetically. Fcer1a positive cells were subsequently labelled with rabbit anti-Fcer1a (Biorbyt, Cat# orb41809) and Alexa Fluor 647-conjugated anti-rabbit

IgG (Invitrogen, Cat# A-31573) while IgE were subsequently labelled with biotinylated anti-mouse IgE (Biolegend, Cat# 406903) and Alexa Fluor 488-conjugated streptavidine (Invitrogen, Cat# S11223). Fcer1a, IgE double positive cells were counted using a ACCURI® C6 flow cytometer (BD).

Cell culture

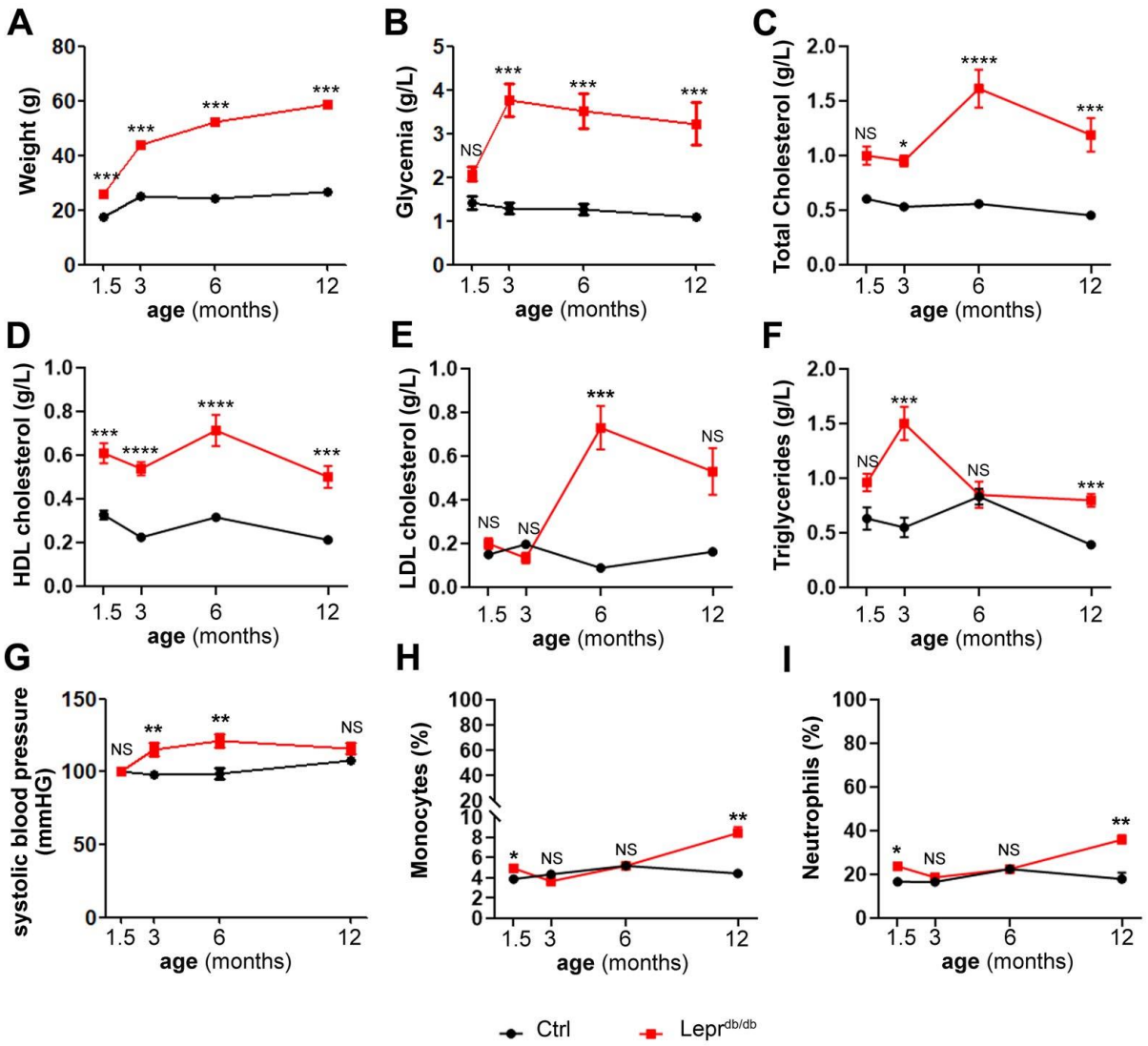
Human umbilical vein endothelial cells (HUVECs) (Lonza) were cultured in endothelial basal medium-2 (EBM-2) supplemented with EGM™-2 BulletKit™ (Lonza). Cell from passage 3 to passage 6 were used.

Mast cells were obtained from mouse bone marrow. Briefly, bone marrow cells were flushed out from mouse femurs and cultured in OptiMEM (Gibco) containing 10 ng/mL Il-3 (R&D systems) and 10 ng/mL Stem cell factor (R&D systems). After 2 week-culture, more than 90% of cells were Fcer1a+ and CD117+.

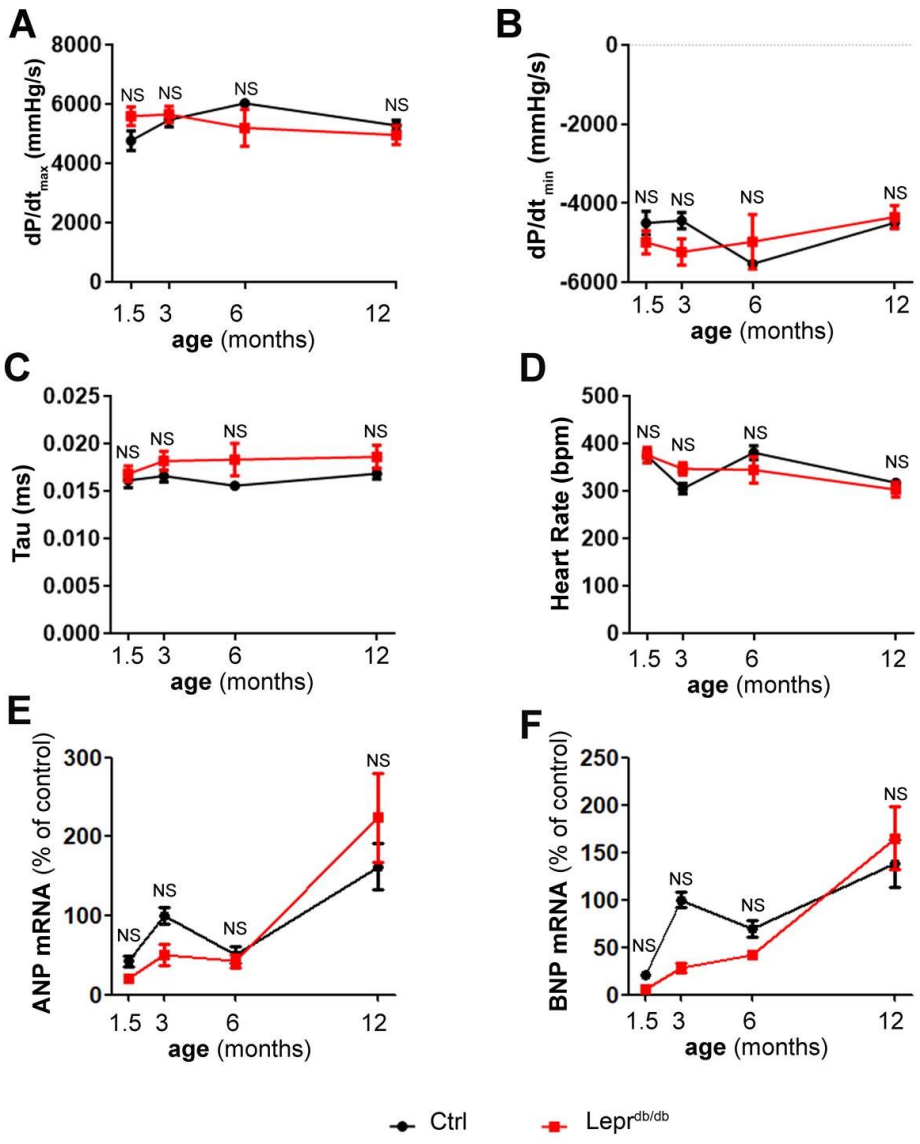
In vitro permeability assay

100 000 cells were seeded in Transwell® inserts. The day after, 0.5 mg/mL 70 kDa FITC-dextran (Sigma) was added to the upper chamber. FITC fluorescence in the lower chamber was measured one hour later.

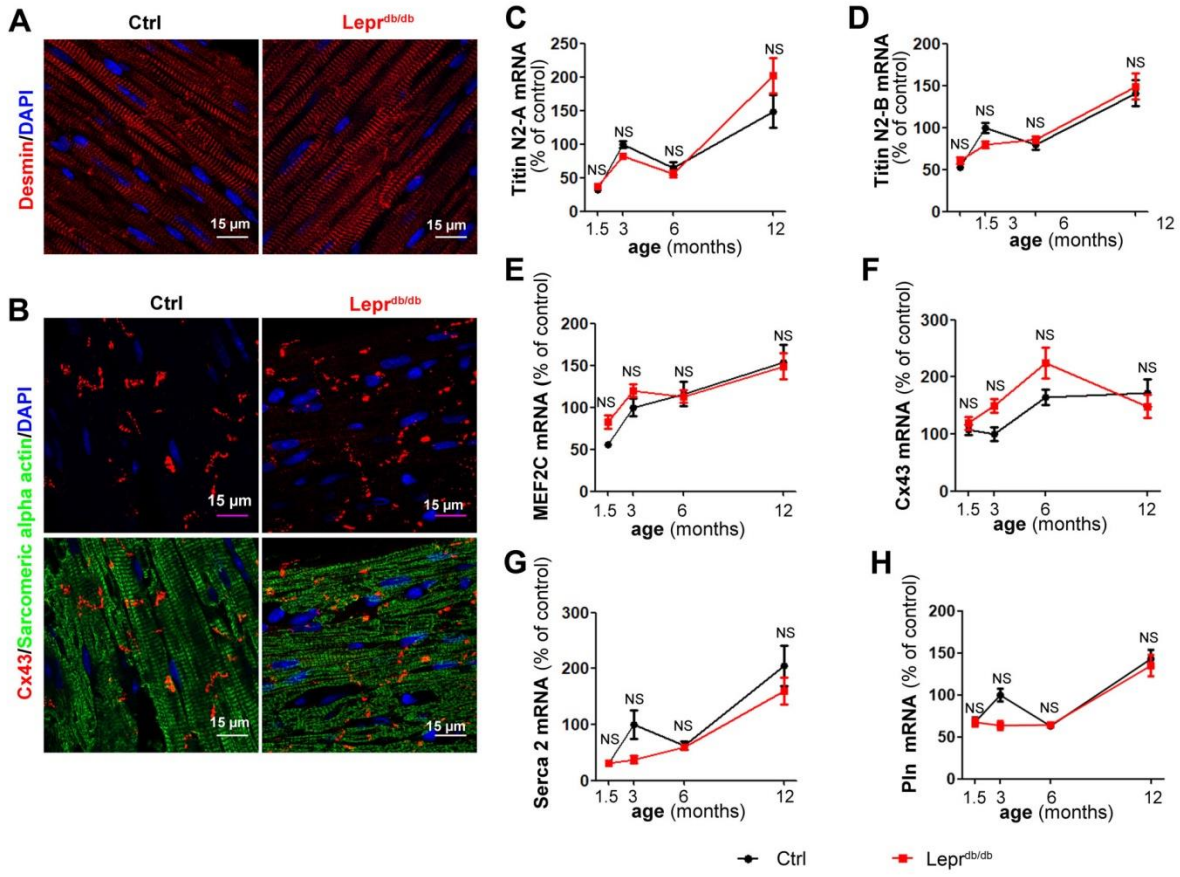
Supplemental Figures and Supplemental Figure legends



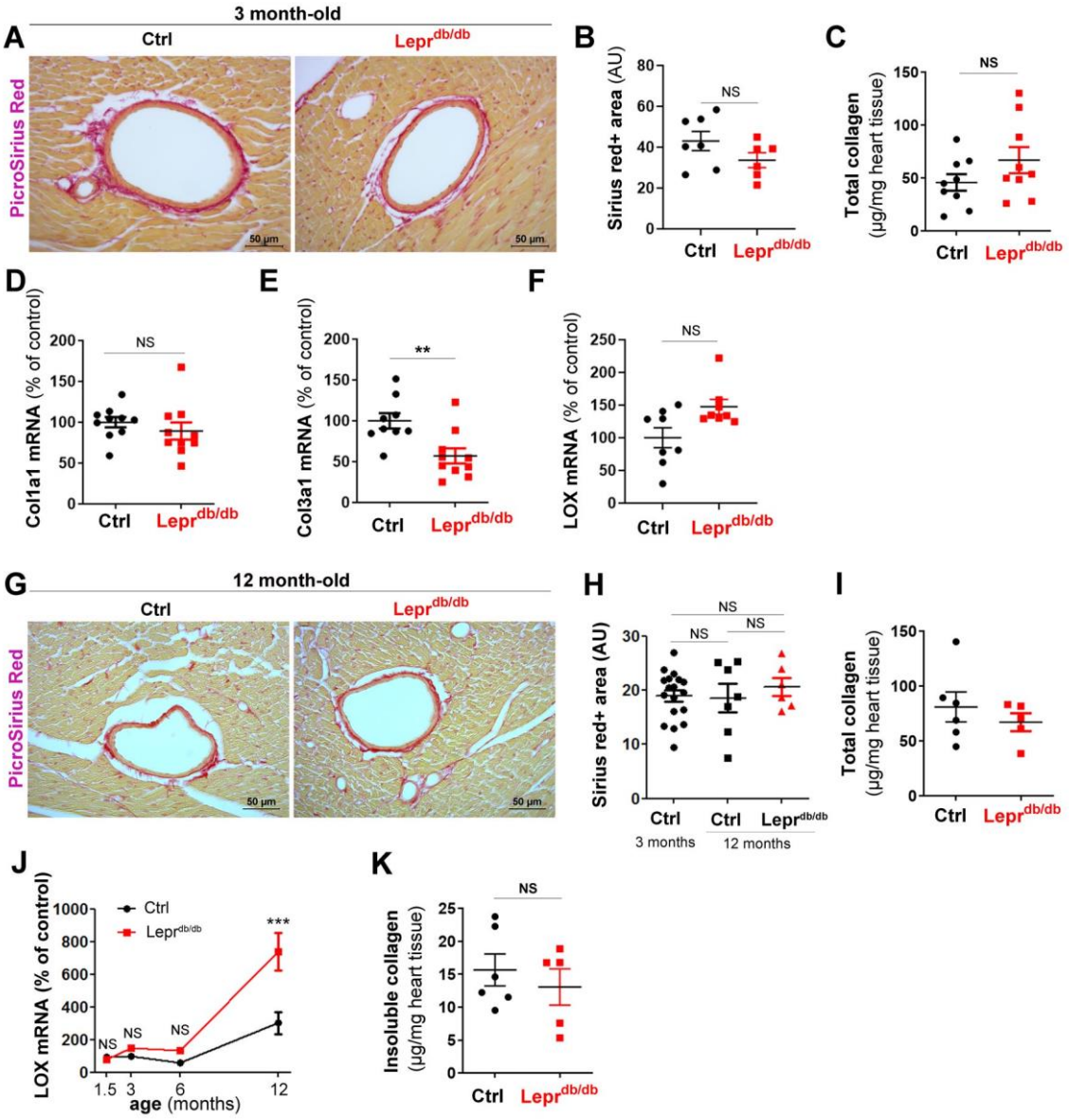
Supplemental Figure I: (A) The weight of Lepr$^{db/db}$ female mice and control Lepr^{db}/+ mice was measured at 1, 5, 3, 6 and 12 months of age. **(B-F, H-I)** Lepr$^{db/db}$ female mice and their control Lepr^{db}/+ littermates were harvested with blood at 1, 5, 3, 6 and 12 months of age. Glycemia (**B**), Total cholesterol (**C**), HDL cholesterol (**D**), LDL cholesterol (**E**) and Triglycerides (**F**) were measured. (**G**) Systolic blood pressure was measured invasively using a pressure catheter in Lepr$^{db/db}$ female mice and control Lepr^{db}/+ mice at 1,5, 3, 6 and 12 months of age. Circulating monocyte (**H**) and neutrophils (I) were counted. *: p≤0.05. **: p≤0.01. ***: p≤0.001. NS: not significant. Two way ANOVA followed by Sidak's multiple comparison test.



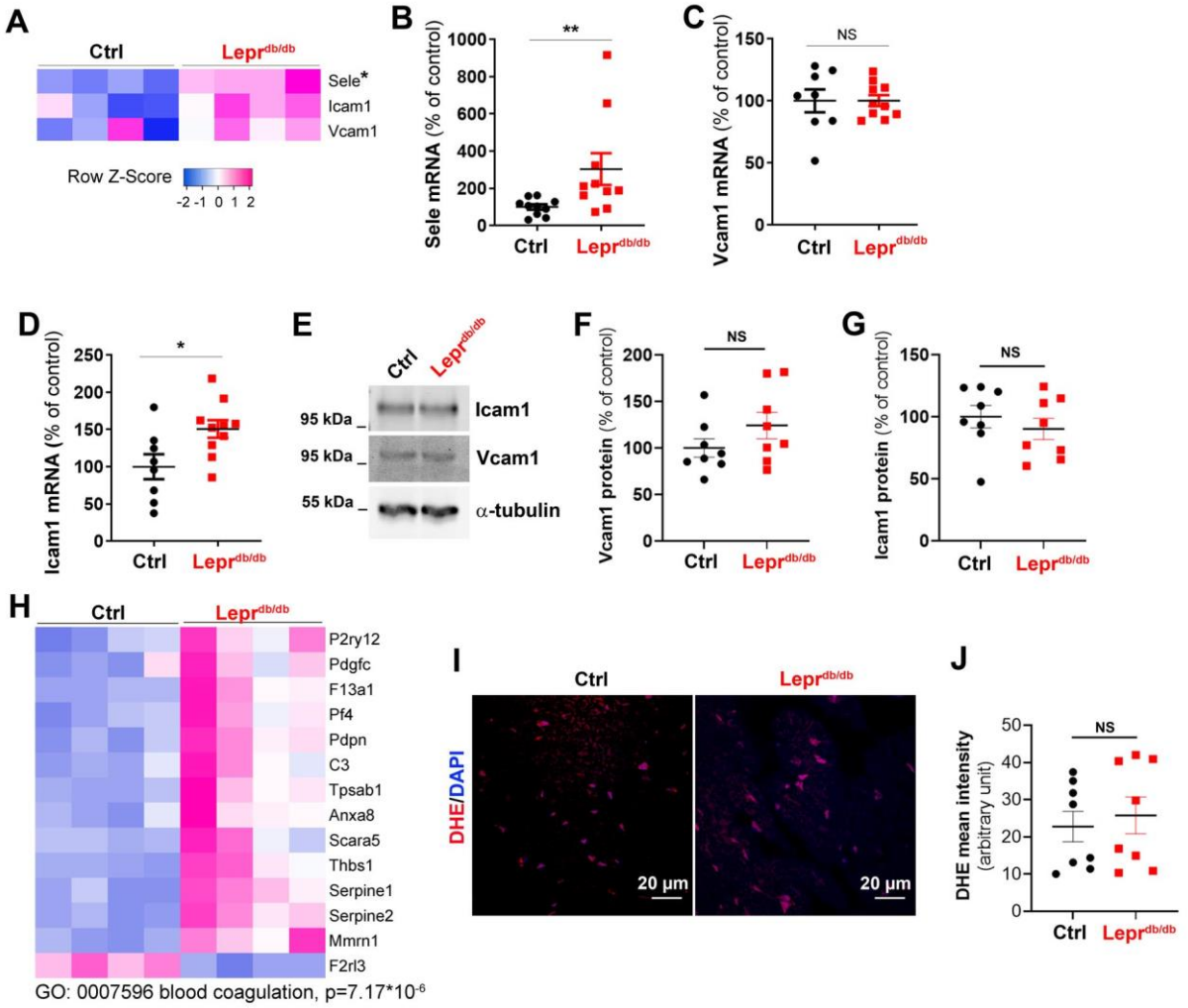
Supplemental Figure II: Lepr$^{db/db}$ female mice and their control Lepr^{db}/+ littermates were subjected LV catheterization and sacrificed at the indicated time points (n=8 to 15 per group). dP/dt maximum (**A**), dP/dT minimum (**B**), Tau (**C**) and the heart rate (**D**) were recorded. ANP (**E**) and BNP (**F**) mRNA expression was measured via RT-qPCR in heart biopsies. NS: not significant. Two way ANOVA followed by Sidak's multiple comparison test.



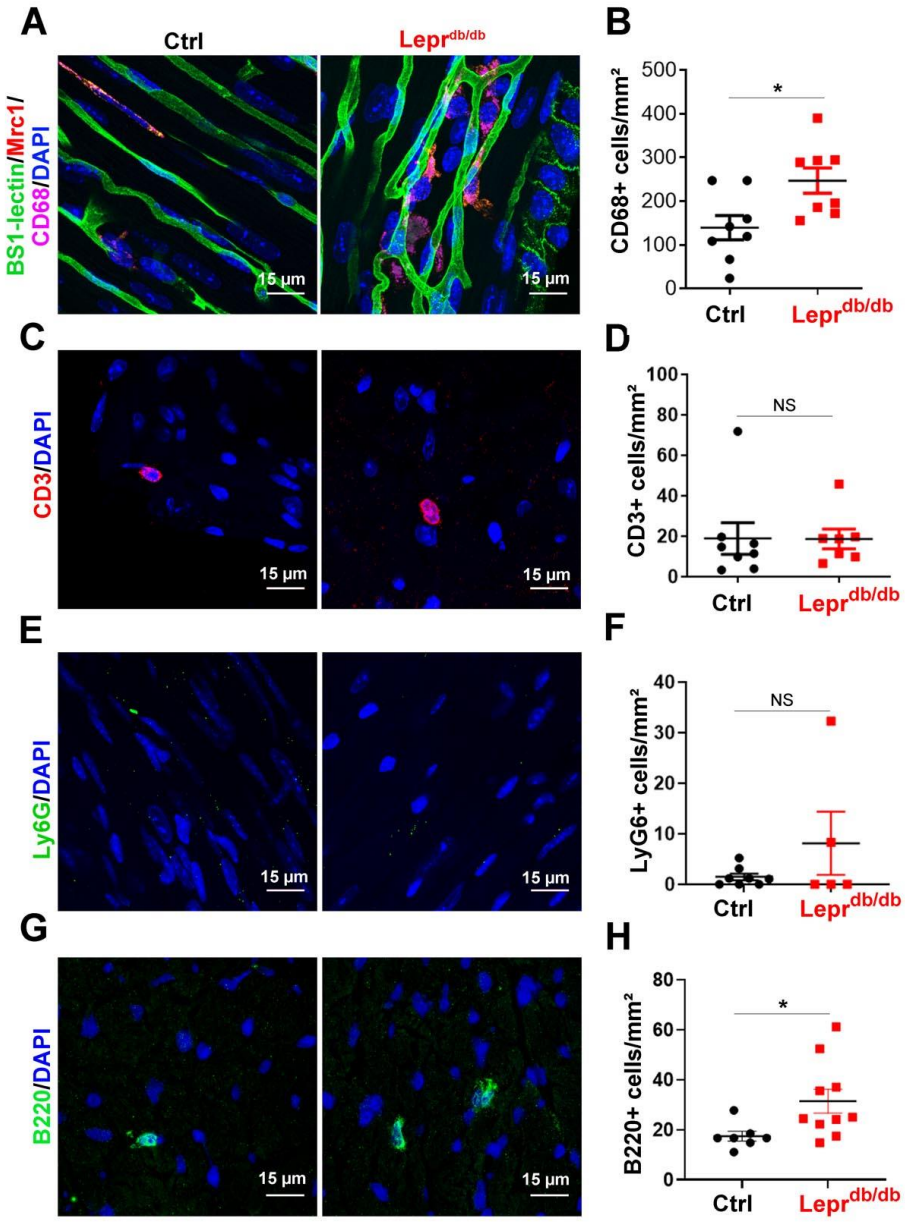
Supplemental Figure III: Lepr$^{db/db}$ female mice and their control Lepr^{db}/+ littermates were sacrificed at 3 months of age. (**A**) Heart cross sections were immunostained with anti-Desmin antibodies to identify cardiomyocyte sarcomeres. Representative pictures are shown (scale bar: 15 µm). (**B**) Heart cross sections were co-immunostained with anti-Cx43 antibodies (in red) to identify cardiomyocyte intercalated discs and anti-Sarcomeric alpha actin antibodies (in green). Representative pictures are shown (scale bar: 15 µm). Titin isoform N2-A (**C**), Titin isoform N2-B (**D**), MEF2C (**E**), Cx43 (**F**), Serca2 (**G**) and Pln (**H**) mRNA expression was measured via RT-qPCR in heart biopsies. NS: not significant. Two way ANOVA followed by Sidak's multiple comparison test.



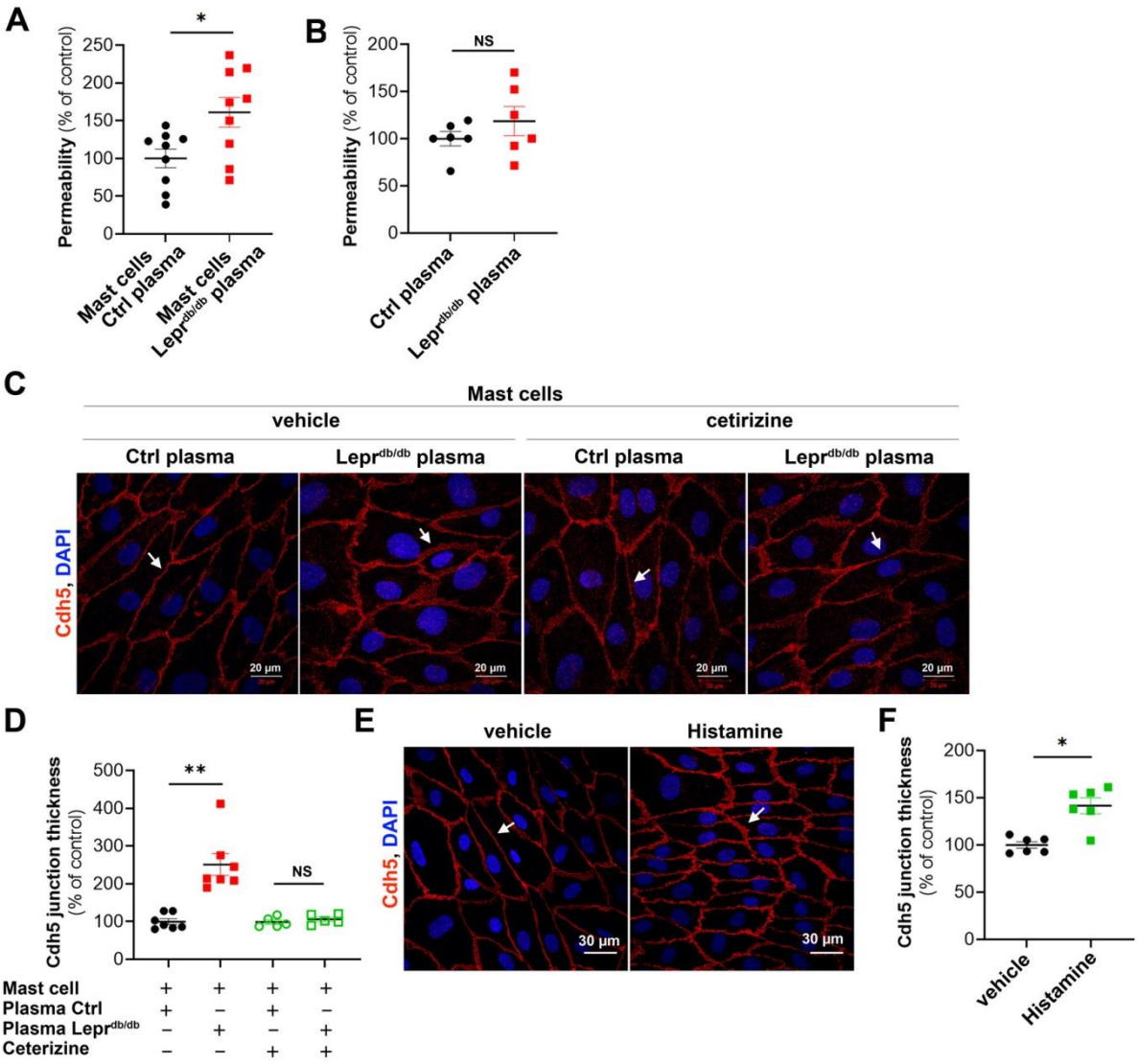
Supplemental Figure IV: Lepr$^{db/db}$ female mice do not display significant cardiac fibrosis. (**A-F**) Lepr$^{db/db}$ female mice and their control Lepr^{db}/+ littermates were sacrificed at 3 months of age. (**A**) Heart cross sections were stained with Sirius red to identify collagen. Representative pictures are shown (scale bar: 50 µm). (**B**) Fibrosis was quantified as the Sirius red+ surface area (n=7 mice/group). (**C**) Total cardiac collagen was assessed using the Sircol™ assay. Col1a1 (**D**) Col3a1 (**E**) and LOX mRNA expression was measured via RT-qPCR in heart biopsies. (**G-I, K**) Lepr$^{db/db}$ female mice and their control Lepr^{db}/+ littermates were sacrificed at 12 months of age. (**G**) Heart cross sections were stained with Sirius red to identify collagen. Representative pictures are shown (scale bar: 50 µm). (**H**) Fibrosis was quantified as the Sirius red+ surface area (n=7 mice/group). (**I**) Total cardiac collagen was assessed using the Sircol™ assay. (**J**) LOX mRNA expression was measured via RT-qPCR in heart biopsies at the indicated time points. (**K**) Unsoluble cardiac collagen was assessed using the Sircol™ assay. **: p≤0.01. ***: p≤0.001. NS: not significant. Two way ANOVA followed by Sidak's multiple comparison test or Mann Whitney test.



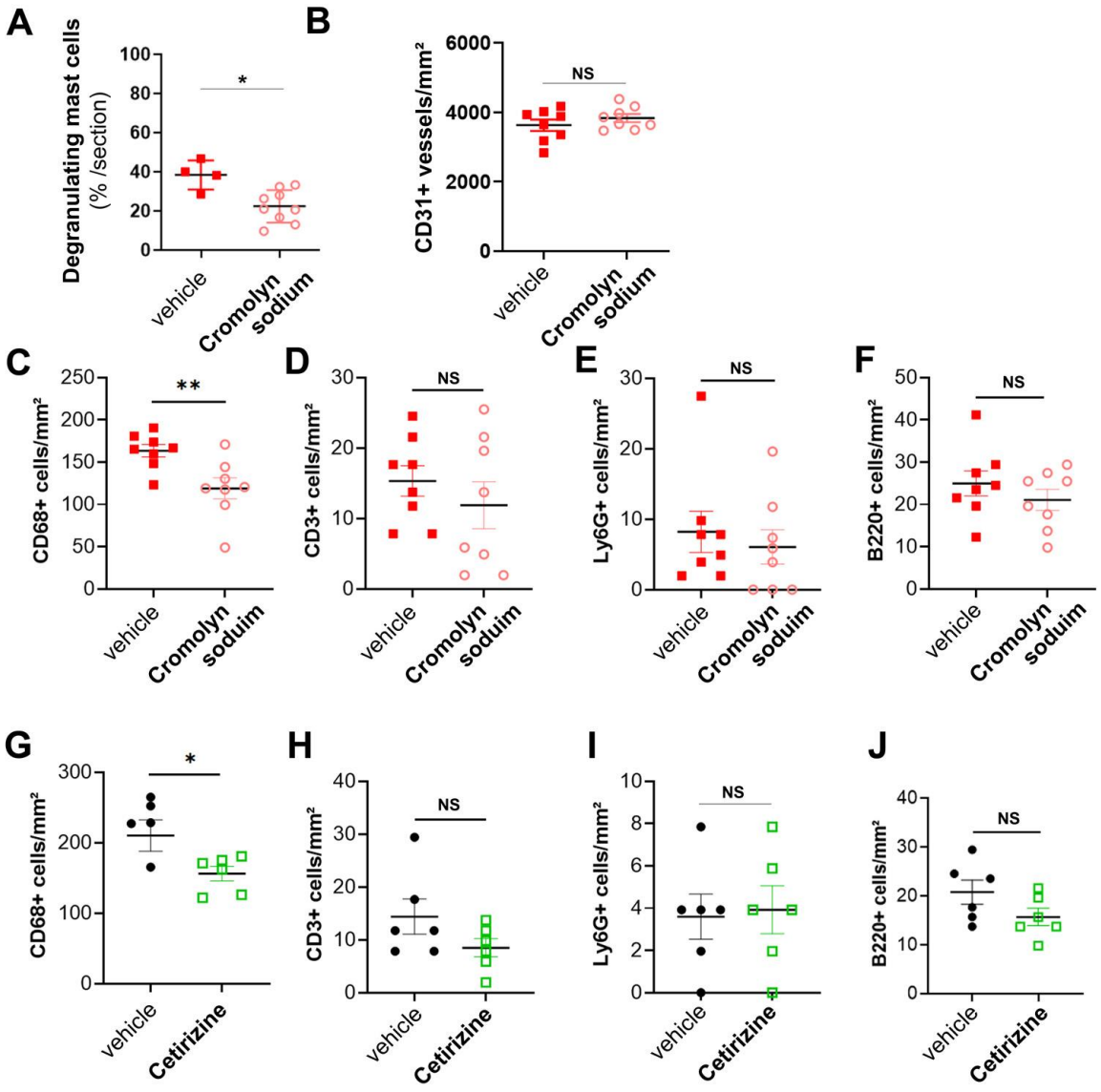
Supplemental Figure V: Lepr$^{db/db}$ female mice and their control Lepr^{db}/+ littermates were sacrificed at 3 months of age. (**A**) RNA sequencing analysis of the cardiac vascular fraction revealed an increased expression of Sele. Sele (**B**), Vcam1 (**C**) and Icam1 (**D**) mRNA expression was measured via RT-qPCR in total heart biopsies (n=10 mice/group). (**E**) Vcam1 and Icam1 protein levels were assessed by western blot analysis and (**F-G**) quantified using image J software. (**H**) RNA sequencing analysis of the cardiac vascular fraction revealed that "Blood coagulation" is one of the biological processes significantly increased in Lepr$^{db/db}$ mice (n=4 mice/group). (**I**) Heart cross sections were stained with dihydroethidium (DHE) to identify oxidative stress. Representative pictures are shown (scale bar: 20 µm). (**J**) Oxidative stress was quantified as the mean red fluorescence intensity. *: p≤0.05, **: p≤0.01. NS: not significant. Mann Whitney test.



Supplemental Figure VI: Lepr$^{db/db}$ female mice and their control Lepr^{db}/+ littermates were sacrificed at 3 months of age (n=6-10 mice/group). (**A**) Heart cross sections were immunostained with anti-CD68 antibodies to identify macrophages. Representative pictures are shown (scale bar: 15 µm). (**B**) Macrophage infiltration was quantified as the number of CD68+ cells/mm². (**C**) Heart cross sections were immunostained with anti-CD3 antibodies to identify T Lymphocytes. Representative pictures are shown (scale bar: 15 µm). (**D**) T-Lymphocyte infiltration was quantified as the number of CD3+ cells/mm². (**E**) Heart cross sections were immunostained with anti-Ly6G antibodies to identify neutrophils. Representative pictures are shown (scale bar: 15 µm). (**F**) Neutrophil infiltration was quantified as the number of Ly6G+ cells/mm². (**G**) Heart cross sections were immunostained with anti-B220 antibodies to identify B Lymphocytes. Representative pictures are shown (scale bar: 15 µm). (**H**) B Lymphocyte infiltration was quantified as the number of B220+ cells/mm². *: p≤0.05. **: p≤0.01. ***: p≤0.001. NS: not significant. Mann Whitney test.

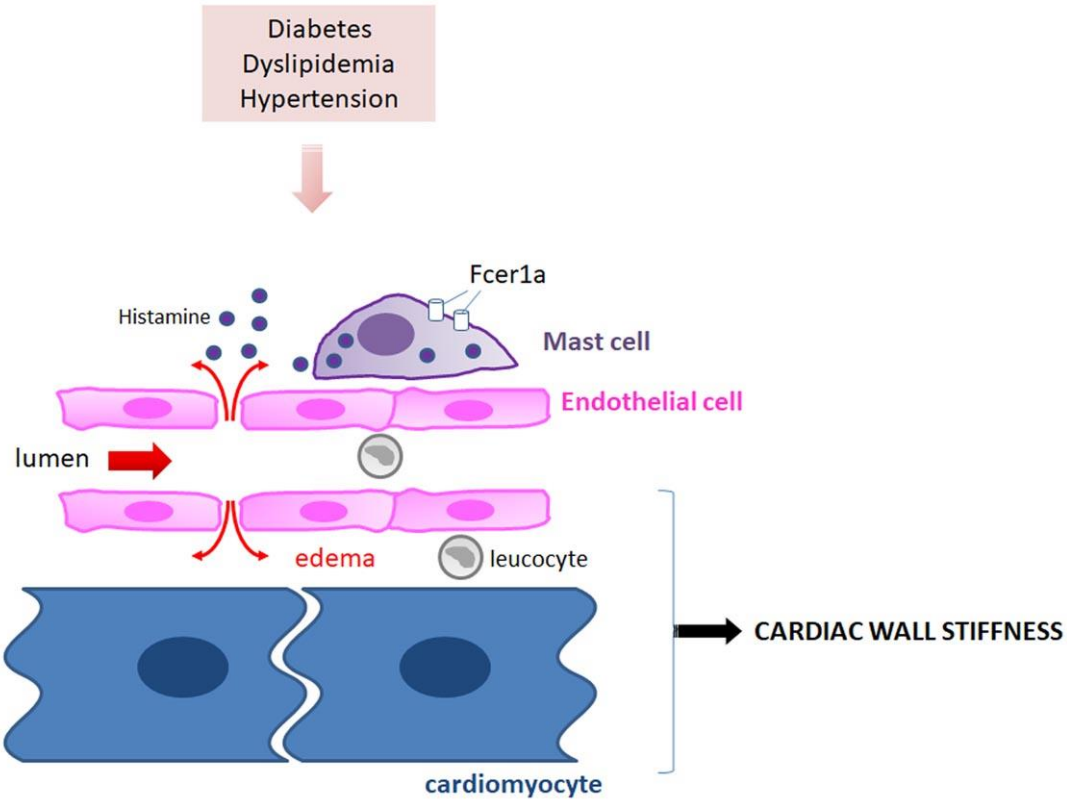


Supplemental Figure VII: (**A**) HUVECs were co-cultured with mast cells activated with plasma from Lepr$^{db/db}$ or control mice. Endothelial monolayer permeability to 70 kDa FITC-dextran was assessed using Transwells. The experiment was repeated 3 times, each experiment included triplicates. (**B**) HUVECs were treated with plasma from Lepr$^{db/db}$ or control mice. Endothelial monolayer permeability to 70 kDa FITC-dextran was assessed using Transwells. The experiment was repeated 3 times, each experiment included duplicates. (**C**) HUVECs were co-cultured with mast cells activated with plasma from Lepr$^{db/db}$ or control mice in the presence or not of 0.1 µg/mL cetirizine. Cdh5 localization was evaluated by immunofluorescent staining (in red) of a confluent cell monolayer and (**D**) quantified as the mean junction thickness using Image J software. The experiment was repeated 5-7 times. (**E**) HUVECs were treated with 200 µmol/L histamine. Cdh5 localization was evaluated by immunofluorescent staining (in red) of a confluent cell monolayer and (**F**) quantified as the mean junction thickness using Image J software. The experiment was repeated 6 times. *: p≤0.05. **: p≤0.01. Mann Whitney test or Kruskal-Willis test followed by Dunn's multiple comparisons tests.



Supplemental Figure VIII: (A-F) 2 month old Lepr$^{db/db}$ female mice were treated or not with 50 mg/kg/day cromolyn sodium for 28 days. Mice were sacrificed at 3 months of age. (**A**) Heart cross sections were stained with Toluidine Blue to identify mast cells degranulation. The percentage of degranulating mast cell was quantified. (**B**) Heart cross sections were immuno-stained with anti-CD31 antibodies to identify endothelial cells. Capillary density was quantified as the number of CD31+ vessels/mm² (n= 8 mice/ group). (**C**) Heart cross sections were immuno-stained with anti-CD68 antibodies to identify macrophages. Macrophage density was quantified as the number of CD68+ cells/mm² (n= 8 mice/ group). (**D**) Heart cross sections were immuno-stained with anti-CD3 antibodies to identify T Lymphocytes. T lymphocyte density was quantified as the number of CD3+ cells/mm² (n= 8 mice/ group). (**E**) Heart cross sections were immuno-stained with anti-Ly6G antibodies to identify neutrophils. Neutrophil density was quantified as the number of Ly6G cells/mm² (n= 8 mice/ group). (**F**) Heart cross sections were immuno-stained with anti-B220 antibodies to identify B Lymphocytes. B lymphocyte density was quantified as the number of B220+ cells/mm² (n= 8

mice/ group). **(G-J)** 2 month old Lepr$^{db/db}$ female mice were treated or not with 4 mg/kg/day cetirizine for 28 days. Mice were sacrificed at 3 months of age. (**G**) Heart cross sections were immuno-stained with anti-CD68 antibodies to identify macrophages. Macrophage density was quantified as the number of CD68+ cells/mm² (n= 6 mice/ group). (**H**) Heart cross sections were immuno-stained with anti-CD3 antibodies to identify T Lymphocytes. T lymphocyte density was quantified as the number of CD3+ cells/mm² (n= 6 mice/ group). (**I**) Heart cross sections were immuno-stained with anti-Ly6G antibodies to identify neutrophils. Neutrophil density was quantified as the number of Ly6G cells/mm² (n= 6 mice/ group). (**J**) Heart cross sections were immuno-stained with anti-B220 antibodies to identify B Lymphocytes. B lymphocyte density was quantified as the number of B220+ cells/mm² (n= 6 mice/ group). *: $p \leq 0.05$, **: $p \leq 0.01$, NS: not significant. Mann Whitney test.



Supplemental Figure IX: Schema recapitulating the findings

Supplemental table

18S	F	5′-CGCGGTTCTATTTTGTTGGT-3′
	R	5′-AGTCGGCATCGTTTATGGTC-3′
Myh7	F	5′-GGATGACGTCACCTCCAACA-3′
	R	5′-AGATCAGAGCCTCCTTCTCGT-3′
Vcam1	F	5′-CGTACACCATCCGCCAGGCA-3′
	R	5′-TAGAGTGCAAGGAGTTCGGGCG-3′
Icam1	F	5′-TGGCCTGGGGGATGCACACT-3′
	R	5′-CCACCGGGCTGTAGGTGGGT-3′
Sele	F	5′-ACGTCCCGGGAAAGATGAAC-3′
	R	5′-GTCAGGAGTGAGGTTCCTGC-3′
Ccl7	F	5′-AAGTGGGTCGAGGAGGCTAT-3′
	R	5′-AGCTCCTATCCCTTAGGACCG-3′
Il6	F	5′-CACTTCACAAGTCGGAGGCT-3′
	R	5′-CTGCAAGTGCATCATCGTTGT-3′
Cpa3	F	5′-GCCCTTGTTTTGAAACGTGCT-3′
	R	5′-TTAAAGTGGGGCTGTTGGGAG-3′
Fcer1a	F	5′-TTCTCCACTGTCAAAGGCCA-3′
	R	5′-GGCAGTGTTTATTGAGTATTTGCTA-3′
Tpsab1	F	5′-CTTGGACTGGATCCACCACT-3′
	R	5′-TTGAGGCATAGCAGAGAGCG-3′
Cma1	F	5′-CAGCCTGTGAGGAAATCTGGAA-3′
	R	5′-GCAGTTGACAATCTGGGTCTTTA-3′
Alox5	F	5′-CATACCACATGCTGAGGTCCA-3′
	R	5′-CTACAAAAGCAGAAAGGGCCAC-3′
ANP	F	5′-CGTCTTGGCCTTTTGGCTTC-3′
	R	5′-GGTGGTCTAGCAGGTTCTTGAAA-3′
BNP	F	5′-AAGCTGCTGGAGCTGATAAGA-3′
	R	5′-GTTACAGCCCAAACGACTGAC-3′
Col1a1	F	5′-CAACCTCAAGAAGGCCCTGC-3′
	R	5′-TGTCCAAGGGAGCCACATCG-3′
Col3a1	F	5′-AGCACGAGGTCTTGCTGGAC-3′
	R	5′-ACCAGCTGTACCAGGCTGAC-3′
Lox	F	5′-CGCTAGGCACTCTTTGTAACAG-3′
	R	5′-AGTAGTTCATGACTAAGGGCTCA-3′
Titin isoform N2-A	F	5′-GAGACATTGCTCCGCTTTTC-3′
	R	5′-GATCTCCAAAGAGGCTGTC-3′
Titin isoform N2-B	F	5′- ACAGTGGGAAAGCAAAGACATC-3′
	R	5′- AGGTGGCCCAGAGCTACTTC-3′
MEF2C	F	5′- TAAGCAGGCAAGGGTCACTG-3′
	R	5′- AAAGTCCAGCTTATGCCGCT-3′
Cx43	F	5′- ATCAGGGAGGCAAGCCATGCTCA-3′
	R	5′- ACGTTGGCCCACACCACAAAGA-3′
Serca2a	F	5′-GATCCTCTACGTGGAACCTTTG-3′
	R	5′-GGTAGATGTGTTGCTAACAACG-3′
Pln	F	5′-TGCTCACTACCACATCAACTTCA-3′
	R	5′-TTCACCAAAATCAAAACTCCATTTG-3′

F forward, R reverse
18S was used as the household gene

Supplemental Table I: List of primers used for reverse transcription (RT) quantitative polymer chain reaction (qPCR)

Major Resources Table

Animals (in vivo studies)

Species	Vendor or Source	Background Strain	Sex	Persistent ID / URL
mouse	Charles River Laboratory	BKS.Cg-Dock7m +/+ Leprdb/J	F	https://www.jax.org/strain/000642

Antibodies

Target antigen	Vendor or Source	Catalog #	Working concentration	Lot # (preferred but not required)	Persistent ID / URL
CD31	Histonova	DIA-310	4 µg/mL		https://search.cosmobio.co.jp/cosmo_search_p/search_gate2/docs/DNV_/DIA310.20110629.pdf
Albumin	Abcam	ab8940	40 µg/mL		https://www.abcam.com/human-serum-albumin-antibody-ab8940.html
mouse CD45	BD Pharmingen Inc	550539	0,625 µg/mL		https://www.bdbiosciences.com/us/applications/research/stem-cell-research/cancer-research/human/purified-rat-anti-mouse-cd45-30-f11/p/550539
Fcer1a	Biorbyt	AF1356	4 µg/mL		https://www.biorbyt.com/fcer1a-antibody-orb41809.html
CD117	R&D systems	AF1356	2 µg/mL		https://www.rndsystems.com/products/human-mouse-cd117-c-kit-antibody_af1356
IgE	R&D systems	MAB9935	4 µg/mL		https://www.rndsystems.com/products/mouse-ige-antibody_mab9935
Desmin	DB Biotech	DB148-0.1	? 1/200		https://www.clinisciences.com/anti-desmine-ce-ivd-pour-ihc-pathologies-4413/rabbit-monoclonal-anti-desmin-clone-45000654.html
Cx43	Sigma	C6219	0,62 µg/mL		https://www.sigmaaldrich.com/catalog/product/sigma/c6219?lang=fr&region=FR&cm_sp=Insite-_-caSrpResults_srpRecs_srpModel_c6219-_-srpRecs3-1
a-sarcomeric actin	Sigma	A2172	? 1/500		https://www.sigmaaldrich.com/catalog/product/sigma/a2172?lang=fr&region=FR&cm_sp=Insite-_-caSrpResults_srpRecs_srpModel_a2172-_-srpRecs3-1
CD68	Biolegend	137001	5 µg/mL		https://www.biolegend.com/en-us/products/purified-anti-mouse-cd68-antibody-6421?GroupID=GROUP20
Mrc1	R&D systems	AF2535	2 µg/mL		https://www.rndsystems.com/products/mouse-mmr-cd206-antibody_af2535
CD3	Santa-Cruz	sc-1127	2 µg/mL		https://www.scbt.com/fr/p/cd3-epsilon-antibody-m-20
Mouse Ly6G	BD Pharmingen Inc	551459	5 µg/mL		https://www.bdbiosciences.com/us/reagents/research/antibodies-buffers/immunology-reagents/anti-mouse-antibodies/cell-surface-antigens/purified-rat-anti-mouse-ly-6g-1a8/p/551459
B220	R&D systems	MAB1217	4 µg/mL		https://www.rndsystems.com/products/mouse-b220-cd45r-antibody-ra3-6b2_mab1217
Podocal	R&D	AF1556	4 µg/mL		https://www.rndsystems.com/products/mouse-

yxin	systems				podocalyxin-antibody_af1556
NOS3	Cell signaling technology	32027	? 1/1000		https://www.cellsignal.com/products/primary-antibodies/enos-d9a5l-rabbit-mab/32027
Phospho-NOS3	Cell signaling technology	9571	? 1/1000		https://www.cellsignal.com/products/primary-antibodies/phospho-enos-ser1177-antibody/9571?_=1608215467641&Ntt=9571&tahead=true
Icam1	R&D systems	AF-796	0,4 µg/mL		https://www.rndsystems.com/products/mouse-icam-1-cd54-antibody_af796
Vcam1	abcam	ab134047	0,47 µg/mL		https://www.abcam.com/vcam1-antibody-epr5047-ab134047.html
Ccl7	R&D systems	AF-456	0,4 µg/mL		https://www.rndsystems.com/products/mouse-ccl7-mcp-3-marc-antibody_af-456-na
Il-6	R&D systems	AF-406	0,4 µg/mL		https://www.rndsystems.com/products/mouse-il-6-antibody_af-406-na
Alpha-tubulin e	Sigma	T5168	? (1/2000)		https://www.sigmaaldrich.com/catalog/product/sigma/t5168?lang=fr&region=FR
SMA	Sigma	C6198	5 µg/mL		https://www.sigmaaldrich.com/catalog/product/sigma/c6198?lang=fr&region=FR
Cdh5	Santa-Cruz	sc-9989	2 µg/mL		https://www.scbt.com/p/ve-cadherin-antibody-f-8?gclid=Cj0KCQiAlsv_BRDtARIsAHMGVSYW8og18blUCA_r4Ke6OQo2iVvdvbUd1deW8gtvlruqT7Qg5_KL2csaApZ9EALw_wcB
IgE	Biolegend	406903	5 µg/mL		https://www.biolegend.com/en-gb/products/biotin-anti-mouse-ige-2517

Cultured Cells

Name	Vendor or Source	Sex (F, M, or unknown)	Persistent ID / URL
HUVEC	Lonza	F	https://bioscience.lonza.com/lonza_bs/CH/en/Primary-and-Stem-Cells/p/000000000000184665/HUVEC-%E2%80%93-Human-Umbilical-Vein-Endothelial-Cells%2C-Pooled%2C-in-EGM-2

Other

Description	Source / Repository	Persistent ID / URL
Cromolyn sodium	Abcam	https://www.abcam.com/cromolyn-sodium-macrophage-blocker-and-mast-cell-stabilizer-ab142857.html
Cetirizine	Arrow	https://www.vidal.fr/medicaments/gammes/cetirizine-arrow-23412.html
WGA	Invitrogen	https://www.thermofisher.com/order/catalog/product/W11261#/W11261
IsoB4	Sigma Aldrich	https://www.sigmaaldrich.com/catalog/search?term=L2895&interface=All&N=0&mode=match%20partialmax&lang=fr&region=FR&focus=product
DHE	Sigma Aldrich	https://www.sigmaaldrich.com/catalog/product/sigma/37291?lang=fr&region=FR&cm_sp=Insite-_-caContent_prodMerch_gruCrossEntropy-_-prodMerch10-1

Alexa-Fluor 488-Streptavidin	Invitrogen	https://www.thermofisher.com/order/catalog/product/S11223#/S11223