SUPPLEMENTAL MATERIAL

Expanded Materials and Methods

Animal studies

Zebrafish were handled in compliance with institutional (MPG) and national animal welfare legislation, and maintained according to standard protocols (https://zfin.org). Procedures involving animals were performed in accordance with and approved by the veterinary department of the Regional Board of Darmstadt. 3 months-old male and female zebrafish of a length of 27-28 mm were used for most of this study. Wild-type (WT) siblings were used in all cases, except for Figure S2E-F, where non-sibling WTs were used. We used the previously published lines *Tg(hsp70l:slft4)bns82* [29], *Tg(hsp70l:vegfaa121-F17A)bns100* [34] (hereafter *Tg(hsp70l:dn-vegfaa)*), *Tg(-0.8flt1:RFP)hu5333* [35], *Tg(myl7:DsRed)s879* [36], *TgBAC(tcf21:NTR-mCherry)pd108* [37] (hereafter *Tg(tcf21:mCherry))*, *TgBAC(tcf21:CreERT2)pd42* [38], *Tg(-5.2lyve1b:DsRed)nz101* [39], *Tg(flt1:Mmu.Fos-GFP)wz2* [40], *vegfc^{hu5055}* [32], *vegfd^{bns257}* [22] and *cxcr1^{sa14414}* [41]. Cryoinjuries were performed as previously described[8,9,10]. Heat shock treatments to induce the expression of *hsp70l*-driven transgenes were performed by housing zebrafish in preheated system water (39°C) for one hour every 12 hours. For *Tg(hsp70l:sflt4); TgBAC(tcf21:CreERT2),* and *Tg(hsp70l:loxP-CFP-loxP-emilin2a-p2A-mCherry); Tg(flt1:Mmu.Fos-GFP)* zebrafish*,* 10 µl of 1.25 mM 4-hydroxytamoxifen (Sigma (Cat#H7904)) or vehicle (ethanol) were injected intraperitoneally (IP) in adult zebrafish 3 consecutive days prior to cryoinjury, followed by daily heat shocks at 39°C for one hour every 12 hours. *Tg(-0.8flt1:RFP)* zebrafish treated with SB225002 (Sigma (Cat#182498-32-4)) or DMSO were injected IP with 20 µl of 0.01 mM DMSO (Sigma (Cat#D4540)) or 20 µl of 0.01 mM SB225002 (dissolved in DMSO and diluted in water). For intramyocardial injections of Qdots 705 (Qtracker Q21061MP, Thermo Fisher Scientific), zebrafish were first anesthetized in 0.02% tricaine. A small incision was made to expose the heart, followed by injecting 5 µl of Qdots 705 into the cardiac tissue using a micro-injector. Zebrafish were left to recover in water. Hearts were then dissected within 90 minutes following injection[23].

Construction of mutant and transgenic lines

To establish the *Tg(hsp70l:emilin2a, cryaa:CFP)bns504* line, WT zebrafish embryos were injected at the one-cell stage with 25 pg/nl of *hsp70l:emilin2a, cryaa:cerulean* plasmid DNA along with I-Sce enzyme (NEB (Cat# R0694S)). Injected embryos were raised to adulthood and founders were screened by examining for CFP expression in larval eyes. To establish the *Tg(hsp70l:loxP-CFP-loxP-emilin2a-p2A-mCherry)bns510* line, (hereafter *Tg(hsp70l:LCL-emilin2a-p2A-mCherry))*, WT zebrafish embryos were injected at the one-cell stage with 30 pg/nl of *hsp70l:loxP-CFP-loxP-emilin2a-p2A-mCherry* plasmid DNA, along with 50 ng/µl Tol2

transposase mRNA. Injected embryos were raised and founders were screened by outcrossing and examining for CFP expression in embryos after performing two heat shocks at 39ºC for one hour each. The CRISPR-Cas9 technology was used to generate the *emilin2a*^{bns556} full locus deletion allele using two gRNAs targeting exons 1 and 9 with the sequences AGCAGTGCGGACCAAGGCCA and TACCCGTCAAGTCTGTTCCA, respectively, as previously described[42]. Using this approach, a genomic region of 31.1 kb was deleted and mutants were identified by PCR using the DyNAmo Flash SYBR Green qPCR master mix (Thermo Fisher Scientific (Cat# F415S)) and the following program: incubation at 50°C for 2 minutes and, preamplification at 95°C for 10 minutes followed by 40 cycles of amplification at 95°C for 10 seconds and 60°C for 30 seconds. Primer sequences are listed in table S2. The *cxcl8a*^{vu660} allele was generated using a gRNA with the sequence CCTTGATGACAACTGGAC leading to a 28 base pair deletion and an insertion of 12 bases in exon 3. For gRNA synthesis, 2.5 µL of 2x NEB buffer 2 with BSA was mixed with 1 µl of 10 µM of the universal oligo TTTTGCACCGACTCGGTGCCACTTTTTCAAGTTGATAACGGACTAGCCTTATTTTAACTT GCTATTTCTAGCTCTAAAA and 1 µl of 10 µM of the site-specific oligo TAATACGACTCACTATAGGCCTTGATGACAACTGGAC GTTTTAGAGCTAGAAATAGCAAG. Oligonucleotides were annealed using the following program: 98°C for 1 minute and cool down to 37°C with a ramp speed of −0.1°C/s. 500 µM of dNTP and T4 DNA polymerase (NEB (Cat# M0203S)) were added to the annealing mix and incubated at 12°C for 20 minutes. T4 DNA polymerase was inactivated by incubating at 75°C for 20 minutes. The gRNA was synthesized using a MEGA short script T7 kit (Invitrogen (Cat# AM1354)) following manufacturer's protocol. Mutants were identified by PCR using PrimeStar (Takara (Cat# R045)) and the following program: preamplification at 95°C for 2 minutes followed by 40 cycles of amplification at 95°C for 10 seconds, 60°C for 10 seconds, and 72°C for 20 seconds (primer sequences are listed in table S2), followed by restriction digest of the amplicon with Ms1I enzyme (NEB (Cat# R0571S)).

Histological analysis, Imaging, and Quantification

Zebrafish hearts were dissected and fixed in 4% paraformaldehyde for 1 hour at room temperature (RT). Hearts were embedded in OCT (Tissue-Tek) and sectioned into 8 µm sections. For each biological replicate, 3 non-superficial non-consecutive midsagittal sections were used for imaging and quantification. Immunostaining was performed as previously described[11]. Primary antibodies used: anti-Mef2 (Santa Cruz Biotechnology (Cat#sc-313, AB_631920), 1:200), anti-N2.261 (DSHB, RRID: AB_531790, 1:20), anti-GFP (Aves Labs (Cat#GFP-1010, AB_2307313), 1:500), anti-RFP (Rockland (Cat# 600-401-379, AB_2209751), 1:200), anti-PCNA (Santa Cruz Biotechnology (Cat#sc-56, AB_628110,

1:500) and anti-Fli1a (Abcam (Cat#ab133485, AB_2722650), 1:100). Secondary antibodies used: Alexa Fluor 488 Goat Anti-chicken IgG (H+L) (Thermo Fisher Scientific (Cat#A-11039, AB_142924), 1:500), Alexa Fluor 488 Goat Anti-mouse IgG (H+L) (Thermo Fisher Scientific (Cat#A-11029, AB_138404), 1:500), Alexa Fluor 568 Goat Anti-mouse IgG (H+L) (Thermo Fisher Scientific (Cat#A-11004, AB_2534072), 1:500), Alexa Fluor 647 Goat Anti-rabbit IgG (H+L) (Thermo Fisher Scientific (Cat#A-21244, AB_2535812), 1:500). Control experiments were performed by omitting primary antibodies from the immunostaining procedure. Confocal images were acquired using a LSM700 microscope (Zeiss). For cEC proliferation analysis, the percentage of proliferating cECs was calculated as a ratio from the total number of cECs in the injured tissue and in 200 μm of the injury border zone using the ZEN Blue software. For CM dedifferentiation and proliferation analysis, the percentage of dedifferentiating/proliferating CMs was calculated as a ratio from the total number of CM in 100 μm of the injury border zone using the ZEN Blue software. For coronary coverage analysis, the percentage fluorescence intensity was calculated as a ratio from background fluorescence in the injured tissue using the ImageJ software in wholemount images of at least 4 biological replicates. For wholemount imaging of Qdots, a Lightsheet Z.1 microscope (Zeiss) was used, and images were processed using the ZEN Blue Software. AFOG staining was performed using the AFOG (Acid Fuschsin Orange G) staining kit (Biognost (Cat#: AFOG100T)) following manufacturer's protocol without H&E staining. Images were taken using a Nikon SMZ25 stereomicroscope. For scar area analyses, scar area was calculated as a ratio from total ventricle area using the ImageJ software in 3 non-consecutive sections per ventricle of at least 4 biological replicates. For *in situ* hybridization, dissected hearts were fixed in sterile 4% paraformaldehyde at 4°C overnight. Hearts were washed in PBS twice for 5 minutes, followed by 15-30 minutes incubation through a gradient of ethanol in DEPC-water: 50%, 70%, 80%, 95% and 100% at RT. Hearts were washed in 50% xylene in ethanol and in 100% xylene for 30 minutes at RT, followed by 3 washes in 100% paraffin at 50°C for one hour. Hearts were embedded in paraffin and stored at 4°C and sectioned into 8 μm sections and stored at RT. Sections were washed twice in xylene for 10 minutes, followed by rehydration in a gradient of ethanol in DEPC-water for 2 minutes: 100%, 95%, 80%, 70% and 50%. Slides were then washed twice for 5 minutes with TBST (Tris 50 mM pH 7.4, NaCl 150mM, 0.05% Tween-20). Slides were then incubated for 20 minutes in sterile 4% PFA, followed by 2 washes in TBST. Slides were incubated in 0.5 μg/mL Proteinase K diluted in TBS (Tris 50 mM pH 7.4, NaCl 150mM) + CaCl2 2 mM) for 15 minutes at 37°C, followed by a 5 minutes wash in cold Tris/Glycine (Tris 50mM pH7.4, 50 mM Glycine) to stop the reaction. Slides were washed twice in TBST, followed by refixation in sterile 4% PFA for 5 minutes and wash with TBST. Slides were immersed in triethanolamine (0.1 M, pH 8.0) and acetic anhydride was added to reach 0.25% under agitation for 12

minutes. This step was followed by two TBST washes and pre-hybridization of sections using a hybridization buffer (50% formamide, 5X SSC, 0.1% Tween-20, 50 µg/ml Heparin, 500 µg/ml yeast t-RNA, 460 µl 1M citric acid) at 60˚C - 65˚C for at least 1 hour. Probes (1 µg/ml in hybridization buffer) were denatured at 60˚C - 65˚C for 15 minutes and applied to sections at 60˚C - 65˚C overnight. Slides were washed with 50% formamide in 2X SSC for 30 minutes at 60˚C - 65˚C. Slides were then washed in 60˚C - 65˚C for 15 minutes once at 2X SSC and twice with 0.1X SSC, followed by TBST wash at RT. Slides were washed in 37˚C for 15 minutes once at 2X SSC and twice at 1X SSC, followed by TBST wash at RT. Slides were incubated in blocking solution (TBST + 0.5% BSA) for at least 1 hour at RT. Anti-digoxigenin (Roche (Cat# 11093274910), 1:1000 in blocking solution) was applied to slides at RT for at least 2 hours. Slides were then washed 5 times with TBST. Pre-filtered BM Purple (Roche (Cat#: 11442074001)) was added to slides and incubated in a dark humid chamber for a maximum of 2 hours. After signal appeared, slides were washed with TBST, fixed in 4% PFA for 5 minutes, and mounted. Images were taken using a Nikon SMZ25 stereomicroscope. Primers used to generate antisense probes are listed in table S2.

***vegfc* mRNA injections**

Full-length *vegfc* cDNA was amplified from 24 hpf embryo cDNA and cloned into PCS2+. After linearization with Not1 enzyme (NEB (Cat# R3189S)), *vegfc* mRNA was *in vitro* transcribed using mMessage mMachine SP6 Transcription Kit (Invitrogen (Cat# AM1340)). WT embryos were injected at the one-cell stage with 100 pg of *vegfc* mRNA. RNA was then extracted from 48 hpf *vegfc*-injected and non-injected embryos for RT-qPCR.

Cell sorting

Adult zebrafish ventricles were dissected and digested using the Pierce Primary Cardiomyocyte Isolation kit (Thermo Fisher Scientific (Cat# 88281)), following manufacturer's instructions with the following modifications: incubation was performed at 30ºC with gentle shaking (300 rpm) for 45 minutes, followed by resuspension of cells in 1X HBSS with 0.25% BSA. Cell suspension was filtered through a 40uM nylon mesh and sorted using the BD FACSAria™ III (BD Biosciences) instrument. Live cells were selected by exclusion of DAPI (Sigma (Cat#D954)) positive cells using 30mW 405nm excitation paired with 450/50 nm band pass filter. For sorting epicardial cells (*Tg(tcf21:*mCherry*)*$^+$), mCherry fluorescence was measured with 50mW 561nm excitation paired with 610/20nm band pass filter; for coronary endothelial cells (*Tg(-0.8flt1:*RFP*)*$^+$), cardiomyocytes (*Tg(myl7:*DsRed*)*$^+$) and non-cardiomyocytes (*Tg(myl7:*DsRed*)*$^-$) RFP and DsRed fluorescence were measured with 50mW 561 nm excitation paired with a and 586/15 band pass filter. Sorted cells were resuspended in 500 µl of Trizol for subsequent RNA extraction.

RT-qPCR

For quantitative RT-PCR (RT-qPCR), RNA was extracted from whole ventricles of sham-operated or cryoinjured hearts, using one ventricle per biological replicate, or when from the injured tissue only using 4 ventricles per biological replicate. RNA extraction was performed using the RNA clean and concentrator extraction kit (Zymo (Cat# R1013)) following manufacturer's protocol. 250 ng of RNA was used for cDNA synthesis using a Maxima first strand cDNA synthesis kit (Thermo Fisher Scientific (Cat# K1641)) following manufacturer's protocol. For sorted cells, RNA extraction was done using an RNeasy Mini kit (Qiagen) and 100 ng of RNA was used for cDNA synthesis. RT-qPCR reactions were performed using DyNAmo Flash SYBR Green qPCR master mix (Thermo Fisher Scientific (Cat# F415S)) and a CFX Connect Real-Time BioRad machine using the following program: preamplification at 95°C for 7 minutes followed by 39 cycles of amplification at 95°C for 5 seconds and 60°C for 20 seconds, using a melting curve from 60 to 92°C with an increment of 1.0°C every 5 seconds. Primer sequences are listed in table S2. mRNA levels were normalized against *rpl13a* as a housekeeping gene and fold changes were calculated using the log 2 ΔΔ Ct method[43]. Average Ct value for *rpl13a* expression for all RT-qPCR on ventricles was 18.5-20. Average Ct value for *rpl13a* expression for all RT-qPCR on sorted cells was 23-25. For HUVECs experiments, mRNA levels were normalized against *GAPDH* as a housekeeping gene. Average Ct values of all RT-qPCR data are listed in table S3.

RNA sequencing

WT and *Tg(hsp70l:sflt4)* hearts were dissected at 24 hpci. The atrium and bulbus arteriosus were removed, and only ventricles were used for RNA sequencing. A pool of 3 ventricles was used per biological replicate. Total RNA was isolated using a miRNeasy micro kit (QIAGEN), combined with on-column DNase digestion (DNase-Free DNase Set, QIAGEN). Total RNA and library integrity were verified with LabChip Gx Touch 24 (Perkin Elmer). 500 ng of total RNA was used as input for VAHTS Stranded mRNA-seq Library preparation following manufacturer's protocol (Vazyme). Sequencing was performed on a NextSeq500 instrument (Illumina) using v2 chemistry, resulting in an average of 43M reads per library with 1x75bp single end setup. The resulting raw reads were assessed for quality, adapter content and duplication rates with FastQC (available online at http://www.bioinformatics.babraham.ac.uk/ projects/fastqc). Trimmomatic version 0.38 was used to trim reads with a quality drop below a mean of Q20 in a window of 10 nucleotides[44]. Only reads between 30 and 150 nucleotides were used in subsequent analyses. Trimmed and filtered reads were aligned versus the Ensembl Zebrafish genome version DanRer11 (GRCz11.92) using STAR 2.6.1d with the parameter "outFilterMismatchNoverLmax 0.1" to increase the maximum ratio of mismatches to mapped length to 10%[45]. The number of

reads aligning to genes was counted with featureCounts 1.6.3 tool from the Subread package[46]. Only reads mapping at least partially inside exons were admitted and aggregated per gene, while reads overlapping multiple genes or aligning to multiple regions were excluded from further analysis. Differentially expressed genes were identified using DESeq2 version 1.18.1[47]. 17221 genes were tested. Only genes with a minimum fold change of ±0.585 and a Benjamini-Hochberg corrected *P*-value ≤0.05 were and a minimum combinedmean of 5 reads deemed to be significantly differentially expressed. Log2 fold change of 0.585 reflects a 1.5 fold up or down regulation and constitutes a robust cut off to eliminate minor changes. The Ensembl annotation was enriched with UniProt data (release 06.06.2014) based on Ensembl gene identifiers (Activities at the Universal Protein Resource (UniProt)). The heat map was constructed showing the top 50 most significantly differentially expressed genes between both conditions. DESeq2 (1.18.1) normalized expression counts of these genes were averaged per condition and plotted in R studio (1.4.1717) using the ComplexHeatmap package, using z-scores with canberra distance and hierarchical clustering. Z-score, the number of standard deviations that a value is above or below the mean of all values, was generated by inserting the normalized reads into R studio, which calculates the Z-score using the scale() function.

Cell Culture

Human umbilical vein endothelial cells (HUVECs) were purchased from Lonza or Life technologies and used until the sixth passage. HUVECs were cultured in endothelial basal medium (EBM-2, Lonza) supplemented with fetal bovine serum, hydrocortisone, human basic fibroblast growth factor, vascular endothelial growth factor, R3-insulin-like growth factor, ascorbic acid, human epidermal growth factor, GA-1000 and heparin (EGM-2 BulletKit, Lonza) and 100 units/ml penicillin and 100 µg/ml streptomycin. The scratch assay was carried out by seeding 30,000 cells per well in a 24-well plate and performing the scratch using a pipette tip 24 hours after seeding. Cells were collected for RNA isolation 6 hours post scratch.

RNA interference

In HUVECs, *VEGFC* was targeted with pools of siRNA duplexes (Mission esiRNA, Sigma (Cat# EHU013781)). Transfections were performed using Lipofectamine RNAiMAX (Invitrogen (Cat# 13778030)) following manufacturer's standard protocol (final siRNA concentration used: 10 pmol/well). For all RNAi experiments, cells were seeded in 24-well plates and transfected when reaching 80% confluence. Cells were lysed with 10% beta mercaptoethanol (Gibco) in lysis buffer (Invitrogen) and RNA was isolated for RT-qPCR 24 hours after transfection.

Statistical Analysis

Distribution of data for cEC proliferation, percentage fluorescence intensity, CM dedifferentiation and proliferation, as well as percentage of scar area relative to ventricular area was assessed using the Shapiro-Wilks normality test (threshold: alpha value= 0.05), and comparative statistics of data was performed using the Student's t-test or the Mann–Whitney nonparametric test, as stated in the Figure legends. For the RT-qPCR analyses, at least 4 biological replicates were used. For cell sorting, at least 15 ventricles were pooled per sample. All RT-qPCR expression data were analyzed by the Mann–Whitney nonparametric test and were considered significant at $P<0.05$. No experiment-wide multiple testing correction was applied. Error bars represent mean ± SD and bars represent median values. All statistical analyses were performed in GraphPad Prism 8.

Randomization and Blinding Procedures

Experiments involving mutant and WT sibling zebrafish were randomized as follows: mutants and WT siblings were housed in the same tank, used for the experiment, imaged, analyzed and then subsequently genotyped. However, blinding was not performed in experiments involving drug treatments (i.e., 4-OHT vs. EtOH, or DMSO vs SB25002) due to the inability to distinguish between control and experimental groups, or with zebrafish carrying heat shock driven transgenes as they were fluorescently sorted during larval stages (i.e., long before the experiment). Zebrafish with injuries smaller than 20% of the ventricle were excluded from the experiments and analyses. Power calculations were not used to determine sample size which was based on previous publications in the field[11,23,48,49,50].

Figure S1

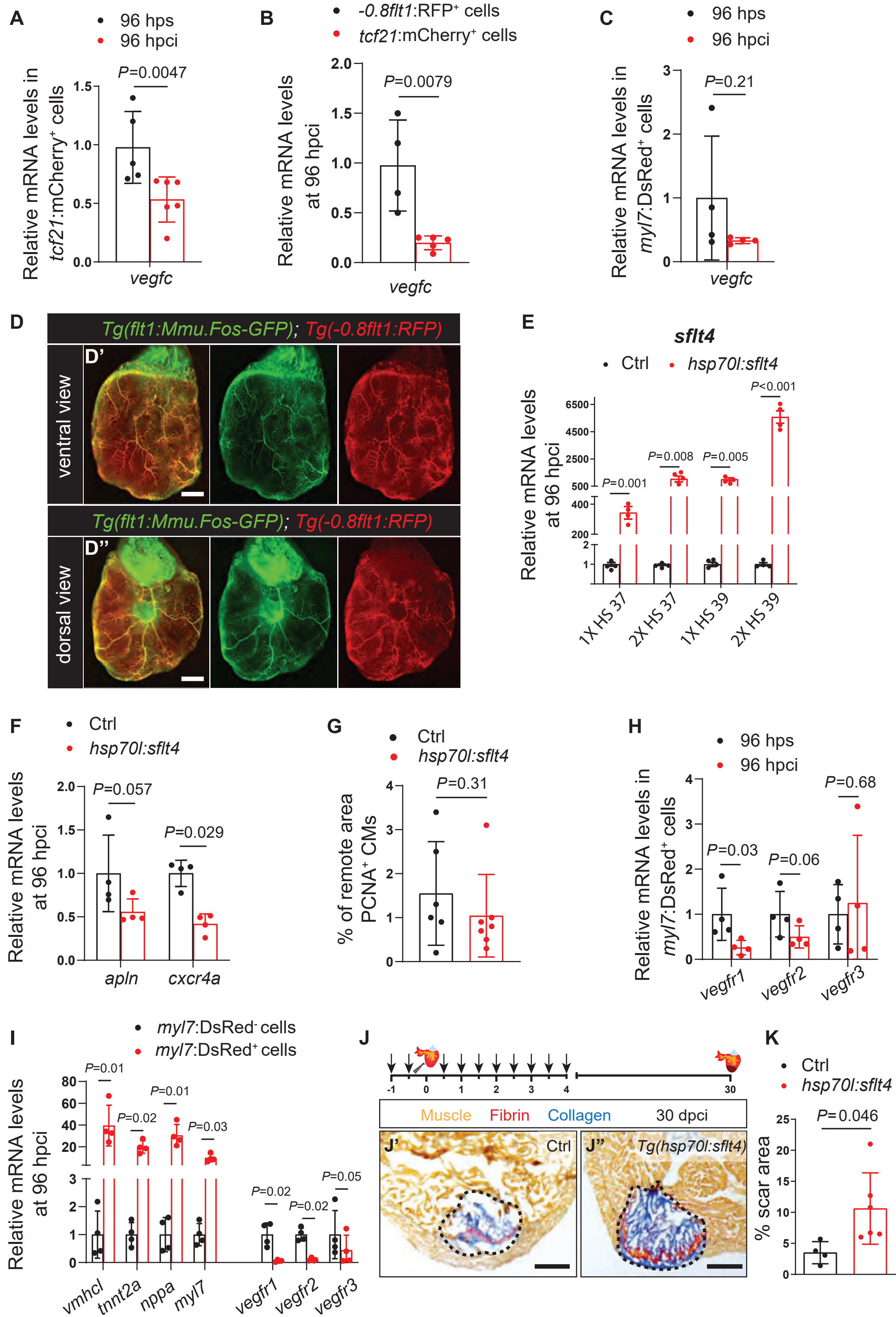

Figure S1: Blocking Vegfc signaling leads to reduced coronary revascularization.
A. RT-qPCR analysis of *vegfc* mRNA levels at 96 hpci in sorted *tcf21*:mCherry$^+$ cells (EPDCs) (n=5) normalized to sham-operated hearts (96 hps) (n=6). **B.** RT-qPCR analysis of *vegfc* mRNA levels at 96 hpci in sorted *tcf21*:mCherry$^+$ cells (EPDCs) (n=5) normalized to sorted *flt1:*RFP$^+$ cells (cECs) (n=4). **C.** RT-qPCR analysis of *vegfc* mRNA levels at 96 hpci in sorted *myl7:*DsRed$^+$ cells (CMs) (n=4) normalized to sham-operated hearts (96 hps) (n=4). **D.** Wholemount images of uninjured ventricle of a *Tg(-0.8flt1:RFP); Tg(flt1:Mmu.Fos-GFP)* zebrafish, showing ventral **(D')** and dorsal **(D'')** views. **E.** RT-qPCR analysis of *sflt4* mRNA levels at 96 hpci in *Tg(hsp70l:sflt4)* ventricles normalized to non-transgenic sibling (Ctrl) ventricles after different heat shock treatments (from left to right): once daily at 37°C, twice daily at 37°C, once daily at 39°C, and twice daily at 39°C (n=4). **F.** RT-qPCR analysis of *apln* and *cxcr4a* mRNA levels at 96 hpci in *Tg(hsp70l:sflt4)* ventricles normalized to non-transgenic sibling (Ctrl) ventricles (n=4). **G.** Percentage of PCNA$^+$ CMs in remote area in non-transgenic sibling (Ctrl) (n=6) and *Tg(hsp70l:sflt4)* (n=7) ventricles at 7 dpci. **H.** RT-qPCR analysis of mRNA levels of *vegfr1*, *vegfr2* and *vegfr3* at 96 hpci in sorted *myl7*:DsRed$^+$ cells (CMs) (n=4) normalized to sham-operated hearts (96 hps) (n=4). **I.** RT-qPCR analysis of mRNA levels of *vegfr1*, *vegfr2* and *vegfr3* at 96 hpci in sorted *myl7:*DsRed$^+$ cells (CMs) (n=4) normalized to *myl7:*DsRed$^-$ cells (non-CMs) (n=4). **J.** Schematic representation of heat shock treatments (arrows) and cardiac cryoinjury. AFOG staining of sections of non-transgenic sibling (Ctrl) **(J')** and *Tg(hsp70l:sflt4)* **(J'')** ventricles at 30 dpci. Orange, Muscle; red, Fibrin; blue, Collagen. **K.** Percentage of scar area relative to ventricular area in non-transgenic sibling (Ctrl) (n=4) and *Tg(hsp70l:sflt4)* (n=6) ventricles at 30 dpci. Black dotted lines delineate the injured tissue **(J)**. Statistical tests: Mann-Whitney test **(A,B,C,E,F,G,H,I)**, Student's t-test **(K)**. Scale Bars: 100 μm **(D,J)**. Ct values of RT-qPCR data are listed in table S3.

Figure S2

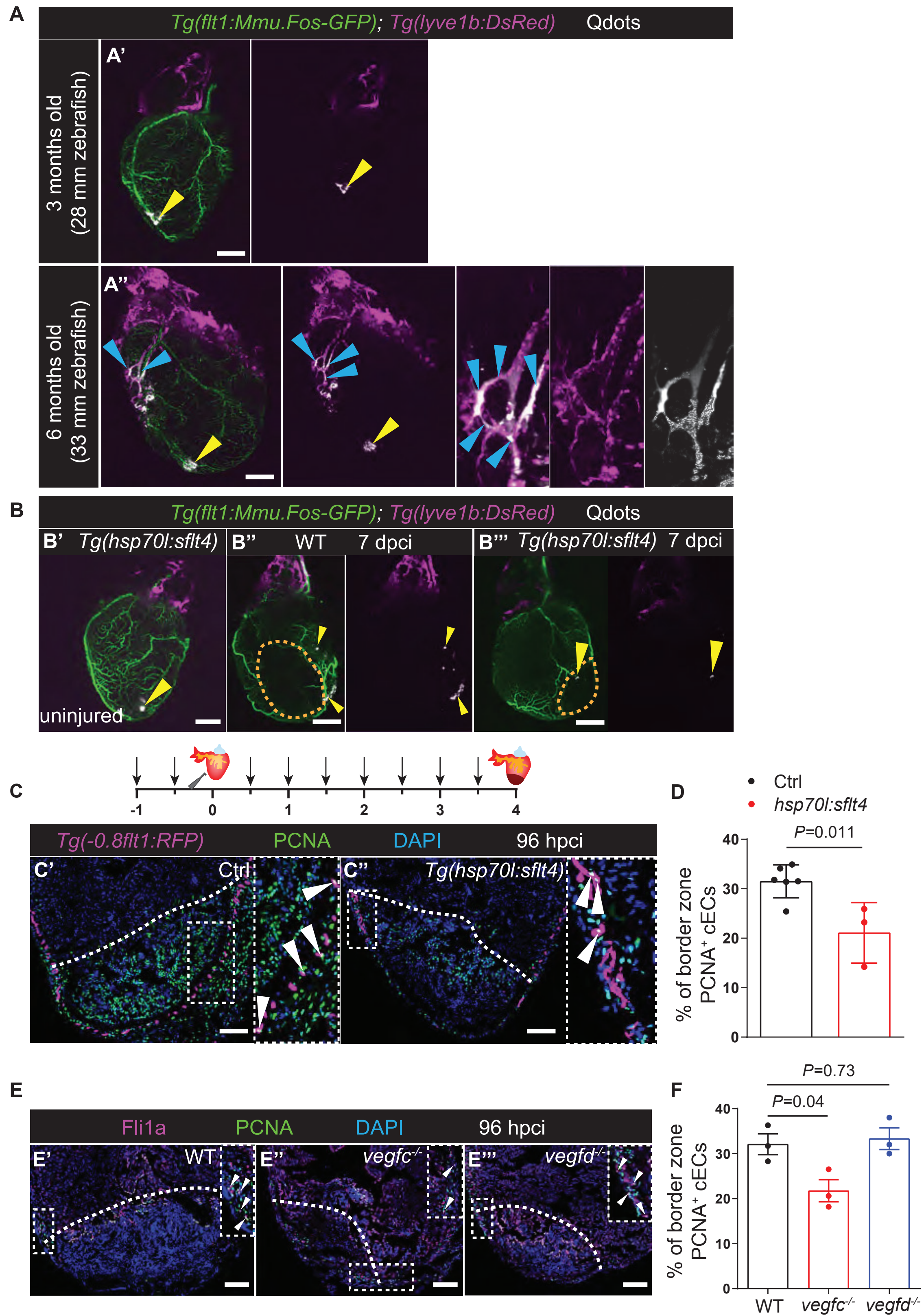

Figure S2: Blocking Vegfc signaling leads to reduced coronary endothelial cell proliferation in the absence of lymphatic endothelial cells.

A. Wholemount images of uninjured ventricles of 3 months old *Tg(lyve1b:DsRed); Tg(flt1:Mmu.Fos-GFP)* zebrafish **(A')** and uninjured ventricles of 6 months old *Tg(lyve1b:DsRed); Tg(flt1:Mmu.Fos-GFP)* zebrafish **(A'')** following intramyocardial injection of Qdots. Yellow arrowheads point to the site of Qdots injection. Blue arrowheads point to Qdots taken up by lymphatic vessels. **B.** Wholemount images of ventricles of 3 months old uninjured *Tg(hsp70l:sflt4)*; *Tg(lyve1b:DsRed); Tg(flt1:Mmu.Fos-GFP)* zebrafish **(B')**, *Tg(lyve1b:DsRed); Tg(flt1:Mmu.Fos-GFP)* zebrafish at 7 dpci **(B'')** and *Tg(hsp70l:sflt4)*; *Tg(lyve1b:DsRed); Tg(flt1:Mmu.Fos-GFP)* zebrafish at 7 dpci **(B''')** following intramyocardial injection of Qdots. Yellow arrowheads point to the site of Qdots injection. **C.** Schematic representation of heat shock treatments (arrows) and cardiac cryoinjury. Immunostaining of sections of cryoinjured hearts of *Tg(0.8flt1:RFP)* (Ctrl) **(C')** and *Tg(hsp70l:sflt4); Tg(-0.8flt1:RFP)* **(C'')** sibling zebrafish at 96 hpci; sections stained for RFP (coronaries, magenta), PCNA (proliferation marker, green), and DNA (DAPI, blue). Arrowheads point to PCNA$^+$ cECs. **D.** Percentage of PCNA$^+$ cECs in the border zone of 3 months old *Tg(-0.8flt1RFP)* (Ctrl) (n=6) and *Tg(hsp70l:sflt4); Tg(-0.8flt1:RFP)* (n=3) ventricles at 96 hpci. **E.** Immunostaining of sections of cryoinjured hearts of WT **(E')**, *vegfc*$^{-/-}$ **(E'')** and *vegfd*$^{-/-}$ **(E''')** zebrafish at 96 hpci; sections stained for Fli1a (endothelial cells, magenta), PCNA (proliferation marker, green), and DNA (DAPI, blue). Arrowheads point to PCNA$^+$ cECs. **F.** Percentage of PCNA$^+$ cECs in the border zone of WT (n=3), *vegfc*$^{-/-}$ (n=3) and *vegfd*$^{-/-}$ (n=3) ventricles at 96 hpci (n=3). Orange **(B)** and white **(C,E)** dotted lines delineate the injured tissue. Statistical test: Student's t-test **(D,F)**. Scale Bars: 200 μm **(A,B)**, 100 μm **(C,E)**.

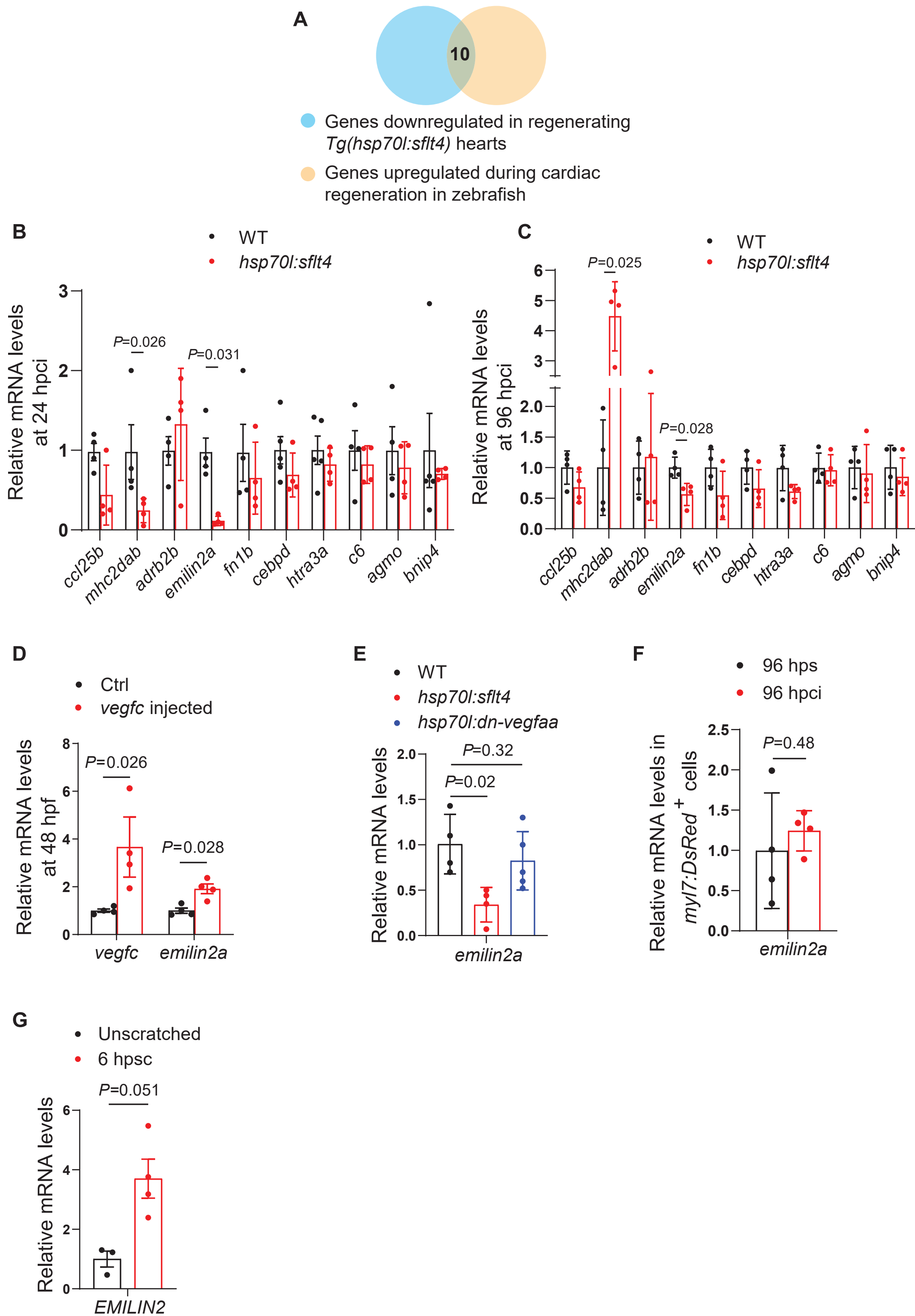

Figure S3
A
10
Genes downregulated in regenerating Tg(hsp70l:sflt4) hearts
Genes upregulated during cardiac regeneration in zebrafish
B
WT
hsp70l:sflt4
Relative mRNA levels at 24 hpci
P=0.026
P=0.031
ccl25b
mhc2dab
adrb2b
emilin2a
fn1b
cebpd
htra3a
c6
agmo
bnip4
C
WT
hsp70l:sflt4
Relative mRNA levels at 96 hpci
P=0.025
P=0.028
D
Ctrl
vegfc injected
Relative mRNA levels at 48 hpf
P=0.026
P=0.028
vegfc
emilin2a
E
WT
hsp70l:sflt4
hsp70l:dn-vegfaa
Relative mRNA levels
P=0.32
P=0.02
emilin2a
F
96 hps
96 hpci
Relative mRNA levels in myl7:DsRed+ cells
P=0.48
emilin2a
G
Unscratched
6 hpsc
P=0.051
Relative mRNA levels
EMILIN2

Figure S3: *emilin2a* is a target of Vegfc signaling.

A. Intersection of significantly downregulated genes in *Tg(hsp70l:sflt4)* at 24 hpci with genes upregulated during cardiac regeneration in zebrafish[15]. **B.** RT-qPCR analysis of mRNA levels of 10 potential targets of Vegfc signaling at 24 hpci in *Tg(hsp70l:sflt4)* ventricles (n=4) normalized to non-transgenic sibling (Ctrl) ventricles (n=4). **C.** RT-qPCR analysis of mRNA levels of 10 potential targets of Vegfc signaling at 96 hpci in *Tg(hsp70l:sflt4)* ventricles (n=4) normalized to non-transgenic sibling (Ctrl) ventricles (n=4). **D.** RT-qPCR analysis of *vegfc* and *emilin2a* mRNA levels at 48 hours post fertilization (hpf) following the injection of 75 pg of *vegfc* mRNA (n=4) normalized to un-injected (Ctrl) embryos (n=4). **E.** RT-qPCR analysis of *emilin2a* mRNA levels in uninjured *Tg(hsp70l:sflt4)* (n=4) and *Tg(hsp70:dn-vegfaa)* adult ventricles (n=5) after daily heat shocks for 3 days, normalized to WT ventricles (n=4). **F.** RT-qPCR analysis of *emilin2a* mRNA levels at 96 hpci in sorted *myl7:*DsRed^{+} cells (CMs) (n=4) normalized to 96 hps (n=4). **G.** RT-qPCR analysis of *EMILIN2* mRNA levels in HUVECs at 6 hours post scratch (6 hpsc) (n=3) normalized to unscratched (n=4). Statistical test: Non-parametric Mann-Whitney test **(B,C,D,E,F,G)**. Ct values of RT-qPCR data are listed in table S3.

Figure S4

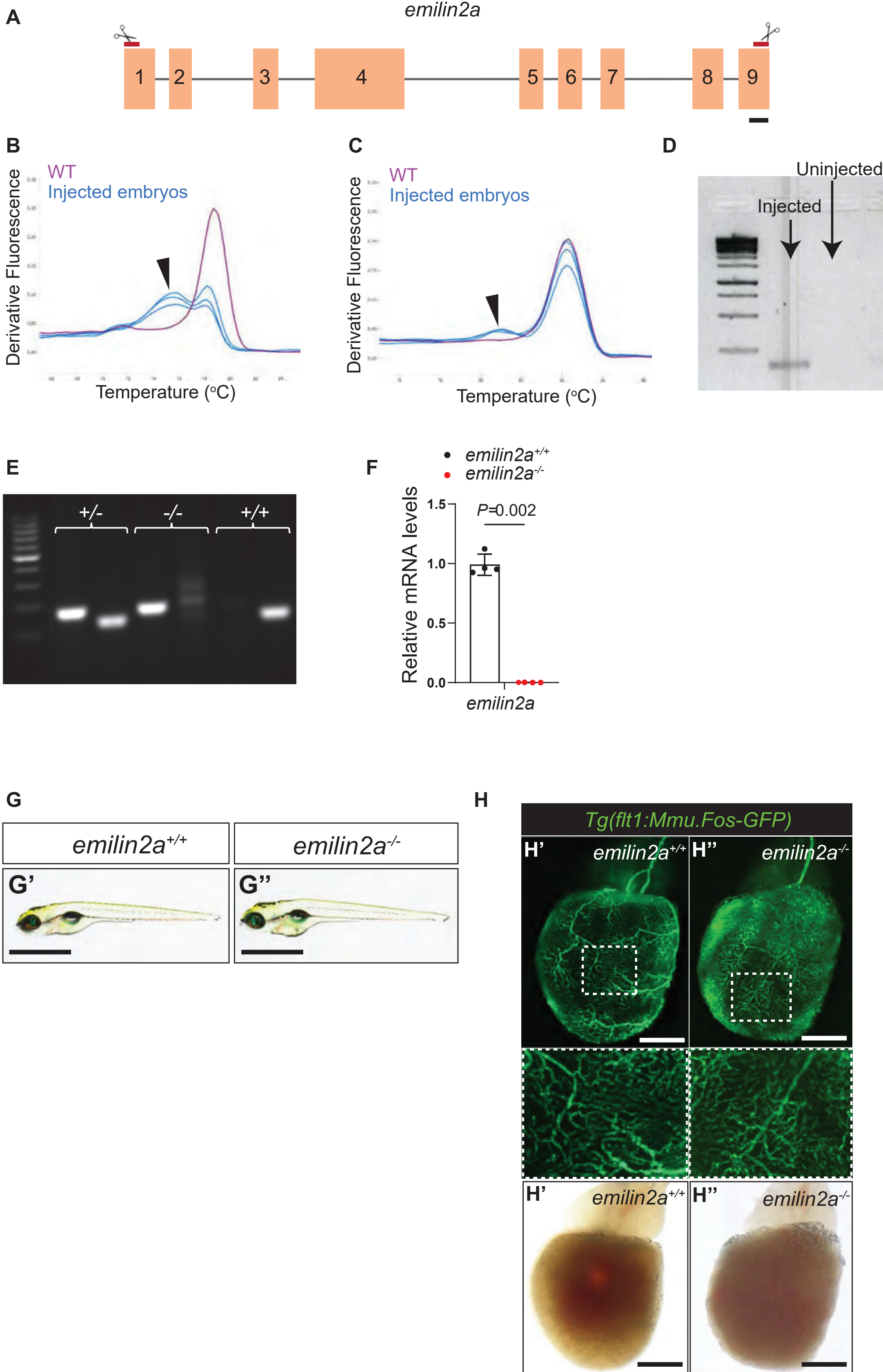

Figure S4: Generation of an *emilin2a* full locus deletion allele.

A. Schematic representation of the *emilin2a* gene showing the location of the gRNAs targeting exons 1 and 9 (red lines). **B.** High resolution melt analysis showing 24 hpf WT embryos, uninjected (purple) and injected with 75 pg of gRNA targeting exon 1 (blue). **C.** High resolution melt analysis showing 24 hpf WT embryos, uninjected (purple) and injected with 75 pg of gRNA targeting exon 9 (blue). **D.** DNA gel electrophoresis analysis of 24 hpf embryos uninjected and injected with the gRNAs targeting exons 1 and 9; DNA ladder =100 bp. **E.** DNA gel electrophoresis assay used to genotype the *emilin2a* full locus deletion allele; DNA ladder = 100 bp. **F.** RT-qPCR analysis of *emilin2a* mRNA levels in uninjured ventricles of *emilin2a*$^{-/-}$ (n=4) normalized to *emilin2a*$^{+/+}$ uninjured ventricles (n=4). **G.** Wholemount images of 5 dpf *emilin2a*$^{+/+}$ and *emilin2a*$^{-/-}$ sibling larvae. **H.** Wholemount images of uninjured adult ventricles of *Tg(flt1:Mmu.Fos-GFP); emilin2a*$^{+/+}$ **(H')** and *Tg(flt1:Mmu.Fos-GFP); emilin2a*$^{-/-}$ **(H'')** sibling zebrafish. Statistical test: Non-parametric Mann-Whitney test **(F)**. Scale bars: 1 kb **(A)**, 500 μm **(G,H)**. Ct values of RT-qPCR data are listed in table S3.

Figure S5

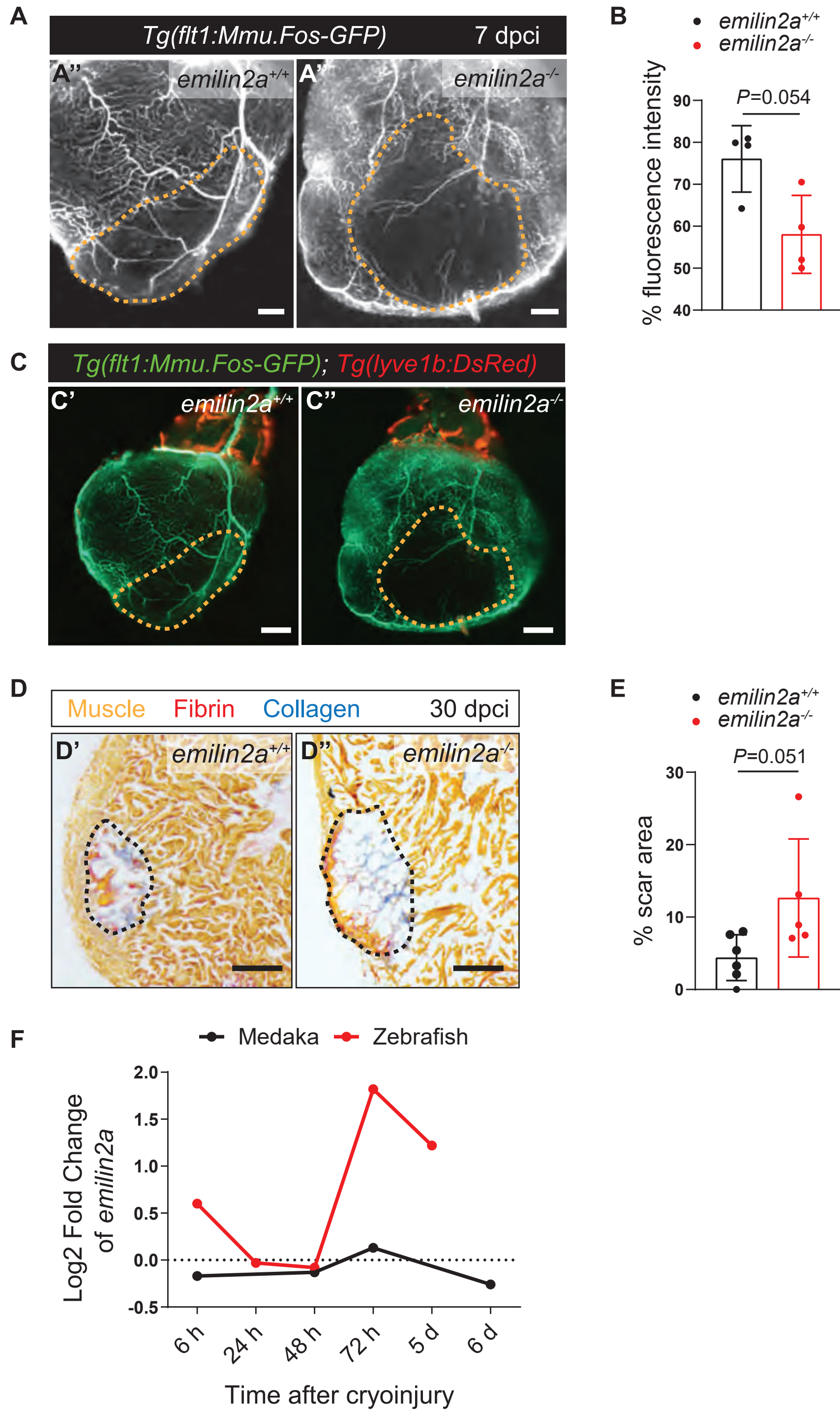

Figure S5: *emilin2a* mutants exhibit reduced coronary endothelial cell coverage after cardiac injury in zebrafish.

A. Wholemount images of ventricles of 7 dpci *Tg(flt1:Mmu.Fos-GFP); emilin2a*$^{+/+}$ **(A')** and *Tg(flt1:Mmu.Fos-GFP); emilin2a*$^{-/-}$ **(A'')** sibling zebrafish. **B.** Percentage of GFP fluorescence intensity in the injured tissue of 7 dpci *Tg(flt1:Mmu.Fos-GFP); emilin2a*$^{+/+}$ (n=4) and *Tg(flt1:Mmu.Fos-GFP); emilin2a*$^{-/-}$ (n=4) ventricles. **C.** Wholemount images of ventricles of 7 dpci *Tg(flt1:Mmu.Fos-GFP); Tg(lyve1b:DsRed)*; *emilin2a*$^{+/+}$ **(C')** and *Tg(flt1:Mmu.Fos-GFP); Tg(lyve1b:DsRed); emilin2a*$^{-/-}$ **(C'')** sibling zebrafish. **D.** AFOG staining of sections of *emilin2a*$^{+/+}$ **(D')** and *emilin2a*$^{-/-}$ **(D'')** ventricles at 30 dpci. Orange, Muscle; red, Fibrin; blue, Collagen. **E.** Percentage of scar area relative to ventricular area in *emilin2a*$^{+/+}$ (n=6) and *emilin2a*$^{-/-}$ (n=5) ventricles at 30 dpci. **F.** *emilin2a* mRNA expression levels in zebrafish and medaka ventricles after cardiac cryoinjury normalized to sham operated ventricles (data from [15]); h: hpci; d: dpci. Orange **(A,C)** and black **(D)** dotted lines delineate the injured tissue. Statistical tests: Non-parametric Mann-Whitney test **(B,E)**. Scale Bars: 200 µm **(A,C,D)**.

Figure S6

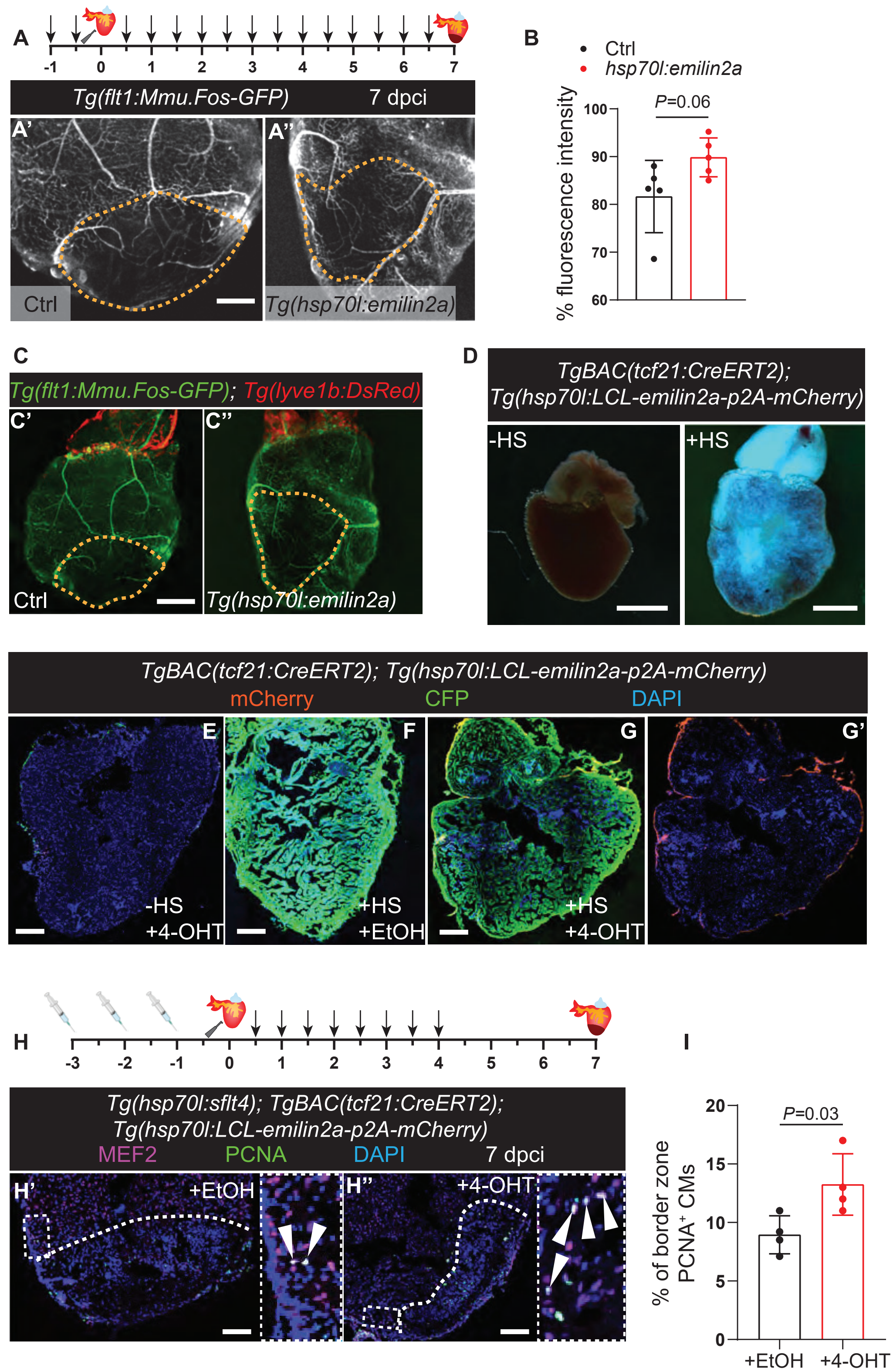

Figure S6: *emilin2a* overexpression increases coronary endothelial cell coverage and cardiomyocyte proliferation after cardiac injury in zebrafish.

A. Schematic representation of heat shock treatments (arrows) and cardiac cryoinjury. Wholemount images of ventricles of 7 dpci *Tg(flt1:Mmu.Fos-GFP)* (Ctrl) **(A')** and *Tg(hsp70l:emilin2a); Tg(flt1:Mmu.Fos-GFP)* **(A'')** sibling zebrafish. **B.** Percentage of GFP fluorescence intensity in the injured tissue of 7 dpci *Tg(flt1:Mmu.Fos-GFP)* (Ctrl) (n=5) and *Tg(hsp70l:emilin2a); Tg(flt1:Mmu.Fos-GFP)* (n=5) ventricles. **C.** Wholemount images of ventricles of 7 dpci *Tg(flt1:Mmu.Fos-GFP); Tg(lyve1b:DsRed)* (Ctrl) **(C')** and *Tg(hsp70l:emilin2a); Tg(flt1:Mmu.Fos-GFP); Tg(lyve1b:DsRed)* **(C'')** sibling zebrafish. **D.** Wholemount images of uninjured hearts of *TgBAC(tcf21:CreERT2); Tg(hsp70l:LCL-emilin2a-p2A-mCherry)* zebrafish without heat shock treatment (-HS) and after heat shock treatment (+HS). **E.** Immunostaining of sections of cryoinjured hearts of *TgBAC(tcf21:CreERT2); Tg(hsp70l:LCL-emilin2a-p2A-mCherry)* zebrafish without heat shock (-HS) and with tamoxifen injection (+4-OHT) **(E)**, with heat shock (+HS) and ethanol injection (+EtOH) **(F)** and with heat shock (+HS) and tamoxifen injections (+4-HT) **(G, G')**; sections stained for mCherry (recombined EPDCs, orange), GFP (non-recombined tissue, green), and DNA (DAPI, blue). **H.** Schematic representation of Ethanol (EtOH) or 4-hydroxytamoxifen (4-OHT) injections followed by cardiac cryoinjury and heat shock treatments (arrows). Immunostaining of sections of cryoinjured hearts of *Tg(hsp70l:sflt4); TgBAC(tcf21:CreERT2); Tg(hsp70l:LCL-emilin2a-p2A-mCherry)* zebrafish injected with EtOH **(H')** or 4-OHT **(H'')** at 7 dpci; sections are stained for MEF2 (CMs, magenta), PCNA (proliferation marker, white), and DNA (DAPI, blue). Arrowheads point to PCNA$^+$ CMs. **I.** Percentage of PCNA$^+$ CMs in the border zone of *Tg(hsp70l:sflt4); TgBAC(tcf21:CreERT2); Tg(hsp70l:LCL-emilin2a-mCherry)* at 7 dpci injected with EtOH (n=4) or 4-OHT (n=4). Orange **(A,C)** and white **(H)** dotted lines delineate the injured tissue. Statistical tests: Student's t-test **(B,I)**. Scale bars: 200 μm **(A,C,D)**, 100 μm **(E,F,G,H)**,

Figure S7

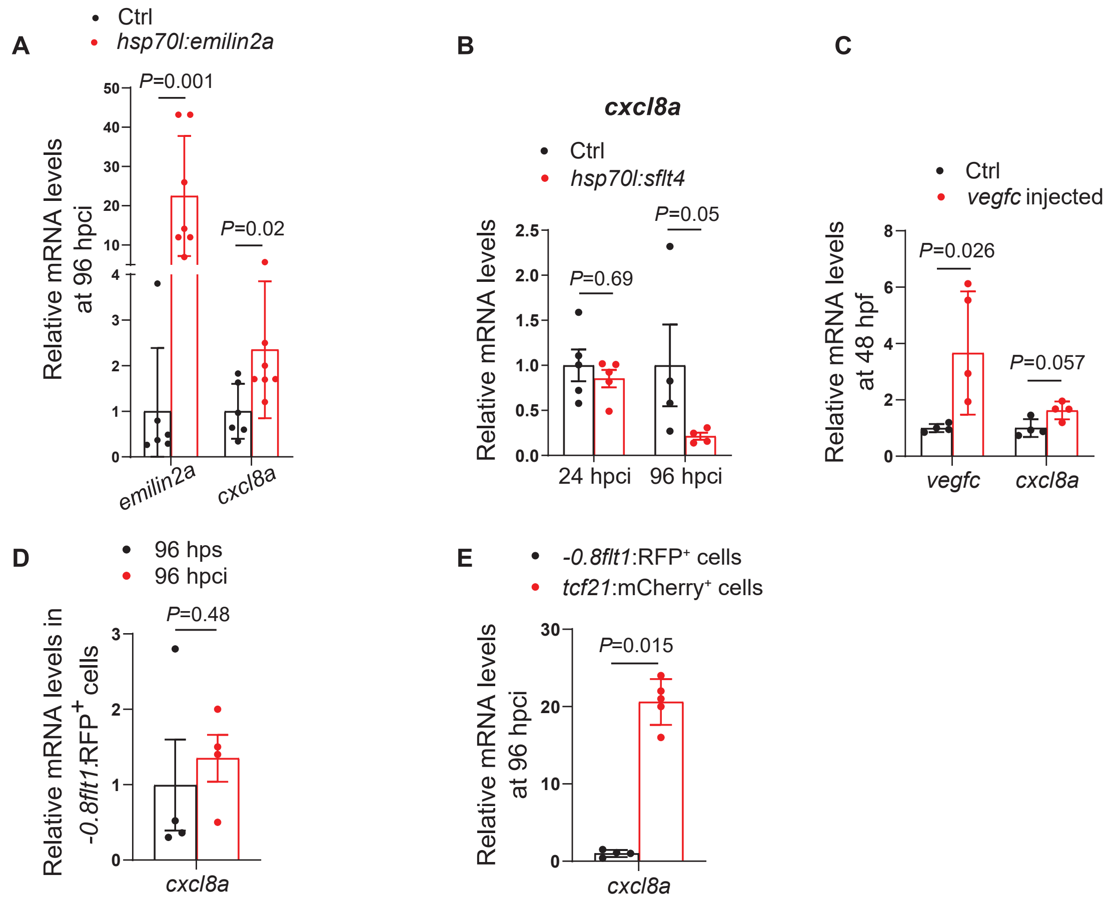

Figure S7: *cxcl8a* expression.

A. RT-qPCR analysis of *emilin2a* and *cxcl8a* mRNA levels at 96 hpci in *Tg(hsp70l:emilin2a)* ventricles (n=7) normalized to non-transgenic sibling (Ctrl) ventricles (n=6). **B.** RT-qPCR analysis of *cxcl8a* mRNA levels at 24 and 96 hpci in *Tg(hsp70l:sflt4)* ventricles (n=4-5) normalized to non-transgenic sibling (Ctrl) ventricles (n=4-5). **C.** RT-qPCR analysis of *vegfc* and *cxcl8a* mRNA levels at 48 hpf following the injection at the one-cell stage of 75 pg of *vegfc* mRNA (n=4) normalized to uninjected embryos (Ctrl) (n=4). **D.** RT-qPCR analysis of *cxcl8a* mRNA levels in sorted *-0.8flt1*:RFP$^+$ cells (cECs) at 96 hpci (n=4) normalized to sham-operated hearts (96 hps) (n=4). **E.** RT-qPCR analysis of *cxcl8a* mRNA levels in sorted *tcf21*:mCherry$^+$ cells (EPDCs) (n=5) at 96 hpci normalized to sorted *-0.8flt1:*RFP$^+$ cells (cECs) (n=4). Statistical test: Non-parametric Mann-Whitney test **(A,B,C,D,E)**. Ct values of RT-qPCR data are listed in table S3.

Figure S8

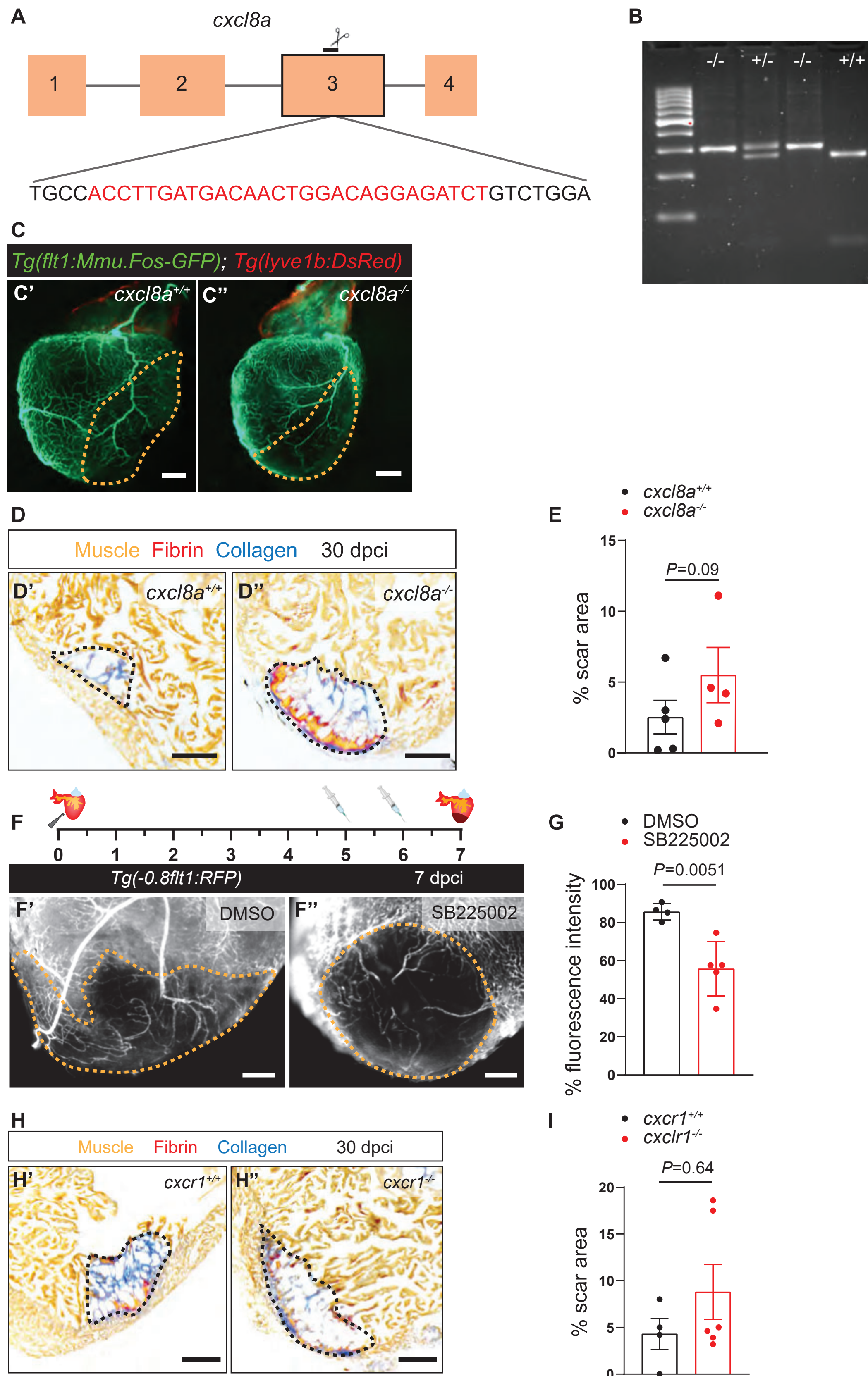

Figure S8: Inhibition of Cxcl8a-Cxcr1 signaling reduces revascularization after cardiac injury in zebrafish.

A. Schematic representation of the *cxcl8a* gene showing the deleted bases in red. **B.** DNA gel electrophoresis assay used to genotype the *cxcl8a* mutation; DNA ladder = 100 bp. **C.** Wholemount images of ventricles of 7 dpci *Tg(flt1:Mmu.Fos-GFP); Tg(lyve1b:DsRed); cxcl8a*$^{+/+}$ **(C')** and *Tg(flt1:Mmu.Fos-GFP); Tg(lyve1b:DsRed); cxcl8a*$^{-/-}$ **(C'')** sibling zebrafish. **D.** AFOG staining of sections of *cxcl8a*$^{+/+}$ **(D')** and *cxcl8a*$^{-/-}$ **(D'')** ventricles at 30 dpci. Orange, Muscle; red, Fibrin; blue, Collagen. **E.** Percentage of scar area relative to ventricular area in *cxcl8a*$^{+/+}$ (n=5) and *cxcl8a*$^{-/-}$ (n=4) ventricles at 30 dpci. **F.** Schematic representation of intraperitoneal injections of DMSO or 0.01 mM SB225002 and cardiac cryoinjury. Wholemount images of 7 dpci *Tg(0.8flt1:RFP)* zebrafish after injections with DMSO as control **(F')** or 0.01 mM SB225002 **(F'')**. **G.** Percentage of RFP fluorescence intensity in the injured tissue of 7 dpci *Tg(-0.8flt1:RFP)* ventricles after injections with DMSO (n=4) or SB225002 (n=5). **H.** AFOG staining of sections of *cxcr1*$^{+/+}$ **(H')** and *cxcr1*$^{-/-}$ **(H'')** ventricles at 30 dpci. Orange, Muscle; red, Fibrin; blue, Collagen. **I.** Percentage of scar area relative to ventricular area in *cxcr1*$^{+/+}$ (n=4) and *cxcr1*$^{-/-}$ (n=6) ventricles at 30 dpci. Orange **(C,F)** and black **(D,H)** dotted lines delineate the injured tissue. Statistical tests: Non-parametric Mann-Whitney test **(I)**, Student's t-test **(G,E)**. Scale bars: 200 μm **(C,D,F,H)**.

Table S1: Average number of coronaries in the border zone (200 μm) + injured area per section in the different experimental manipulations

Experiment	Average # of coronaries per section
Ctrl (Fig. 1E)	58
Tg(hsp70l:sflt4)	50
emilin2a$^{+/+}$	56
emilin2a$^{-/-}$	54
Ctrl (Fig. 4B)	60
Tg(hsp70l:emilin2a)	54
Tg(hsp70l:sflt4); TgBAC(tcf21:CreERT2); Tg(hsp70l:LCL-emilin2a-mCherry) – EtOH	39
Tg(hsp70l:sflt4); TgBAC(tcf21:CreERT2); Tg(hsp70l:LCL-emilin2a-mCherry) – 4-OHT	40
cxcl8a$^{+/+}$	54
cxcl8a$^{-/-}$	59
cxcr1$^{+/+}$	47
cxcr1$^{-/-}$	40
3 months old Ctrl (Fig. S2D)	66
3 months old *Tg(hsp70l:sflt4)*	57
WT (Ctrl) (Fig. S2F)	60
vegfc$^{-/-}$	54
vegfd$^{-/-}$	56

Table S2: Primer sequences

Target Gene	Primer sequence
RT-qPCR	
rpl13a	**Fwd:** TCTGGAGGACTGTAAGAGGTATG **Rev:** AGACGCACAATCTTGAGAGCAG
vegfc	**Fwd:** TCGAGTCAAGTCACGACTACTATG **Rev:** ATCCACACTACCCGCTGAAC
sflt4	**Fwd:** CGCCAGCTCCTACGTGTTCGTGAGAG **Rev:** CGTCTGGCCACAGCACCGAGC
apln	**Fwd:** GCTGTGTTCAGCCAGTGCT **Rev:** TTCTGCCGCAAAGGAGTC
cxcr4a	**Fwd:** AGAGTGAGCACAAACAGAAGG **Rev:** GGCTTATTACGAACACATCGTC
vegfr1	**Fwd:** GCTCATTCAGGTGAAGTGGACAG **Rev:** AGAAGATCGCCTTCATAATGTGG
vegfr2	**Fwd:** ACCTCAGTCAAAGCCTTCTTCAC **Rev:** AGCAGTTGTGGATCAGGCAGAC
vegfr3	**Fwd:** GACCCAGAGCATCCATTCAT **Rev:** AGGCTCTGGATACGGCACTA
vmhcl	**Fwd:** GCGATGCTGAAATGTCTGTT **Rev:** CAGTCACAGTCTTGCCTCCT
tnnt2a	**Fwd:** CAACGAAGAAGTGGAAGAGTACGAG **Rev:** TTCTCCATCGTGTTCCTGAGTG
nppa	**Fwd:** GATGTACAAGCGCACACGTT **Rev:** TCTGATGCCTCTTCTGTTGC
myl7	**Fwd:** GGCTCTTCCAATGTCTTCTCC **Rev:** GGACTCCAGCTCTTCATCAC

emilin2a	**Fwd:** CCTCCTGTCAACCCTGTCTCATATGATACC **Rev:** GGCAATGATGCCGAAGTCCCCAGAG
ccl25b	**Fwd:** GGCTTTGCTCCTGCTGTTGGCTTGC **Rev:** ACAGCGGGAATGTTGCATCCTCCGT
mhc2dab	**Fwd:** GTCCTGGCTGAGAGATGGTAAAGAGGTG **Rev:** GGTTGAGTTAAGCTGGCGTGCTCC
adrb2b	**Fwd:** GCGCTGGTCATCAGTGCCATTGTACGATTCC **Rev:** GCCGAAGGGCACCACCATAAGACCC
fn1b	**Fwd:** CTCTTCCAAATGGTGTCACG **Rev:** CACACTTGAACTCTCCTTTGC
cebpd	**Fwd:** CCAAAGGGATTCATTCACAA **Rev:** CTGTTGTTGTTGTTGTTCTC
htra3a	**Fwd:** TGACAAGAAATCAGACATCG **Rev:** AGAGCGACCTAATGATAAGA
c6	**Fwd:** GTCACTTAAGATTCAACAAGCGTC **Rev:** GTTTCTCCCATGTACCATCCTG
agmo	**Fwd:** CTCTCCGTTGGCGCTGCTGATTCCTCCT **Rev:** TTCGCCCGTGGTGAACTCTGTGGTGGC
bnip4	**Fwd:** GCTGTCTTCAACACACATAC **Rev:** TATAAATGCTGTGGGTGGTA
cxcl8a	**Fwd:** TGTGTTATTGTTTTCCTGGCATTTC **Rev:** GCGACAGCGTGGATCTACAG
cxcr1	**Fwd:** GTGATCGTACGCGCTATGGA **Rev:** ATTCGGGTTGCTAATCGCCA
cxcr2	**Fwd:** GTCACTGGCCGTTCGGCACCATCATG **Rev:** CCCAATCAGATGGAGCTTTCGGTTGAGG
GAPDH	**Fwd:** TGTTGCCATCAATGACCCCTT **Rev:** CTCCACGACGTACTCAGCG

VEGFC	**Fwd:** CACACTTCCTGCCGATGC **Rev:** GTTCGCTGCCTGACACTG
EMILIN2	**Fwd:** AAAGCCACAGATAATGAACC **Rev:** CCTCTAGCACCTGTATCTTC
in situ hybridisation	
vegfc	**Fwd:** ATGCACTTATTTGGATTTTCTGTC **Rev:** TTAGTCCAGTCTTCCCCAGTATGTGGG
emilin2a	**Fwd:** TTGAGGAATTGCGGGGAACAGTG **Rev:** AATCTGGTTGTGGCTTGGTCTGC
cxcl8a	**Fwd:** ATGACCAGCAAAATCATTTCAGTG **Rev:** TCATGGTTTTCTGTTGACAATG
cxcr1	**Fwd:** GCAACTTCACGTTTGTTCCCGACGAG **Rev:** CTGTAGCAGCTCAACATGACCACTAGG
Genotyping	
emilin2a	**Out-Out primers to detect the deletion** **Fwd:** CCATACACGATTGTTTGGAGCAAAGTTAT **Rev:** GGCGAGTAGAGGAATATAGCGCTGAA **In-In primers to detect the gene** **Fwd:** CTGGAGTAGCTGAGGGTGTCTCT **Rev:** GTTATTCATTTGTGCCACCTTCCTCTCC
cxcl8a	**Fwd:** GCTTTCAGGAATGAGCTTGAGAG **Rev:** TCTTAACCCATGGAGCAGAGG

Table S3: RT-qPCR Ct Values

Average Ct values of RT-qPCR reactions			
Figure 1A - *vegfc*			
	48 h	**96 h**	**7 d**
Sham	27.7	28.5	30.12
Cryoinjury	24.10	25.71	27.83
Figure 1C - *vegfc* in sorted *-0.8flt1*:RFP$^+$			
96 hps	32.4		
96 hpci	30.1		
Figure 3B - *emilin2a* in *Tg(hsp70l:slft4)*			
	Ctrl	***Tg(hsp70l:sflt4)***	
24 hpci	29.43	30.72	
96 hpci	24.03	25.82	
Figure 3C - *VEGFC* and *EMILIN2* after *siVEGFC*			
	Ctrl	***siVEGFC***	
VEGFC	25.58	28.57	
EMILIN2	35.78	38.04	
Figure 3D - *emilin2a*			
	48 h	**96 h**	**7 d**
Sham	25.69	26.69	28.61
Cryoinjury	25.36	25.30	27.01
Figure 3F - *emilin2a* in sorted *-0.8flt1*:RFP$^+$			
96 hps	31.0		

96 hpci	29.4	
Figure 3F - *emilin2a* in sorted *tcf21*:mCherry⁺		
96 hps	29.3	
96 hpci	27.1	
Figure 3G - *emilin2a* at 96 hpci		
***-0.8flt1*:RFP⁺**	29.4	
***tcf21*:mCherry⁺**	27.1	
Figure 5A - *emilin2a* and *cxcl8a* at 96 hpci		
	emilin2a	***cxcl8a***
emilin2a $^{+/+}$	26.42	27.26
emilin2a $^{-/-}$	N.D	29.07
Figure 5C - *cxcl8a* in *tcf21*:mCherry⁺		
96 hps	29.5	
96 hpci	27.2	
Figure 6B - *cxcr1* and *cxcr2* in *-0.8flt1*:RFP⁺		
	cxcr1	***cxcr2***
96 hps	36.2	38.3
96 hpci	34.6	38.8
Figure S1A - *vegfc* in sorted *tcf21*:mCherry⁺		
96 hps	32.1	
96 hpci	33.1	
Figure S1B - *vegfc* at 96 hpci		
***-0.8flt1*:RFP⁺**	30.1	

<table>
<tr><td>tcf21:mCherry⁺</td><td colspan="7">33.1</td></tr>
<tr><td colspan="8">Figure S1C - vegfc in sorted myl7:DsRed⁺</td></tr>
<tr><td>96 hps</td><td colspan="7">36.6</td></tr>
<tr><td>96 hpci</td><td colspan="7">35.9</td></tr>
<tr><td colspan="8">Figure S1E - sflt4 at 96 hpci</td></tr>
<tr><td></td><td colspan="4">Ctrl</td><td colspan="3">Tg(hsp70l:sflt4)</td></tr>
<tr><td>1x HS 37</td><td colspan="4">36.15</td><td colspan="3">27.83</td></tr>
<tr><td>2x HS 37</td><td colspan="4">35.7</td><td colspan="3">25.91</td></tr>
<tr><td>1x HS 39</td><td colspan="4">34.91</td><td colspan="3">24.38</td></tr>
<tr><td>2x HS 37</td><td colspan="4">35.32</td><td colspan="3">23.01</td></tr>
<tr><td colspan="8">Figure S1F - apln and cxcr4a at 96 hpci</td></tr>
<tr><td></td><td colspan="4">Ctrl</td><td colspan="3">Tg(hsp70l:sflt4)</td></tr>
<tr><td>apln</td><td colspan="4">24.0</td><td colspan="3">25.1</td></tr>
<tr><td>cxcr4a</td><td colspan="4">28.3</td><td colspan="3">29.1</td></tr>
<tr><td colspan="8">Figure S1H – vegfr1, vegfr2 and vegfr3 in sorted myl7:DsRed⁺</td></tr>
<tr><td></td><td colspan="3">vegfr1</td><td colspan="2">vegfr2</td><td colspan="2">vegfr3</td></tr>
<tr><td>96 hps</td><td colspan="3">34.86</td><td colspan="2">31.49</td><td colspan="2">37.01</td></tr>
<tr><td>96 hpci</td><td colspan="3">35.10</td><td colspan="2">31.07</td><td colspan="2">36.50</td></tr>
<tr><td colspan="8">Figure S1I – vegfr1, vegfr2 and vegfr3 at 96 hpci</td></tr>
<tr><td></td><td>vmhcl</td><td>tnnt2a</td><td>nppa</td><td>myl7</td><td>vegfr1</td><td>vegfr2</td><td>vegfr3</td></tr>
<tr><td>myl7:DsRed⁻</td><td>26.09</td><td>27.00</td><td>27.39</td><td>22.62</td><td>30.32</td><td>28.04</td><td>33.13</td></tr>
<tr><td>myl7:DsRed⁺</td><td>20.87</td><td>23.28</td><td>22.92</td><td>19.92</td><td>35.10</td><td>31.07</td><td>36.50</td></tr>
<tr><td colspan="8">Figure S3B – Validation of Vegfc potential targets at 24 hpci</td></tr>
</table>

	WT	*Tg(hsp70l:sflt4)*
ccl25b	25.50	25.75
mhc2dab	27.91	27.68
adrb2b	30.91	30.48
emilin2a	29.43	30.47
fn1b	22.58	23.01
cebpd	23.36	22.91
htra3a	27.86	27.16
c6	23.22	22.92
agmo	29.20	29.42
bnip4	26.14	25.21
Figure S3C – Validation of Vegfc potential targets at 96 hpci		
	WT	***Tg(hsp70l:sflt4)***
ccl25b	23.10	24.60
mhc2dab	24.70	23.23
adrb2b	28.55	29.54
emilin2a	28.55	29.54
fn1b	18.83	19.73
cebpd	22.88	24.15
htra3a	26.68	27.87
c6	22.16	22.85
agmo	28.30	29.05
bnip4	26.07	26.83

Figure S3D - *vegfc* and *emilin2a* at 48 hpf

	Ctrl	*vegfc* injected
vegfc	27.9	25.9
emilin2a	30.6	29.7

Figure S3E – *emilin2a* in *Tg(hsp70l:sflt4)* and *Tg(hsp70l:dn-vegfaa)*

	WT	*Tg(hsp70l:sflt4)*	*Tg(hsp70l:dn-vegfaa)*
emilin2a	26.0	27.7	26.5

Figure S3F – *emilin2a* in sorted *myl7*:DsRed$^+$

96 hps	35.6
96 hpci	34.9

Figure S3G - *EMILIN2* after scratch assay

Unscratched	36.06
6 hpsc	34.69

Figure S4F - *emilin2a* in uninjured hearts

emilin2a $^{+/+}$	28.9
emlin2a $^{-/-}$	38.2

Figure S7A - *emilin2a* and *cxcl8a* in *Tg(hsp70l:emilin2a)*

	Ctrl	*Tg(hsp70l:emilin2a)*
emilin2a	32.53	25.99
cxcl8a	28.82	26.67

Figure S7B - *cxcl8a* in *Tg(hsp70l:sflt4)*

	Ctrl	*Tg(hsp70l:sflt4)*
24 hpci	25.28	25.40

96 hpci	25.27	26.85
Figure S7C - *vegfc* and *cxcl8a* at 48 hpf		
	Ctrl	***vegfc* injected**
vegfc	27.9	25.9
cxcl8a	28.9	27.9
Figure S7D - *cxcl8a* in sorted *-0.8flt1*:RFP$^+$		
96 hps	33.7	
96 hpci	32.8	
Figure S7E – *cxcl8a* at 96 hpci		
***-0.8flt1*:RFP$^+$**	32.8	
***tcf21*:mCherry$^+$**	27.2	

Table S4

List of ECM genes downregulated in *Tg(hsp70l:sflt4)* ventricles at 24 hpci
prelp
frem1a
fn1b
htra3a
emilin2a
fgl2b
igfbp1b