Supplemental Material

Expanded Materials and Methods

Porcine experiments

Immunohistochemistry of porcine heart tissue

Five μm-thick transections from porcine heart biopsies were prepared for histology as previously described.[1] In short, biopsies were stored in Zinc Formalin Fixative (#Z2902, Sigma-Aldrich), deparaffinized, and incubated in neutral antigen retrieval solution (Agilent Technologies, S1699) for 20 minutes at 95°C. The rabbit anti-human MPO (Dako, A0398) primary antibody for neutrophil staining was applied ON at room temperature (1:100 in blocking buffer). Next, the secondary goat anti-rabbit antibody was applied for 45 minutes (1/50, Dako, P0488) and peroxidase activity was detected with 0.01% H2O2/3,3'-diaminobezidine (DAB, Sigma-Aldrich D8001). Images were obtained using an inverted bright-field AxioVert 200 microscope (Carl Zeiss, Jena, Germany) and AxioVision software. Per animal, two random sections of ischemic zone tissue were analyzed (4 microscopy fields per section) with at least 50 μm between the sections.

Porcine CLEC4E primer design

CLEC4E forward and reverse primers were designed using NCBI Primer-Blast and were scanned for uniqueness using NCBI BLAST search engine. The SYBR-green primer oligos were synthetized by Bio-Rad (Belgium) and were designed to have a primer length of 19-23 nucleotides, GC-content of 35-65% and a melting temperature (Tm) between 60-68°C, according to Qiagen guidelines (see Supplemental Table 1).[2]

Porcine and murine qRT-qPCR

Heart tissue was first pulverized in liquid nitrogen, followed by mRNA extraction using the RNeasy fibrous Tissue Mini Kit (Qiagen 74704) according to the manufacturer's protocol. Neutrophil extraction was performed using the RNeasy Micro Kit (Qiagen 74004). The concentration and quality of the extracted mRNA was determined with a spectrophotometer (NanoDrop, Thermo Scientific). The mRNA was reverse transcribed using the GoScript Reverse Transcription System (Promega A5004). For tissue, CLEC4E transcript levels were determined in duplicate by RT-qPCR (QuantStudio 3 System, Thermo Scientific) using PrimePCR Gene Expression Assays (SYBR Green probes, Bio-Rad, Belgium) for porcine tissue and TaqMan Gene Expression Assays (Mm01183703_m1, Thermo Fisher Scientific,

Belgium) for murine tissue. On isolated murine neutrophils we determined Clec4e and Grk2-expression levels (Mm00804778, Thermo Fisher Scientific, Belgium). The mRNA expression levels were quantified by determining the threshold cycle level (Ct) and values were normalized to mRNA levels of a housekeeping gene (peptidyl-prolyl cis-trans isomerase (*PPIA,* BioRad*,* qSscCED0020254) for pigs and glyceraldehyde-3'-phosphate-dehydrogenase (*Gapdh,* Thermo Fisher Scientific, Mm99999915_g1) for mice. Normalized mRNA levels were expressed relative to the control group according to Livak's method ($2^{-\Delta\Delta Ct}$).[3]

Murine model of ischemia-reperfusion injury

Clec4e$^{-/-}$ C57Bl6/J (MMRRC, 031936-UCD) and wild-type C57Bl6/J control mice between 12-14 weeks of age were randomly allocated to 24h, 72h or 4 weeks' time point. Mice were anesthetized with an intraperitoneal (IP) injection of ketamine/xylazine (100 mg/kg and 10 mg/kg, respectively), intubated and mechanically ventilated with a tidal volume of 7 µl/g BW at 140 strokes/min) (Minivent, Hugo Sachs Elektronik, March-Hugstetten, Germany). A small incision over the thoracic region was made and the muscles were prepared by loosening the skin with a Kocher. The muscles overlying the ribs were divided and a thoracotomy was performed in the intercostal space between the third and fourth rib. A small moisturized triangle was inserted into the thorax to retract the ribs. Once the heart was exposed and the Left Anterior Descending coronary artery was located, it was ligated with 6-0 Prolene suture. The left anterior descending coronary artery (LAD) remained ligated for 60 minutes and heart rate was monitored. After 60 minutes, the ligation was released to restore blood flow, the triangle was removed and the ribs closed gently (6-0 Ti-Cron, non-absorbable suture material). The animal was extubated and allowed to recover in a warm environment. The skin was disinfected with iodine dissolved alcohol and Xylocaine was applied for pain relief. Blood sampling was performed at 90 minutes reperfusion via the tail vein (150 µl). Once fully awake, mice were transferred to their original cage and returned to the animalium. Buprenorphine (100 mg/kg) was administered IP on a daily basis during the first three days for post-operative pain relief. The mice were euthanized via cervical translocation at indicated time points.

Murine cardiac MRI

All CMRI data were acquired on a Bruker BioSpec 70/30 MRI system (Bruker BioSpin, Ettlingen, Germany) operating at a magnetic field strength of 7 Tesla and equipped with actively shielded gradients (200 mT/ m). A dedicated mouse quadrature volume coil with a 40

mm internal diameter (Bruker BioSpin) was used. Animals were anesthetized through inhalation of 1-1.5% isoflurane carried by 100% oxygen. Body temperature, respiration and cardiac rate were monitored during the measurements and maintained at 36-37˚C, 60-70 min^{-1} and 500-600 min^{-1}, respectively. For planning of the CMRI scans, localizer scans and three orthogonal 2D MRI scans (axial, sagittal and coronal orientation) were acquired. Next, a self-gated (IntraGate, Bruker BioSpin) [4] four-chamber view scan was acquired with the following parameters: echo time (TE) 4.2 ms, repetition time (TR) 14.7 ms, flip angle 20 degrees, matrix 192 x 192, field of view (FOV) 30 x 30 mm, 1 single slice of 1mm thickness, oversampling 200. Finally, a self-gated gradient echo sequence (igFLASH) in axial orientation was acquired with the following parameters: TE 3.8 ms, TR 13.5 ms, flip angle 20 degrees, matrix 128 x 128, FOV 25 x 25 mm, 8 contiguous slices of 1 mm thickness, oversampling: 200. The total acquisition time was approximately 45 minutes. Images were reconstructed using the ParaVision software (Bruker BioSpin) version 6.0.1 to 20 frames after verifying retrospective triggering accuracy. To assess global LV-functionality and remodeling, ejection fraction (EF), end-systolic volume (ESV) and end-diastolic volume (EDV) were calculated.

Immunohistochemistry of murine heart tissue

Six µm-thick transversal sections from mouse LV were prepared from paraffin-embedded tissue after fixation in Zinc Formalin Fixative (Sigma-Aldrich, St. Louis, USA), deparaffinized, and incubated in a neutral antigen retrieval solution (Agilent Technologies, S1699) for 20 minutes at 95°C. Endogenous peroxidase was quenched by incubation in 3% H2O2 in methanol. To determine which cell type show Clec4e-expression, sections were incubated ON with rat anti-Clec4e (MBL, D292-3M2, dilution 1/50) together with mouse anti-alpha-smooth muscle actin-FITC (Sigma-Aldrich, F3777 dilution 1/100), rabbit anti-CD68 (Cell Signaling, E3O7V, dilution 1/300) or rabbit anti-myeloperoxidase (Dako, A039829-2, dilution 1/100). Next day, the sections were washed and incubated with biotinylated donkey anti-rat-Cy3 (Jackson ImmunoResearch, #712-065-153, dilution 1/250) (Clec4e) and donkey anti-rabbit-Alexa488 (Thermofisher, #A-21206, dilution 1/250) (CD68 and MPO). The biotinylated donkey anti-rat staining arm was amplified with Str-PO TCA Biotin System (Perkin Elmer, NEL700001KT) and detected using a Cy3 dye. Additionally, TO-PRO-3 (Life Technologies, # 3605, diluted 1/400) staining was performed to visualize nuclei. To evaluate myocardial structures, Clec4e-stained sections were incubated with TO-PRO-3 together with Alexa488-labeled wheat germ agglutinin (Thermo Fisher, W11261, dilution 1/100). Images were

obtained using a laser scanning confocal microscope (Carl Zeiss, Jena, Germany, LSM 700) and ZEN software. At least 2 images where obtained from remote zone, border zone and infarct zone.

To evaluate leukocyte infiltration into the myocardium, sections were incubated with a rat anti-mouse Ly-6G (Gr-1) (eBioscience, 14-5931-81, dilution 1/50) or Mac-3 (BD Biosciences, BD553322, dilution 1/50) for over-night, washed and incubated with biotinylated rabbit anti-rat (Agilent Technologies, E0433, dilution 1/300) and amplified with Str-PO TCA Biotin System (PerkinElmer, NEL700001KT). Cell infiltration was visualized with 3,3'-diaminobenzidine tetrahydrochloride hydrate (DAB) (Alfa Aesar, j62216). Images were obtained using an inverted bright-field AxioVert 200 microscope (Carl Zeiss, Jena, Germany) at three different sections distal to the site of LAD ligation. The number of infiltrating Ly-6G positive cells were counted and expressed per mm^2 using ImageJ software (NIH).

To determine fibrosis in a semi-quantitative manner, Sirius Red staining was performed on three different sections and mosaic scans were obtained using AxioVert 200 microscope (Carl Zeiss, Jena, Germany). The quantity of collagen was determined using a color thresholding (ImageJ software, NIH) and expressed as the percentage of Sirius Red stained area per total tissue area.

To determine infarct size in a semi-quantitative manner, sections were stained with hematoxylin and eosin. Infarct area was visualized by exposing the slides to 488nm wave length light using an AxioVert 200 microscope (Carl Zeiss, Jena, Germany).[5] Mosaic scans were acquired on 5 sections, over a total distance of 600 μm, and analyzed using color thresholding (ImageJ software, NIH). Infarct area was expressed as the percentage of infarct area per total tissue area.

High-sensitivity Troponin I ELISA

Hs-TnI levels were determined on plasma samples collected at 90 minutes reperfusion and at euthanasia by ELISA (CTNI-1-HSP, Life Diagnostics, Stoke-on-Trent, United Kingdom) according to manufacturer's instructions.

Neutrophil isolation and immunocytochemistry

Peripheral blood neutrophils were isolated from WT and *Clec4e*$^{-/-}$ mice. First, blood was collected via cardiac puncture, followed by red blood cells (RBC)-lysis to remove RBCs. Next,

cells were isolated using the Neutrophil Isolation Kit for mice (130-097-658, Miltenyi, Gladbach, Germany), according to the manufacturer's instructions. The purity of the isolated neutrophil fraction was confirmed at 99% with flow cytometry (FACSAria III Cell Sorter, BD Biosciences), using antibodies for the neutrophil markers CD11b (PE-Cy7, 561098, BD Bioscience, New Jersey, United States, dilution 1:300) and LY6-G (APC-H7, 565369, BD Bioscience, dilution 1:100). Dead cells were excluded by using the viability dye 7-aminoactinomycin D (7'AAD).

To evaluate baseline Cxcr2-expression levels, wild-type and *Clec4e*$^{-/-}$ neutrophils were fixed with 4% PFA for 5 minutes at RT, washed and permeabilized using 0.2% Triton-X100. Next, cells were blocked with donkey serum (Sigma, D9663, dilution 1/10) and incubated ON with rabbit anti-Cxcr2 (Fisher Scientific, 16659124, dilution 1/100). Next day, the cells were washed and incubated with biotinylated donkey anti-rabbit-Cy3 (Thermo Fisher, A10042, dilution 1/100). To visualize the cell structures, the cells were incubated with TO-PRO-3 (Life Technologies, #3605, diluted 1/400) and Alexa488-labeled wheat germ agglutinin (Thermo Fisher, W11261, dilution 1/100). Images were obtained using a laser scanning confocal microscope (Carl Zeiss, Jena, Germany, LSM 700) and ZEN software.

In vitro neutrophil migration assay

Twenty-four-well microchambers with a 5 µm pore polycarbonate membrane (3421, Corning, New York, United states) were used for the chemotaxis assay. Freshly isolated peripheral blood WT and *Clec4e*$^{-/-}$ neutrophils were resuspended in RPMI-1640 medium (21875-034, Gibco, Massachusetts, United States) supplemented with 0.5% fetal bovine serum and 1% P/S, and were allowed to migrate towards 10 ng/mL Cxcl2 (452-M2-010, R&D Systems, Minnesota, United States) or medium alone for 120 minutes at 37°C. Afterwards, the migrated cells were collected and incubated with 8µM calcein-acetoxymethyl (calcein-AM, C1359-100UL, Sigma-Aldrich, Missouri, United States) for 30 min. The number of migrated cells was determined based on the intensity of the fluorescent calcein-AM signal on a spectrophotometer (EnSight, PerkinElmer, Massachusetts, United States) (excitation: 485 nm, emission 530 nm).

Bone marrow isolation and Cxcr2-expression assays

Bone marrow cells were collected as previously described.[7] In short, mice were euthanized by cervical translocation and the femurs were collected. Both ends of the femurs were cut-off with sharp sterile scissors. By using a 23-gauge needle and a 10 cc syringe filled with ice cold PBS,

the bone marrow was flushed out onto a 70µm nylon cell strainer placed on a 50mL falcon tube. The collected cells were centrifuged at 1500 rpm for 7 min at 4°C. Supernatant was removed, the cell pellet was resuspended in 1 mL RBC lysis buffer and incubated for 5 min at RT. Next, 5 mL of DMEM medium (41965062, Gibco) containing 10% FBS (F6765, Sigma-Aldrich) was added to neutralize the RBC lysis buffer and the cells were centrifuged at 1500 rpm for 7 min at 4°C. The supernatant was discarded, the cells were immediately put on ice and were washed with ice cold PBS (Gibco, 10010002,) containing phosphatase inhibitors (1 mM sodium orthovanadate and 2 mM sodium fluoride, Sigma). Next, the cells were centrifuged at 1500 rpm for 7 min at 4°C, the supernatant was discarded and the pellet was placed on ice for protein-extraction using RIPA-buffer, supplemented with protease inhibitors (cOMPLETE Protease Inhibitor Cocktail, 11697498001, Roche, Basel, Switzerland). Afterwards, the samples were stored at -80°C before further analysis.

Western Blot Analysis

Prior to electrophoresis, the protein concentration was determined using the BCA-method (Pierce BCA Protein Assay Kit, 23227, Thermo Scientific). The samples were run on NuPAGE 4-10% Bis-Tris Gels **(**Invitrogen, #NP0326BOX) for 3h30 at 100V. Two protein standards were run in the first and last lane of each gel: See Blue (LC5625, ThermoFisher) and Precision Plus (1610374, Bio-Rad). Sample lysates were loaded in the following order: WT cell lysate (n=3) followed by *Clec4e*$^{-/-}$ cell lysate (n=3). After electrophoresis, the proteins were transferred to a Nitrocellulose membrane via dry transfer by using an iBlot 2 dry blotting system (Thermo Fisher Scientific) for 7 minutes. After transfer, the membranes were incubated in TBTS + 5% non-fat milk for 1h30, followed by washing with TBST and incubation with the primary antibody anti-Cxcr2 (PA5-10662, ThermoFisher, 1:800), diluted in TBST+5% milk at 4°C. Afterwards, the membranes were quickly washed 3 times for 10 min with TBST, followed by incubation with goat anti-rabbit secondary antibody (PO44801-2, Agilent Technologies, 1:1000) diluted in TBST + 5% milk at RT. The membranes were then washed 3 times for 10 min with TBST and the bands were evaluated using SuperSignal West Femto Maximum Sensitivity Substrate (34094, Thermo Fisher Scientific). As a loading control, one gel was dedicated for staining with Coomassie Brilliant Blue (50% w/v Coomassie Brilliant Blue, 45% v/v ethanol and 10% v/v acetic acid). The gel was incubated in the staining solution for 1h and destained ON in destaining solution (5% v/v methanol and 7,5% v/v acetic acid in distilled water). All obtained images were analyzed with ImageJ software (NIH). Each band was

normalized to the total protein content determined with Coomassie blue. Results are expressed relatively to baseline Cxcr2 expression levels in WT cells.

RNA-sequencing of murine LV-tissue

RNA-extraction

Total RNA was extracted from left-ventricle tissue with the RNeasy plus mini kit (Qiagen, 74134) according to manufacturer's protocol. Subsequent RNA-sequencing was performed by GENEWIZ (Leipzig, Germany).

RNA Library Preparation and NovaSeq Sequencing

RNA samples were quantified using Qubit 4.0 Fluorometer (Life Technologies, Carlsbad, CA, USA) and RNA integrity was checked with RNA Kit on Agilent 5300 Fragment Analyzer (Agilent Technologies, Palo Alto, CA, USA).

RNA sequencing library preparation was prepared using NEBNext Ultra II Directional RNA Library Prep Kit for Illumina following manufacturer's instructions (NEB, Ipswich, MA, USA). Briefly, mRNAs were first enriched with Oligo (dT) beads. Enriched mRNAs were fragmented. First strand and second strand cDNA were subsequently synthesized. The second strand of cDNA was marked by incorporating dUTP during the synthesis. cDNA fragments were adenylated at 3'ends, and indexed adapter was ligated to cDNA fragments. Limited cycle PCR was used for library amplification. The dUTP incorporated into the cDNA of the second strand enabled its specific degradation to maintain strand specificity. Sequencing libraries were validated using NGS Kit on the Agilent 5300 Fragment Analyzer (Agilent Technologies, Palo Alto, CA, USA), and quantified by using Qubit 4.0 Fluorometer (Invitrogen, Carlsbad, CA).

The sequencing libraries were multiplexed and loaded on the flow cell on the Illumina NovaSeq 6000 instrument according to manufacturer's instructions. The samples were sequenced using a 2x150 Pair-End (PE) configuration. Image analysis and base calling were conducted by the NovaSeq Control Software v1.6 on the NovaSeq instrument. Raw sequence data (.bcl files) generated from Illumina NovaSeq was converted into fastq files and de-multiplexed using Illumina bcl2fastq program version 2.20. One mismatch was allowed for index sequence identification.

Data Analysis

After investigating the quality of the raw data, sequence reads were trimmed to remove possible adapter sequences and nucleotides with poor quality using Trimmomatic v.0.36. The trimmed reads were mapped to the Mus musculus (GRCm38) reference genome available on ENSEMBL using the STAR aligner v.2.5.2b. The STAR aligner is a splice aligner that detects splice junctions and incorporates them to help align the entire read sequences. BAM files were generated as a result of this step. Unique gene hit counts were calculated by using feature Counts from the Subread package v.1.5.2. Only unique reads that fell within exon regions were counted. Since a strand- specific library preparation was performed, the reads were strand-specifically counted.

After extraction of gene hit counts, the gene hit counts table was used for downstream differential expression analysis. Using DESeq2, a comparison of gene expression between the groups of samples was performed. The Wald test was used to generate *P*-values and Log_2 fold changes.

Gene ontology, pathway and network analysis

Genes with adjusted *P*-values < 0.1 (corrected for multiple testing with Benjamini-Hochberg) and absolute log_2-fold changes above 0.58 (Fold change > 1.5) were called as differentially expressed genes and resulted in 398 genes in total. Gene ontology (biological processes), pathway and network analysis were performed on the statistically significant set of genes using the DAVID functional gene annotation tool 6.8, Reactome Pathway Database and STRING database, respectively. For STRING network analysis, the confidence level was set at 0.7 (high confidence), text-mining was disabled and clustering was performed with the MCL-algorithm with inflation parameter 3.

Supplemental Tables

Supplemental Table 1: Porcine CLEC4E qPCR primer

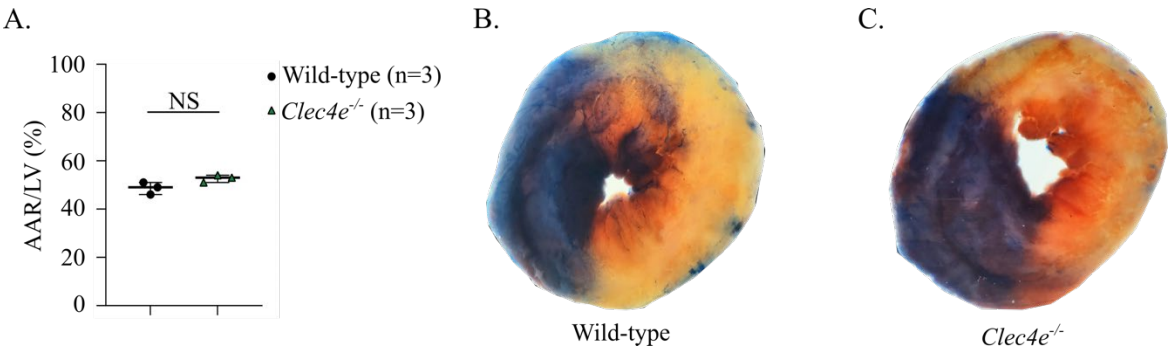
Gene Name	Ensemble ID	Forward Primer	Reverse Primer	Hybrid. Temp. (°C)	PCR effic. (%)
CLEC4E	ENSSSCG00000038418	GACGGACCAGGTGATTGAGGG	GGCACAGTCCTCCACACTAACT	62.2	92.2

Supplemental Table 2: Top 20 DEG in LV-tissue from *Clec4e*$^{-/-}$ (n=5) versus wild-type mice (n=5) 24 hours after I/R. FC= Fold Change (Log_2), FDR=False Discovery Rate.

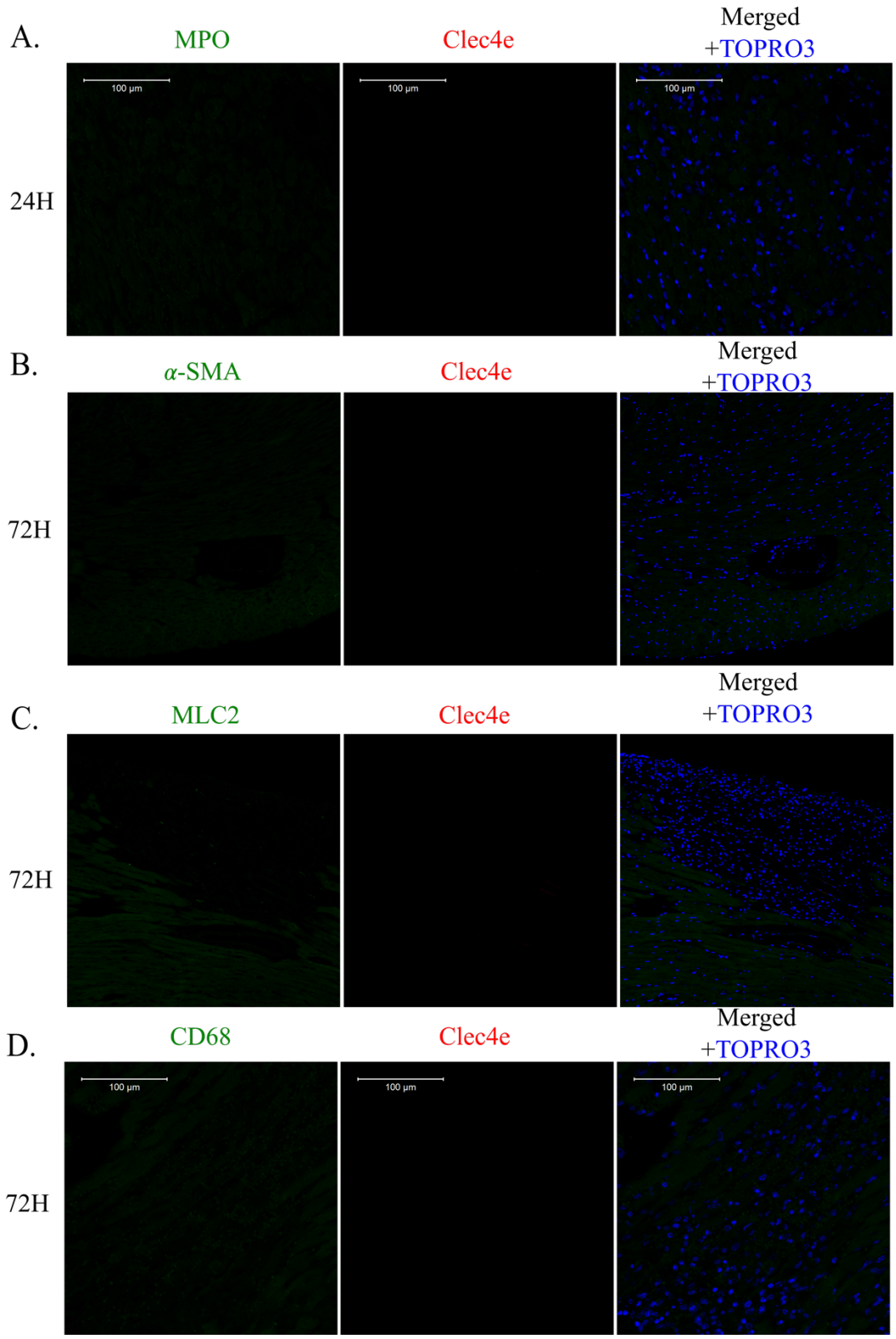
Gene	Name	Function	FC	-Log_{10}(FDR)
Alb	Albumin	Regulation of blood plasma colloid osmotic pressure, carrier protein for endogenous molecules including hormones, fatty acids, and metabolites, as well as exogenous drugs.	6,23	0,026
Pck1	Phosphoenolpyruvate Carboxykinase 1	Main control point for the regulation of gluconeogenesis	2,19	5,953E-04
Ubxn10	UBX Domain Protein 10	Required for ciliogenesis	1,86	0,004
Efna2	Ephrin A2	Cell surface GPI-bound ligand for Eph receptors, a family of receptor tyrosine kinases which are crucial for migration, repulsion and adhesion during neuronal, vascular and epithelial development.	1,69	0,003
Adcy8	Adenylate Cyclase 8	Membrane bound enzyme that catalyzes the formation of cyclic AMP from ATP	1,65	0,044
Lingo3	Leucine Rich Repeat And Ig Domain Containing 3	Protein Coding gene	1,63	2,395E-04
Car3	Carbonic Anhydrase 3	Metalloenzymes that catalyze the reversible hydration of carbon dioxide and are differentially expressed in a number of cell types. Associated with AMI	1,60	0,004
Sost	Sclerostin	Negative regulator of bone growth that acts through inhibition of Wnt signaling and bone formation	1,48	0,038
Col6a6	Collagen Type VI Alpha 6 Chain	Cell-binding protein that may regulate epithelial cell-fibronectin interactions	1,43	0,011
Fam124b	Family With Sequence Similarity 124 Member B	Protein Coding gene	1,37	0,009
Clec4e	C-Type Lectin Domain Family 4 Member E	A calcium-dependent lectin that acts as a pattern recognition receptor of the innate immune system	-4,75	2,304E-06
Mir22hg	MIR22 Host Gene	lncRNA	-2,26	1,141E-08

Hbb-bt	Hemoglobin Subunit Beta	Involved in oxygen transport from the lung to the various peripheral tissues	-2,26	3,769E-12
Hbb-bs		Involved in oxygen transport from the lung to the various peripheral tissues	-2,24	3,479E-13
Hba-a1	Hemoglobin Subunit Alpha 1	Involved in oxygen transport from the lung to the various peripheral tissues	-2,23	4,291E-16
Ciart	Circadian Associated Repressor Of Transcription	Transcriptional repressor which forms a negative regulatory component of the circadian clock	-2,23	1,405E-07
Alas2	5'-Aminolevulinate Synthase 2	Erythroid-specific mitochondrially located enzyme that catalyzes the first step in the heme biosynthetic pathway	-2,20	5,090E-09
Hba-a2	Hemoglobin Subunit Alpha 2	Involved in oxygen transport from the lung to the various peripheral tissues	-2,19	2,196E-13
Slc6a12	Solute Carrier Family 6 Member 12	GABA transporter	-2,16	0,015
Atcayos	ataxia, cerebellar, Cayman type, opposite strand	LncRNA	-2,13	0,006

Supplemental Figures

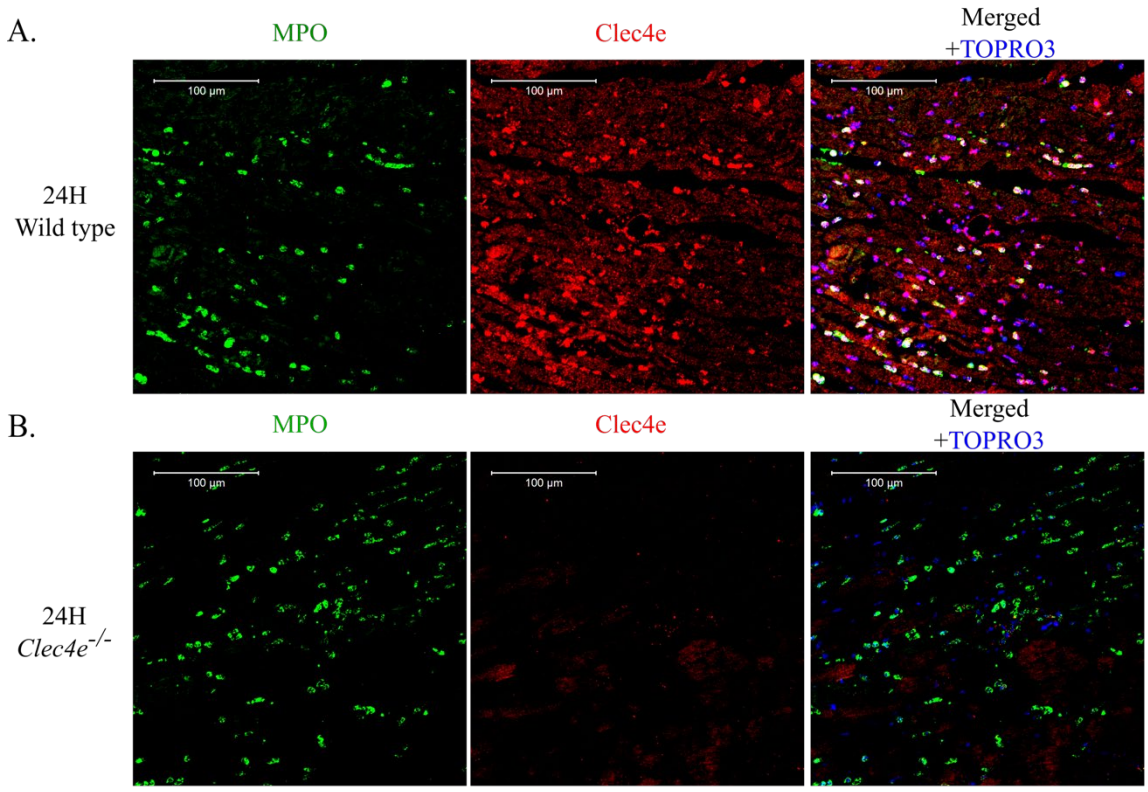


Supplemental Figure 1: Area at risk (AAR) assessment by Evans blue perfusion after re-occlusion of the left-descending artery 24 hours after I/R injury. A. Percentage of AAR per total LV-area in wild-type (n=3) and *Clec4e*$^{-/-}$ mice (n=3), Mann-Whitney test, results are shown as median with interquartile range. Representative images of transversal heart sections 24 hours after I/R in **B.** Wild-type and **C.** *Clec4e*$^{-/-}$ mouse. AAR=area at risk, NS= not significant, LV=left ventricle.

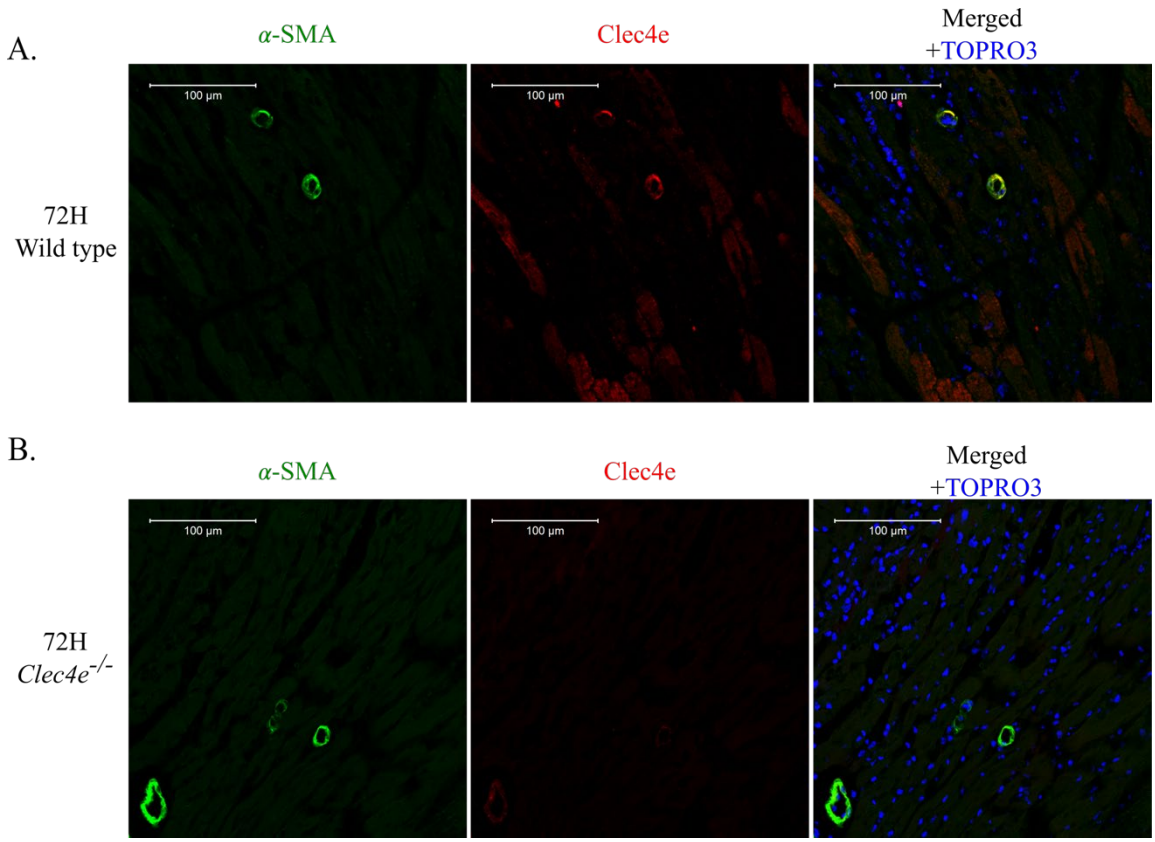


Supplemental Figure 2. Negative control images of Heart tissue by omitting primary antibody for Clec4e (red) in combination with antibodies against **A.** MPO to identify neutrophils (green) at 24 hours post I/R **B.** α-SMA to highlight smooth muscle cells (green)

72 hours after I/R, **C.** MLC2 to visualize cardiomyocytes (green), **D.** CD68 identified infiltrating monocytes/macrophages at 72 hours post I/R. Nuclei are counter-stained with TO-PRO-3 (blue). Scale bar is 100μm.

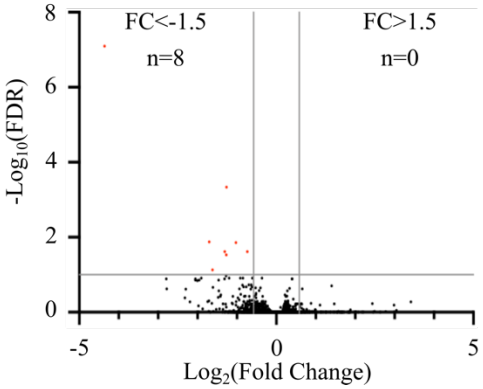


Supplemental Figure 3. Clec4e-antibody specificity IHC staining in ischemic heart tissue after I/R from wild-type and *Clec4e*$^{-/-}$ mice. **A.** MPO-staining to identify neutrophils (green) in combination with Clec4e-staining (red) in WT border zone heart tissue 24 hours post I/R **B.** MPO-staining to identify neutrophils (green) in combination with Clec4e-staining (red) in *Clec4e*$^{-/-}$ heart tissue 24 hours post I/R. Nuclei are counter-stained with TO-PRO-3 (blue). Scale bar is 100µm.



Supplemental Figure 4. Clec4e-antibody specificity IHC staining in ischemic heart tissue after I/R from wild-type and *Clec4e*$^{-/-}$ mice. **A.** α-SMA to highlight smooth muscle cells (green) in combination with Clec4e-staining (red) in WT heart tissue 72 hours post I/R, B**.** α-SMA to highlight smooth muscle cells (green) in combination with Clec4e-staining (red) in *Clec4e*$^{-/-}$ heart tissue 72 hours post I/R. Nuclei are counter-stained with TO-PRO-3 (blue). Scale bar is 100µm.

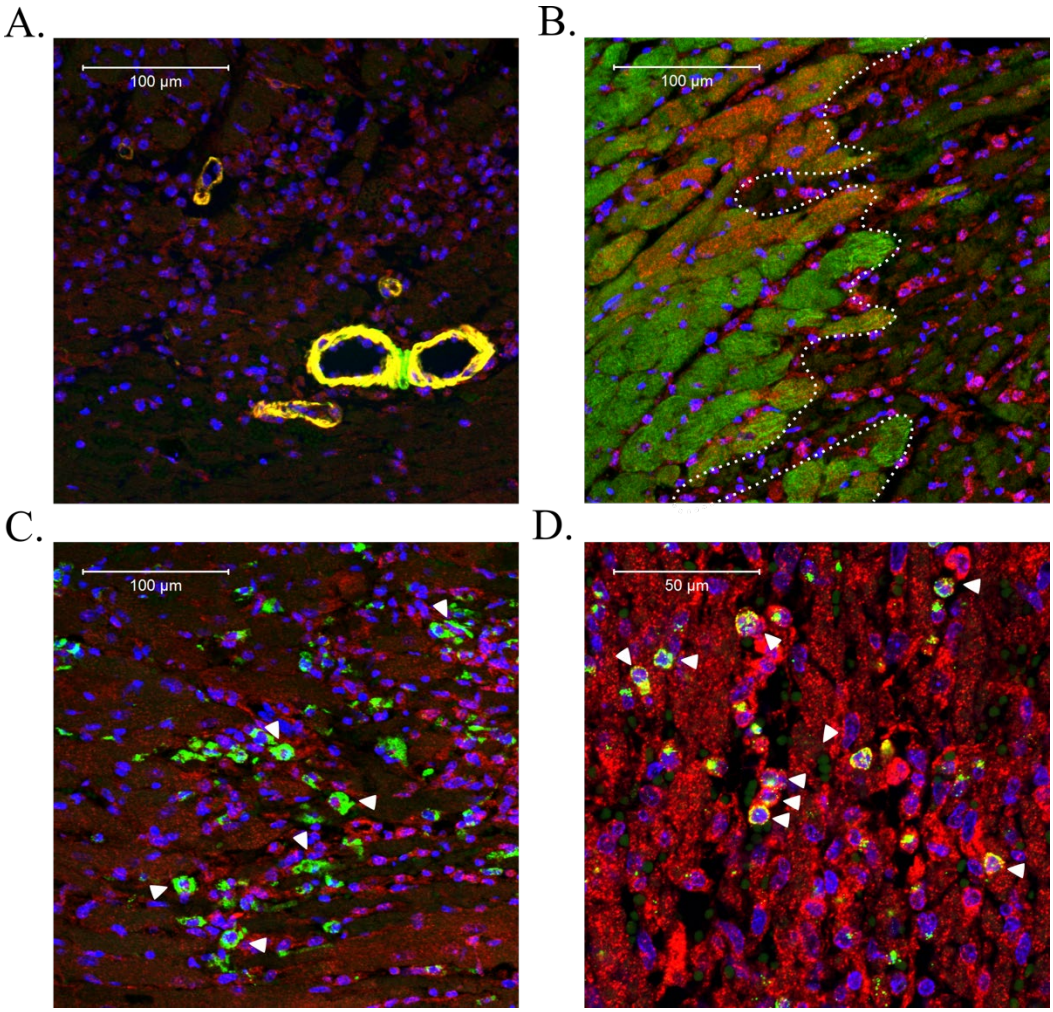
A.



B.

Gene name	Function	FC	-Log$_{10}$(FDR)
Clec4e	Pattern Recognition receptor	-4,36	$8.0x10^{-8}$
Cnn1	Regulation of SMC contraction	-1,26	$4.6x10^{-4}$
Susd5	Hyaluronic acid binding	-1,70	0.013
Myh11	Muscle contraction	-1,03	0.014
Lmod1	Nucleation of actin filaments	-1,31	0.024
Cntn2	Organization of axonal domains	-0,74	0.024
Slc2a13	H^+-myo-inositol cotransporter	-1,27	0.029
Col4a6	ECM structural constituent	-1,62	0.074

Supplemental Figure 5. RNA-sequencing of WT vs. *Clec4e*$^{-/-}$ LV-tissue 72 hours after I/R. A. Volcano-plot and **B.** Table shows 8 differentially expressed genes. Red dots reflect significantly downregulated transcripts (Adjusted *P*-value<0.1). C-Type Lectin Domain Family 4 Member E (*Clec4e*), Calponin 1 (*Cnn1*), Sushi Domain Containing 5 (*Susd5*), Myosin Heavy Chain 11 (*Myh11*), Leiomodin 1 (*Lmod1*), Contactin 2 (*Cntn2*), Solute Carrier Family 2 Member 13 (*Slc2a13*), Collagen Type IV Alpha 6 Chain (*Col4a6*). Fold Change (FC), False Discovery Rate (FDR).



Supplemental Figure 6. Composite image of the performed immunohistochemical stainings on ischemic heart tissue after I/R injury. Heart tissue stained for Clec4e (Red) in combination with **A.** antibody against α-SMA (green) at 72 hours post I/R, to show co-localization of Clec4e with smooth muscle cells (yellow) in both arteries, venules and capillaries. **B.** WGA-staining, delineates plasmatic membranes and endothelium. White dotted line highlights the border between ischemic tissue (right) and viable heart tissue (left). **C.** antibody against CD68 to show infiltrating monocytes/macrophages (green), indicated by white arrow heads (yellow) at 72 hours post I/R. **D.** antibody against myeloperoxidase to show infiltrating neutrophils (green), indicated by white arrow heads (yellow) at 24 hours post I/R. hours post I/R. Nuclei are counter stained with TO-PRO-3 (blue). Scale bar is 100µm in A-C and 50µm in D.

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