Supplemental Material

Different DOACs control inflammation in cardiac ischemia-reperfusion differently

Ihsan Gadi[1]*, Sameen Fatima[1]*, Ahmed Elwakiel[1], Sumra Nazir[1], Moh'd Mohanad Al-Dabet[1,2], Rajiv Rana[1], Fabian Bock[3], Jaykumar Manhoran[1], Dheerendra Gupta[1], Ronald Biemann[1], Bernhard Nieswand[4], Ruediger Braun-Dullaeus[5], Christian Besler[6], Markus Scholz[7], Robert Geffers[8], John H. Griffin[9], Charles T. Esmon[10], Shrey Kohli[1], Berend Isermann[1#], Khurrum Shahzad[1#]

Running headline: Differential effects of DOACs in myocardial IRI

[1] Institute of Laboratory Medicine, Clinical Chemistry and Molecular Diagnostic, University Hospital, Leipzig, 04103 Leipzig, Germany
[2] Department of Medical Laboratories, Faculty of Health Sciences, American University of Madaba (AUM), Amman 11821, Jordan
[3] Department of Medicine, Vanderbilt University Medical Center, 37232 Nashville, Tennessee, United States
[4] Institute of Experimental Biomedicine, University Hospital and Rudolf Virchow Centre, University of Würzburg, 97080 Würzburg, Germany
[5] Clinics of Cardiology and Angiology, Otto-von-Guericke-University, 39120 Magdeburg, Germany
[6] Department of Cardiology, Leipzig-Heart Center, University of Leipzig, Germany
[7] Institute of Medical Informatics, Statistics and Epidemiology, University of Leipzig, Germany
[8] RG Genome Analytics, Helmholtz Center for Infection Research, 38124 Braunschweig, Germany
[9] Department of Molecular Medicine, The Scripps Research Institute, La Jolla, CA, US 92037, United States
[10] Laboratory of Coagulation Biology, Oklahoma Medical Research Foundation, 73104 Oklahoma City, United States

* Equally contributing first authorship.
# Equally contributing last authorship.

Corresponding authors: Khurrum Shahzad, PhD and Berend Isermann, MD
Institute of Laboratory Medicine, Clinical Chemistry
and Molecular Diagnostic (ILM),
Paul-List-Straße 13/15, 04103
Leipzig, Germany

Phone: 49 – (0)341 –9722200

Fax: 49 – (0)341 –9722209

e-mail: khurrum.shahzad@medizin.uni-leipzig.de and berend.isermann@medizin.uni-leipzig.de

Detailed Material and Methods:

Reagents

Antibodies and reagents used in the current study were as follows: rabbit anti-NF-κB p65 (8242s), rabbit anti-phospho-IκBα (Ser32/36; 9246s), rabbit anti-IκBα (4812s), rabbit anti-NLRP3 (12446), rabbit anti-DNMT1 (5032s), rabbit anti- VE-Cadherin (2500), rabbit anti-SIRT1 (8469), rabbit anti-H3K9me3 (13969), rabbit anti-caspase-1 (detecting pro and cleaved caspase-1; 24232), rabbit anti IL-1β (detecting pro and cleaved IL-1β; 12242), anti-rabbit IgG horseradish peroxidase-conjgated (HRP, 7074) linked antibody, anti-mouse IgG horseradish peroxidase-conjugated (7076), (Cell Signaling Technology, Germany), rat anti- monocyte + macrophage antibody (MOMA-2, ab33451, abcam, Germany), rabbit anti-GAPDH (G9545), 2,3,5-triphenyltetrazolium chloride (TTC) and Masson's trichrome staining kit (Sigma-Aldrich, Germany), anti-rabbit IgG (H+L) highly cross-adsorbed secondary antibody, Alexa Fluor Plus 647 (A32733) and anti-rabbit IgG (H+L), superclonal™ recombinant secondary antibody, Alexa Fluor 488 (A27034) (Invitrogen; Germany). Other reagents used in the current study are as follows: protease inhibitor cocktail (Roche Diagnostics, Germany), BCA reagent (Prebioscience, Germany); PVDF membrane Immobilon western chemiluminescent HRP substrate (Merck, Millipore, United States), mouse IL-1β and mouse PF4 ELISA kits (R & D Germany), mouse TAT ELISA kit (Novus Biologicals, LLC, USA), mouse IL-18 ELISA kit (Medical and Biological Laboratories Co. Ltd.), PBS (Life Technologies, Germany), powdered milk, albumin fraction V, hematoxylin Gill II, acrylamide, agarose (Carl ROTH, Germany), aqueous mounting medium (ZYTOMED, Germany), RevertAid™ H Minus First Strand cDNA Synthesis kit (Invitrogen, Germany), SYBR Green (Takyon, Germany), Rompun 2 % (Bayer, Germany), and ketamine 10 % (Beta-Pharm, Germany).

Tail bleeding time

Twelve hours after the last anticoagulant treatment, the mice were anesthetized, and the tail tip was amputated at a given diameter with a scalpel, as described. Bleeding time was determined using a timer. Tail bleeding was monitored by gentle absorption of the blood with filter paper (Whatman paper, No. 1001-185) at 20 sec intervals without making direct contact with the wound. When no blood was observed on the paper, the bleeding was determined to have ceased; otherwise, experiments were stopped after 30 min. None of the control or treated mice showed rebleeding once bleeding had ceased. Procedure and analyses were done by two independent blinded investigators.

***In vivo* thrombus formation**

The mice were pretreated as outlined above (*in vivo* interventions). To enable subsequent visualization of platelets, the mice were intravenously injected with GP1bβ-488-conjugated fluorophore antibodies (0.1 µg/g body weight, X488, Emfret Analytics). After 1 h, the mice were anesthetized with sodium ketamine (100 mg/kg body weight, i.p.) and xylazine (10 mg/kg body weight, i.p.). A midline abdominal incision was made, the mesentery was exteriorized, and arterioles free of fat tissue were visualized using a microscope. Arterioles were injured by topical application of filter paper saturated with 20 % $FeCl_3$ for 10 sec. Thrombus formation in arterioles was monitored for 40 min or until complete occlusion (defined as a stop in blood flow for > 1 min). Digital images were recorded with a Nikon eclipse intravital microscope and analyzed off-line with NIS Elements software (Nikon). Procedure and analyses were done by an independent blinded investigator.

Myocardial ischemia reperfusion injury

Myocardial ischemia reperfusion injury (IRI) was conducted as previously described.[27] Mice were anesthetized with sodium ketamine (100 mg/kg body weight, i.p.) and xylazine (10 mg/kg body weight, i.p.). After endotracheal intubation, ventilation was performed using 0.3 l/min oxygen and 1.5% isoflurane. The mice were placed on a 37°C thermostatically controlled operating platform. The surgical procedure did not differ among the experimental groups. A left parasternal incision was made between the third and fourth ribs. The epicardium was removed to expose the heart. Blood flow in the lateral anterior descending (LAD) artery was temporarily interrupted by carefully tightening an 8-0 silk suture around the LAD. After LAD ligation, the wound was temporally closed using tape. After 90 min of ischemia, the ligation was removed to restore blood flow through the LAD artery. Recovery of blood flow was visually ensured (as indicated by the reappearance of a reddish color). The chest was closed

with continuous 4-0 polypropylene sutures. All animals received subcutaneous analgesics (buprenorphine 0.1 mg/kg) post-surgery. The mice were placed in a temperature-controlled (~35°C) environment during the recovery phase and were regularly inspected. After full recovery, the animals were returned to their cages with free access to food and water. No differences in the recovery times between the different experimental groups were noticed. Sham surgery consisted of an identical procedure without LAD ligation. After 24 h (for short-term model) or 28 days (for long-term model) of reperfusion, the animals were sacrificed. Blood samples were obtained from the inferior vena cava, and the heart was isolated for further analyses. Different mice were used for infarct size determination and protein expression. To determine the animals group size, a priori power calculations were done using G*Power software (3.1.9.7 version). Briefly, first an appropriate test was chosen (here: ANOVA) and mean infarct size obtained from pilot data and the number of groups were entered. Based on an alpha value of 0.05 and a power of 0.80, group sizes were calculated. Surgical procedures were done by an independent blinded investigator.

Determination of infarct size

After 24 h of reperfusion, the animals were sacrificed, the hearts were removed, and the part of the heart from the tip to the point where the ligature was set was cut into 5 slices of approximately equal thickness.[27] The heart slices were placed in tubes containing 1% triphenyltetrazolium chloride (TTC) solution at 37 °C for 5 min. The slices were then fixed in 10 % formalin solution and incubated for 10 min at room temperature. Photos of heart slices were taken using a digital camera (Nikon D750 KIT2), and the infarct area was determined using ImageJ software.[27]

RNA-seq, functional annotation and pathway analysis

RNA was extracted from heart tissues (comprising the infarcted and surrounding tissue) using an RNeasy mini kit (QIAGEN, Germany), and RNA concentration was measured using a nanodrop (2000C, Peq lab, Germany). The quality and integrity of RNA were controlled with an Agilent Technologies 2100 Bioanalyzer (Agilent Technologies, Waldbronn, Germany). Expression profiling was performed using RNA sequencing (RNA-seq). RNA sequencing library was generated from 500 ng total RNA using Dynabeads® mRNA DIRECT(tm) Micro Purification Kit (Thermo Fisher) for mRNA purification followed by NEBNext® Ultra(tm) II Directional RNA Library Prep Kit (New England BioLabs) according to manufacturer's protocols. The libraries were sequenced on Illumina NovaSeq 6000 using NovaSeq 6000 S2 Reagent Kit (100 cycles, paired end run) with an average of 3×10^7 reads per RNA sample. A quality report is generated by the FASTQC (version 0.11.8) tool for each FASTQ file. Before alignment to the reference genome, each sequence in the raw FASTQ files was trimmed on base call quality and sequencing adapter contamination using the Trim Galore! wrapper tool (version 0.4.4). Reads shorter than 20 bp were removed from the FASTQ file. Trimmed reads were aligned to the reference genome using the open source short read aligner STAR (version 2.5.2b, https://code.google.com/p/rna-star/) with settings according to the log file. Feature counts were determined using the R package Rsubread (version 1.32.4). Only genes showing counts greater than 5 at least two times across all samples were considered for further analysis (data cleansing). Gene annotation was performed using the R package bioMaRt (version 2.38.0). Before starting the statistical analysis steps, expression data were log2 transformed and normalized according to the 50th percentile (quartile normalization using edgeR, version 3.24.3). Differential gene expression was calculated by the R package edgeR. Statistically significant DEGs (p<0.05 and FDR <0.05) were sorted and categorized after correcting for multiple hypothesis testing by Benjamini-Hochberg method. The threshold to identify differentially expressed genes (DEGs) was set to a logFc value of ± 0.58 (IRI group), resulting in a 1.5-fold expression change.[72-74] To identify genes differentially regulated by either treatment (fIIai or fXai), DEGs between IRI (control) group and either treatment group were identified based on a minimum logFc difference value of ±0.58. Heatmapper (http://www.heatmapper.ca/) was used to generate heatmaps of gene expression data.[71] Genes shown in the heatmap were sorted based on DEGs in the control group (myocardial IRI without anticoagulant treatment) based on their logFc values. For representation purpose, no clustering method was applied and z-score was used.[71] Venny (version 2.1), an online interactive tool was used for comparison and identification of overlapping DEGs between different groups.[75]

Significance of overlaps was determined by hypergeometric testing and disparity of genes regulated specifically by fIIai or fXai was determined by exact binomial test, as indicated in the figure legend. Gene ontology was performed using online tool, Database for Annotation, Visualization and Integrated Discovery (DAVID) version 6.8 bioinformatics package and Benjamini Hochberg adjustment was applied to all enriched p-values to control for multiple testing. All RNAseq analyses were done by an independent blinded investigator.

Masson's trichrome staining

At the end of the study period (28 days; long-term model), the animals were sacrificed, and the hearts were perfused with PBS (5 ml) and quick frozen (on dry ice). Heart samples were then stored at -80°C before analysis. Cryosections were used for Masson's trichrome staining (Sigma-Aldrich, Germany) according to the manufacturer's instructions. Cryopreserved sections (4 µm thickness) were postfixed in preheated Bouin's solution (explain) at 56°C for 15 min, Weigert's iron hematoxylin solution for 5 min, Biebrich scarlet acid/fuchsin solution for 5 min, 5% phosphotungstic/phosphomolybdic acid for 5 min, aniline blue solution for 20 min and 1% acetic acid for 2 min. Between every staining step, the tissue sections were washed with distilled water. For histological analysis, images were captured with an Olympus Bx43 microscope (Olympus, Hamburg, Germany), and ImageJ software was used for image analysis. All histological analyses were done by an independent blinded investigator.

Immunofluorescence staining

For immunofluorescence staining, frozen sections of hearts including infarcted area were fixed in ice-cold acetone for 8 min, washed twice with ice-cold PBS and incubated in 2% BSA in PBS-Tween (PBST) for 1 h. Sections were then incubated for overnight at 4°C with primary antibodies against MOMA-2 (1:50) or VE-Cadherin (1:50). Sections incubated without primary or secondary antibodies were used as negative controls and for background correction. After overnight incubation the sections were washed three times with 1 × PBS 5 min each time followed by incubation with fluorescently labelled corresponding secondary antibodies (anti-rabbit IgG H+L highly cross-adsorbed secondary antibody, Alexa Fluor Plus 647; 1:300 and anti-rabbit IgG H+L, superclonal™ recombinant secondary antibody, Alexa Fluor 488; 1:300). After washing, nuclear counterstaining was conducted using mounting medium with DAPI. Images were visualized, captured, and analysed using a fluorescence microscope (Olympus Bx43-Microscope, Olympus, Hamburg, Germany) using the same settings in experimental and control groups. Analyses were performed by two independent blinded investigators. Image J software was used for image analysis.

Transmission electron microscopy

Transmission electron microscopy (TEM) was performed at the Institute of Anatomy, University Leipzig, Germany. Hearts tissues were fixed with 2.5 % glutaraldehyde, 2.5 % polyvidone 25, 0.1 M sodium cacodylate pH 7.4. After washing with 0.1 M sodium cacodylate buffer (pH 7.4), samples were post-fixed in the same buffer containing 2 % osmium tetroxide and 1.5 % potassium ferrocyanide for 1 h, washed in water, contrasted en bloc with uranyl acetate, dehydrated using an ascending series of ethanol and embedded in Durcupan ACM-based resin. Ultrathin sections were cut with a Reichert Ultracut S ultramicrotome (Science Service, Munich, Germany), contrasted with lead citrate. Images were viewed and captured with an EM 10 CR electron microscope (Carl Zeiss Gemini, Germany). Analyses were performed by an independent blinded investigator.

Immunoblotting

Proteins were isolated, and immunoblotting was performed as described.[27,44] Cell lysates were prepared in RIPA buffer (50 mM Tris at pH 7.4, 1% Nonidet P-40, 0.25 % sodium deoxycholate, 150 mM NaCl, 1 mM EDTA, 1 mM Na_3VO_4, and 1 mM NaF supplemented with a protease inhibitor cocktail). Lysates were centrifuged (10.000 × g, 10 min at 4°C), and insoluble debris was discarded. The protein concentration in supernatants was quantified using BCA reagent. Equal amounts of protein were electrophoretically separated on 7.5 %, 10 % (vol/vol), or 12.5 % (vol/vol) SDS polyacrylamide gels, as appropriate, transferred to PVDF membranes, and probed with the desired primary antibodies overnight at 4°C. Membranes were then washed with PBS-Tween (PBST) and incubated with anti-

mouse IgG, anti-rat IgG, or anti-rabbit IgG (each 1:4,000) horseradish peroxidase-conjugated antibodies, as indicated. Blots were developed with an enhanced chemiluminescence system. To compare and quantify the levels of proteins, the density of each band was measured using ImageJ. Equal loading was confirmed by immunoblotting with a GAPDH antibody.

Reverse transcriptase polymerase chain reaction (RT-PCR) and quantitative PCR (qPCR)
RNA was extracted from heart tissues (comprising the infarcted and surrounding tissue) using the RNeasy Mini Kit (QIAGEN, Germany)[27], and RNA concentration was measured with a nanodrop (2000 C, Peq lab, Germany). A 1.8 % agarose gel was run to verify the purity and integrity of the RNA. cDNA was synthesized according to the manufacturer's protocol (SuperScript First-Strand Synthesis System for RT-PCR, Fermentas, Germany). Primers were custom synthesized by Thermo Fisher Scientific, and quantitative PCR (qPCR) for IL-6, TNF-α, MIF, and MCP-1 was performed in triplicate using SYBR Green (Takyon qRT-PCR (CFX 96 TouchTM, CFX 96 Touch deep well™ Bio-Rad, Germany). The qPCR reaction mix and thermocycle conditions used for gene amplification are listed in Online Table I and Table II. Relative gene expression was normalized to GAPDH and compared using the ΔΔC*t* method. Reactions lacking reverse transcriptase served as negative controls. Primers used in the current study are listen in the Online Table III.

***In vivo* protein C generation assay**
In vivo activated protein C (aPC) generation was determined as previously described.[44] Mice were intravenously injected with 20 μg of human protein C (PC) and 50 mU of human α-thrombin. Blood samples were collected after 10 min from the vena cava into 0.38 % sodium citrate and 50 mM benzamidine HCl (final concentration). Human PC/APC was captured from plasma samples using an antibody specific for human PC/aPC (HAPC 1555), and the activity of the captured human PC was determined using a chromogenic substrate (PCa).

IL-1β and IL-18 immunoassay
Mouse blood samples were obtained from the inferior vena cava in 0.38 % sodium citrate. Plasma was obtained by centrifugation of blood samples for 10 min at 2000 g and RT. Plasma samples were stored at -80°C until analyses. We measured the concentrations of mouse IL-1β (R & D system) and mouse IL-18 (Medical & Biological Laboratories Co., Ltd) by ELISA, according to the manufacturer's instructions.[27]

Analyses of blood coagulation
Thrombin-antithrombin complex (TAT) and platelet activation factor 4 (PF4) were determined using commercially available ELISA systems that recognized mouse TAT and mouse PF4, respectively (Novus Biologicals, LLC, USA and R & D system, USA). Prothrombin time (PT) was determined using RecombiPlasTin 2G (Instrumentation Laboratory), anti-fXa activity in plasma samples was determined using a commercially available on-step chromogenic assay (HemosIL Anti-Xa Testkit, Instrumentation Laboratory), and anti-fIIa activity was measured using the HEMOCLOT THROMBIN INHIBITORS (Hyphen BioMed, Germany) testkit using a routine coagulation analyzer (ACL TOP 750, Werfen, Vienna, Austria).

Online Table I. Composition of qPCR reaction mixture.

qPCR Reagent	Volume per reaction
SYBR Green Mix	10uL
Primers (5uM/primer)	2.4uL
gDNA (20ng/ul)	0.5uL
Water	7.1uL
Total volume	20uL

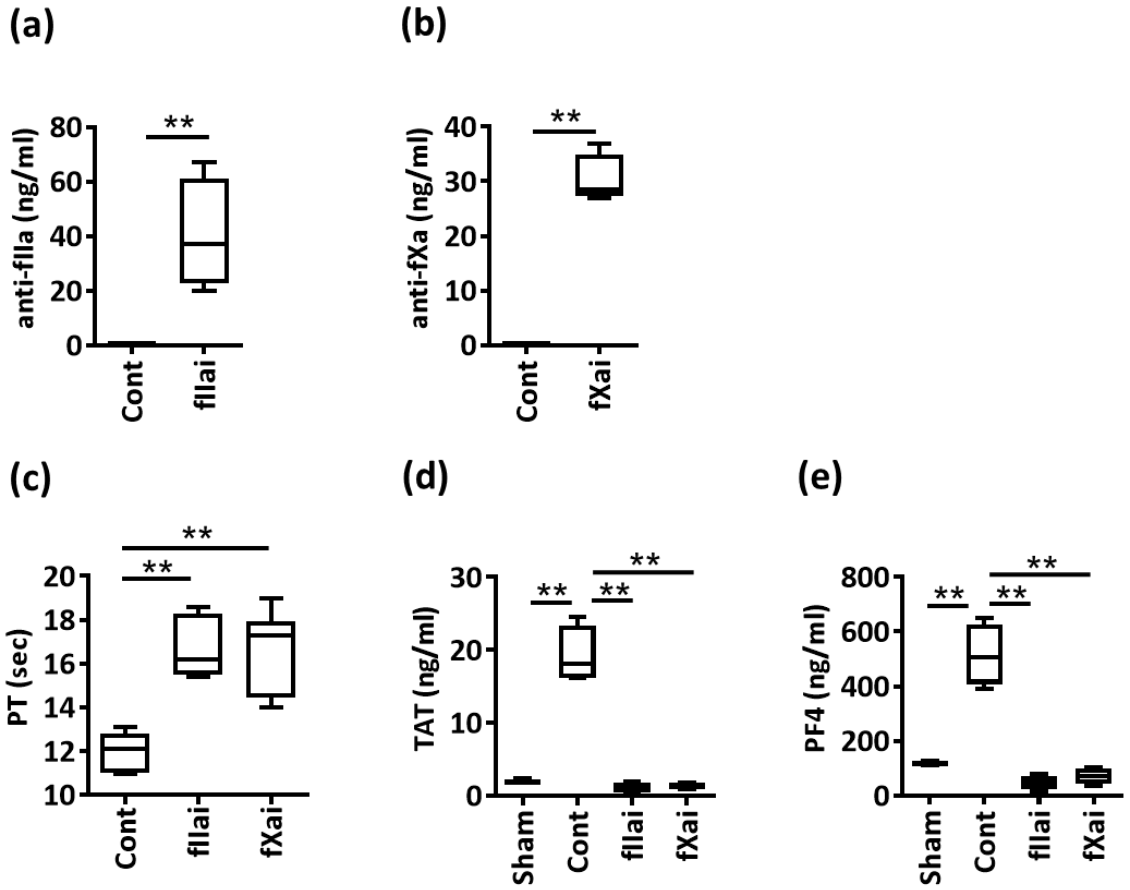
Online Table II. qPCR amplification conditions.

Stage	Temperature (°C)	Time
Initial denaturation	95	10 minutes
40 cycles	95	15 seconds
	60	60 seconds
Final extension	72	05 minutes

Online Table III. List of primers used in current study.

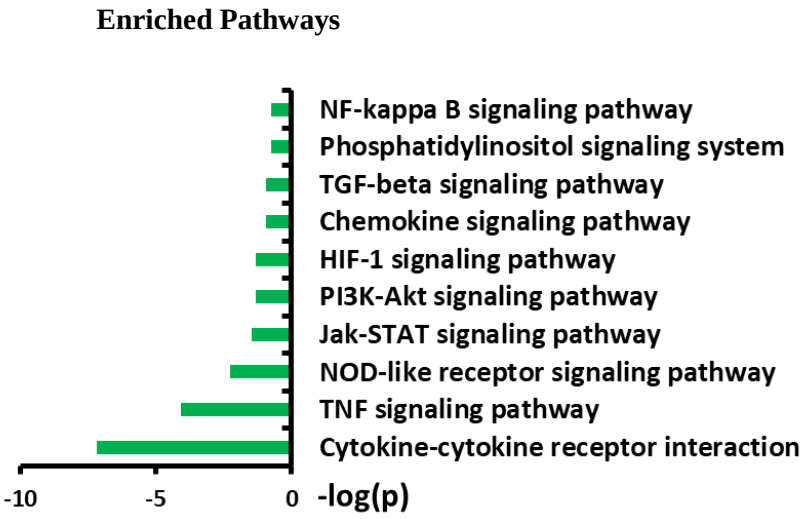
Target	Primer Sequence
IL-6	5'GTCCTTCCTACCCCAATTTCCA 3' 5'TAACGCACTAGGTTTGCCGA 3'
TNF-α	5'ACAGAAAGCATGATCCGCGA3' 5'TCCACTTGGTGGTTTGCTACG3'
MCP1	5'GCTGTAGTTTTTGTCACCAAGC3' 5'AAGGCATCACAGTCCGAGTC3'
MIF	5'GACTTTTAGCGGCACGAACG 3' 5'AAGAACAGCGGTGCAGGTAA 3'
β-actin	5'CTAGACTTCGAGCAGGAGATGG3' 5'GCTAGGAGCCAGAGCAGTAATC3'
IL-1β	5'TGCCACCTTTTGACAGTGATG3' 5'AAGGTCCACGGGAAAGACAC3'
Collagen 1α	5'GGGGCAAGACAGTCATCGAA3' 5'GAGGGAACCAGATTGGGGTG3'
Collagen 3α1	5'ACGTAAGCACTGGTGGACAG3' 5'GGAGGGCCATAGCTGAACTG3'
α-SMA	5'GCCGAGATCTCACCGACTAC3' 5'ATAGGTGGTTTCGTGGATGC3‘
Prkcg	5'ATATTACAATGTACCGGTGGCCGA3' 5'GCATCGCTTGGAGTCCGTA3'
Pik3r5	5'TCACCCCAACTGCTGAGAGTC3' 5'GTCCATGCAAGCATCGCTCC3'
Ucp3	5'GACCCACGGCCTTCTACAAA3' 5'TCAAAACGGAGATTCCCGCA3'

Online Fig. I



Online Fig. I (corresponding to Fig. 1): Characterisation of anticoagulation by fXai and fIIai.
a, b: Anti-fIIa (a) and anti-fXa (b) activity in plasma of mice fed with chow diet containing fIIai or fXai, respectively, compared with untreated control mice.
c-e: Prothrombin time (PT) and thrombin anti-thrombin complex (TAT) and platelet factor 4 (PF4) levels in plasma samples were comparable in fIIai and fXai treated mice; box-plots summarizing data (a, b: n=6; c–e: n=7; for each group). Mice without (Cont) or with fXai- or fIIai-treatment; **P<0.01 (ANOVA).

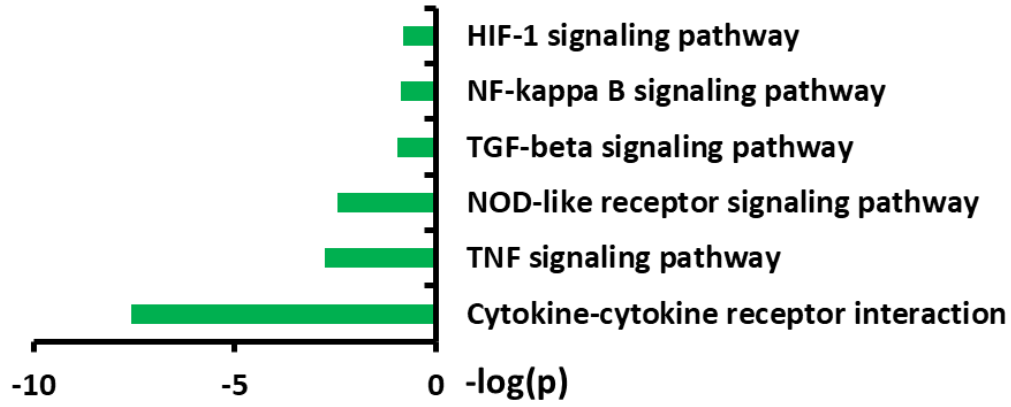
Online Fig. II



Online Fig. II (corresponding to Fig. 2): Pathway analyses
Top enriched pathways (KEGG analysis) for gene expression changes seen in myocardial IRI hearts of control mice (without treatment) compared to sham operated mice. The Log_{10} of p values ($p<0.05$) obtained after correcting for multiple testing (Benjamini-Hochberg correction) is shown.

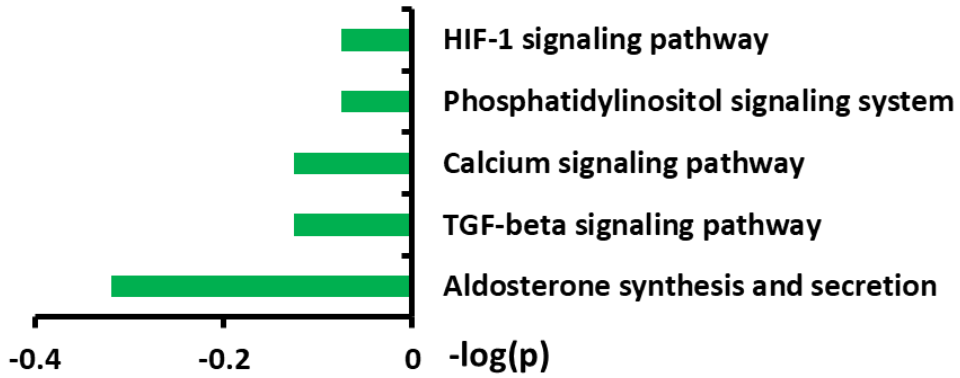
Online Fig. III

Enriched pathways targeted by fXai



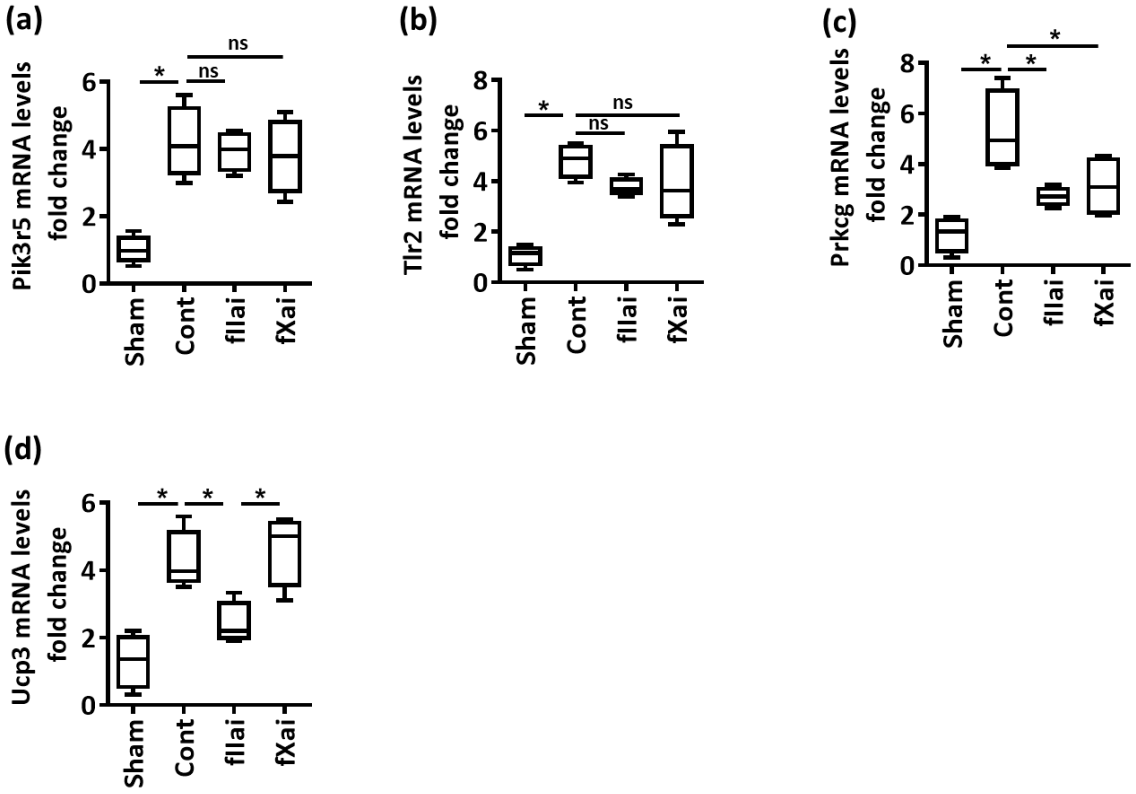
Online Fig. III (corresponding to Fig. 2): Pathway analyses
Top enriched pathways (KEGG analysis) for gene expression changes seen in myocardial IRI hearts of control mice (without treatment) and of fXai-treated mice. The Log_{10} of p values ($p<0.05$) obtained after correcting for multiple testing (Benjamini-Hochberg correction) is shown.

Online Fig. IV
Enriched pathways targeted by fIIai



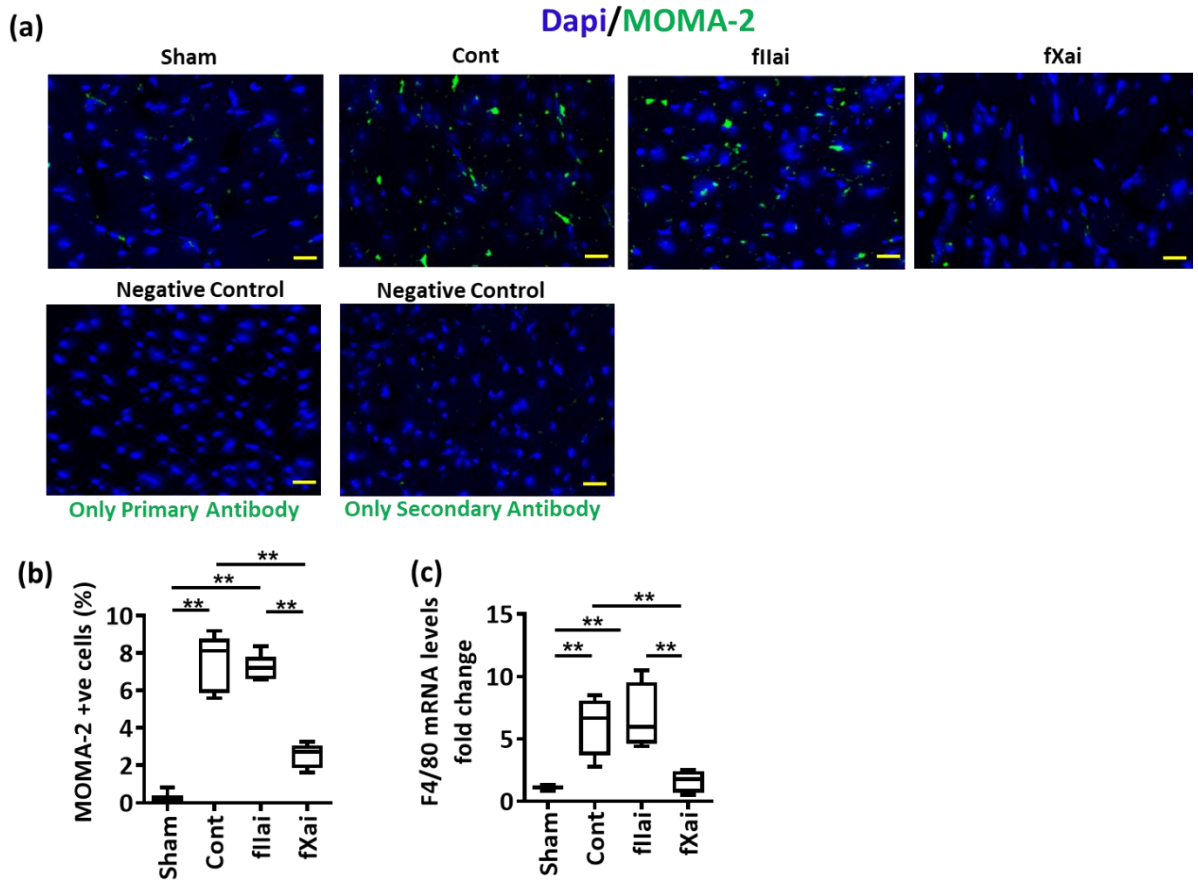
Online Fig. IV (corresponding to Fig. 2): Pathway analyses
Top enriched pathways (KEGG analysis) for the gene expression changes seen in myocardial IRI hearts of control mice (without treatment) and of fIIai-treated mice. The Log_{10} of p values ($p<0.05$) obtained after correcting for multiple testing (Benjamini-Hochberg correction) is shown.

Online Fig. V



Online Fig. V (corresponding to Fig. 2): fIIai- and fXai-dependent gene expression in myocardial tissue post IRI.
mRNA expressions (qRT-PCR) of phosphoinositide-3-kinase regulatory subunit 5 (Pik3r5, a), Toll like receptor-2 (Tlr2, b), protein kinase C gamma (prkcg, c), and mitochondrial uncoupling protein 3 (Ucp3, d). Box-plots summarizing data of qRT-PCR; GAPDH was used for normalization.
Sham operated mice (Sham), mice without (Cont) or with fXai- or fIIai-pretreatment. a-d: n=6 for each group; *P<0.05 (ANOVA).

Online Fig. VI

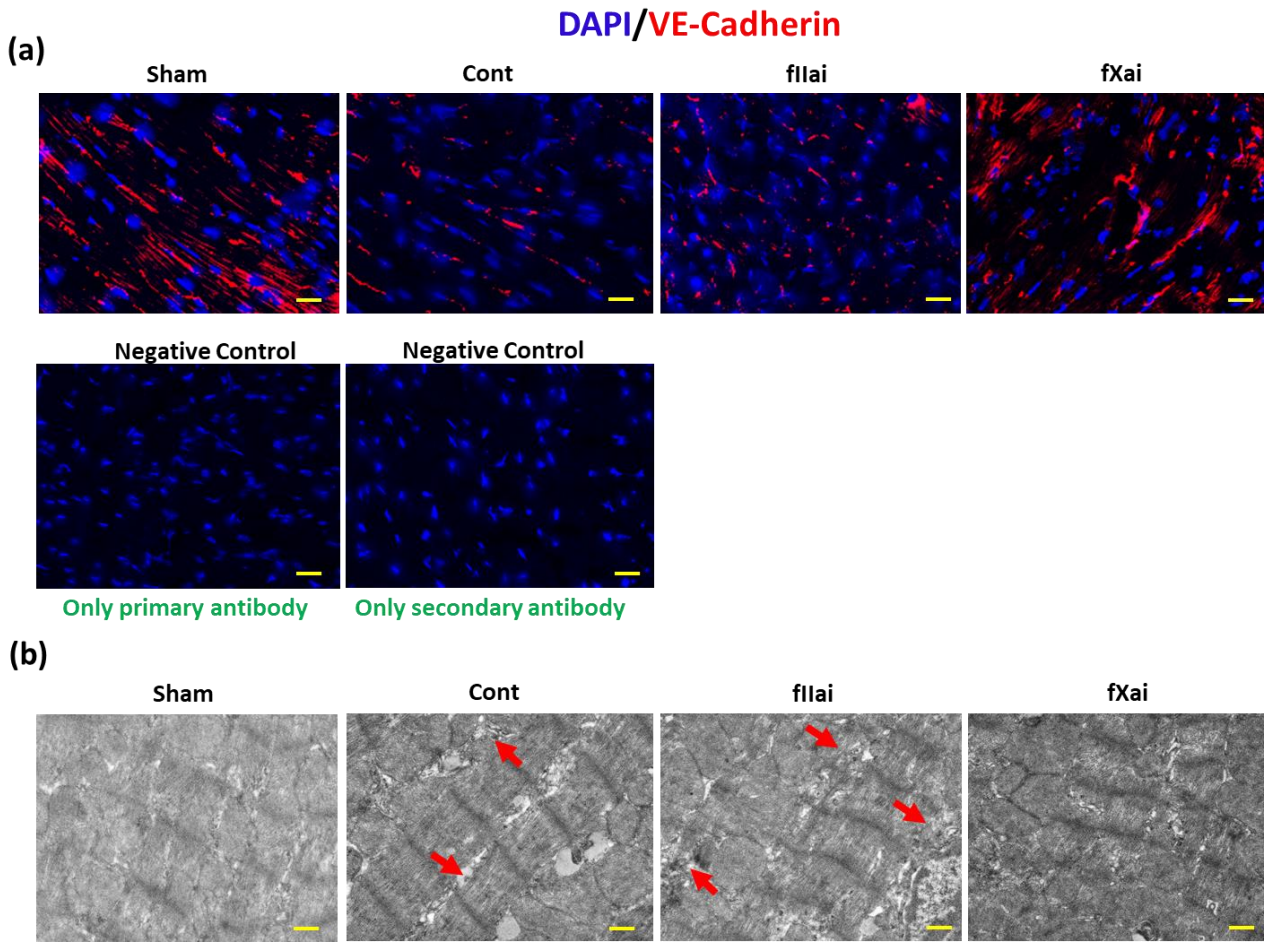


Online Fig. VI: fXai but not fIIai treatment reduces immune cell infiltration following myocardial IRI.

Representative immunofluorescence images showing cardiac infiltration by monocyte/macrophage (green: MOMA-2 positive cells; blue: DAPI nuclear counterstain; size bar, 20 µm). Box-plots summarizing data of MOMA-2 positive cells (b) and mRNA expressions (c; qRT-PCR) of F4/80. Heart sections incubated only with primary or only with secondary antibody served as negative controls for MOMA-2 immunofluorescence staining. GAPDH was used for normalization.

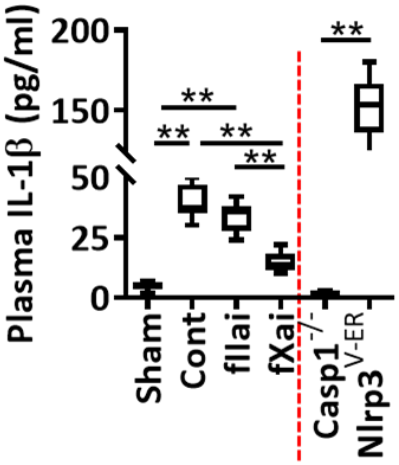
Sham operated mice (Sham), mice without (Cont) or with fXai- or fIIai-pretreatment. b-c: n=6 for each group), **P<0.01 (ANOVA).

Online Fig. VII



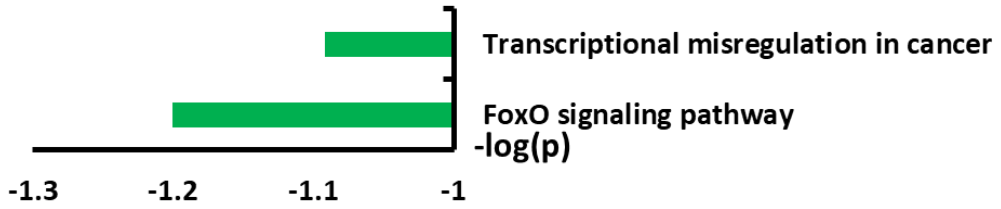
Online Fig. VII: fXai maintains endothelial barrier following myocardial IRI.
Representative immunofluorescence images for VE-Cadherin immunostaining in heart sections from fIIai-and fXai-treated mice (**a;** scale bar: 20 µm). Heart sections incubated only with primary or only with secondary antibody served as negative controls for VE-Cadherin immunofluorescence staining. Myocardial ultrastructure changes following reperfusion determined by electron microscopy (**b**); red arrows indicate interstitial edema formation; scale bar: 0.5 µm.

Online Fig. VIII



Online Fig. VIII: fIIai prevents inflammasome activation in infarcted myocardial tissue.
Box-plot summarizing data of plasma IL-1β levels following myocardial IRI in mice without (Cont) or with fXai or with fIIai pretreatment. $Casp1^{-/-}$ and $Nlrp3^{V\text{-}ER}$ mice were used as negative or positive controls, respectively. n: 6 mice per group; **P<0.01 (ANOVA).

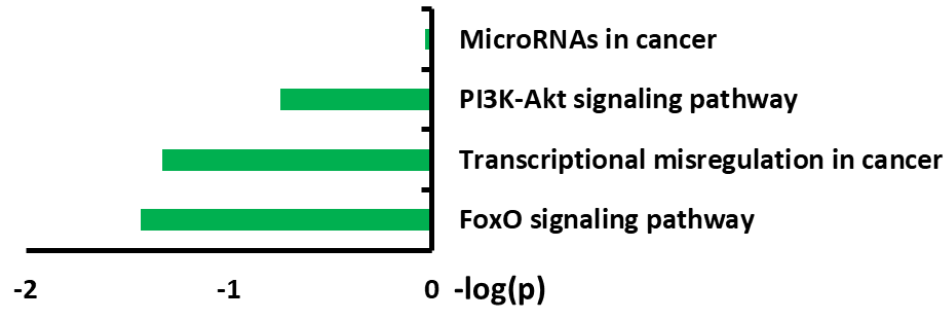
Online Fig. IX
Enriched pathways regulated by fIIai in resting mice



Online Fig. IX (corresponding to Fig. 4): Pathway analyses
Top enriched pathways (KEGG analysis) for the gene expression changes seen in myocardial hearts of control mice (without treatment) and of fIIai-treated mice. The Log_{10} of p values ($p<0.05$) obtained after correcting for multiple testing (Benjamini-Hochberg correction) is shown.

Online Fig. X

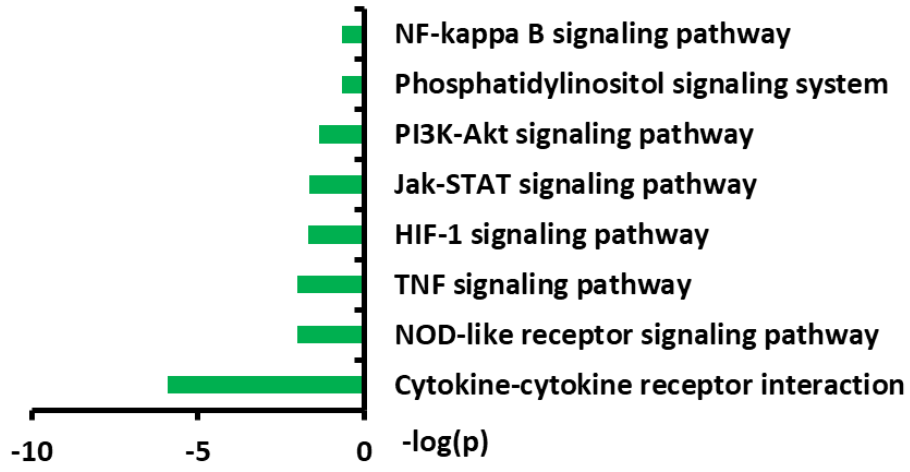
Enriched pathways regulated by fXai in resting mice



Online Fig. X (corresponding to Fig. 4): Pathway analyses
Top enriched pathways (KEGG analysis) for the gene expression changes seen in myocardial hearts of control mice (without treatment) and of fXai-treated mice. The Log_{10} of p values ($p<0.05$) obtained after correcting for multiple testing (Benjamini-Hochberg correction) is shown.

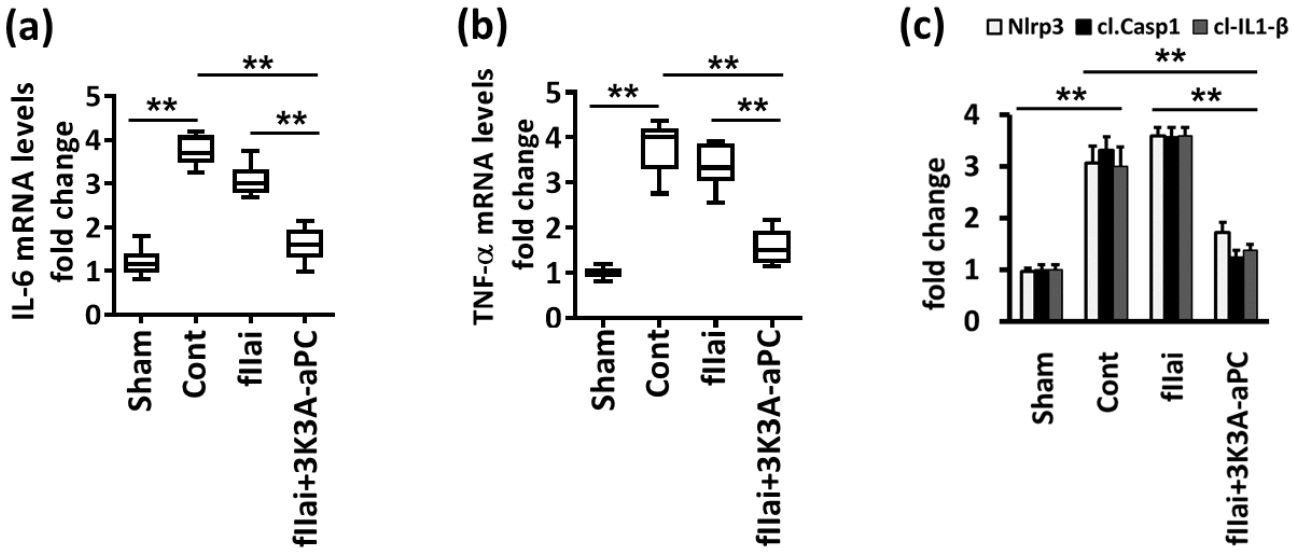
Online Fig. XI

Enriched pathways targeted by aPC



Online Fig. XI (corresponding to Fig. 5): Pathway analyses
Top enriched pathways (KEGG analysis) for the gene expression changes seen in myocardial IRI hearts of control mice (without treatment) and of aPC treated mice. The Log_{10} of p values ($p<0.05$) obtained after correcting for multiple testing (Benjamini-Hochberg correction) is shown.

Online Fig. XII

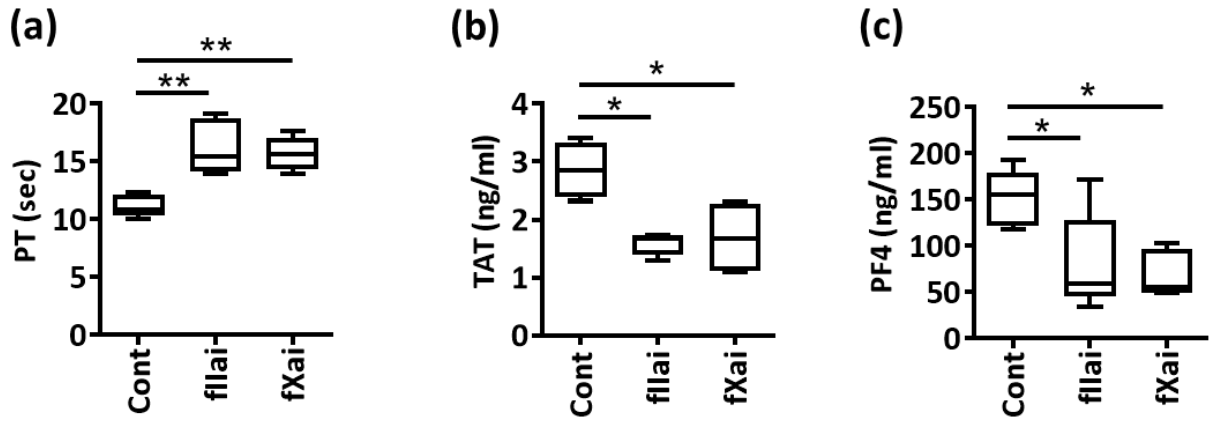


Online Fig. XII: Concomitant treatment of fIIai and 3K3A-aPC reduces the myocardial inflammatory response (corresponding to Fig. 5).

mRNA expression of pro-inflammatory cytokines IL-6 and TNF-α following myocardial IRI is reduced in mice concomitantly treated with fIIai and 3K3A-aPC; box-plots summarizing data of qRT-PCR; β-actin was used for normalization (**a, b**). Bar graphs corresponding to Fig. 5f (**c**). Bar graphs represent mean±SEM of NLRP3 (open bars) and cleaved caspase- 1 (cl-Casp1; black bars) and cleaved IL-1β (cl-IL-1β, grey bars).

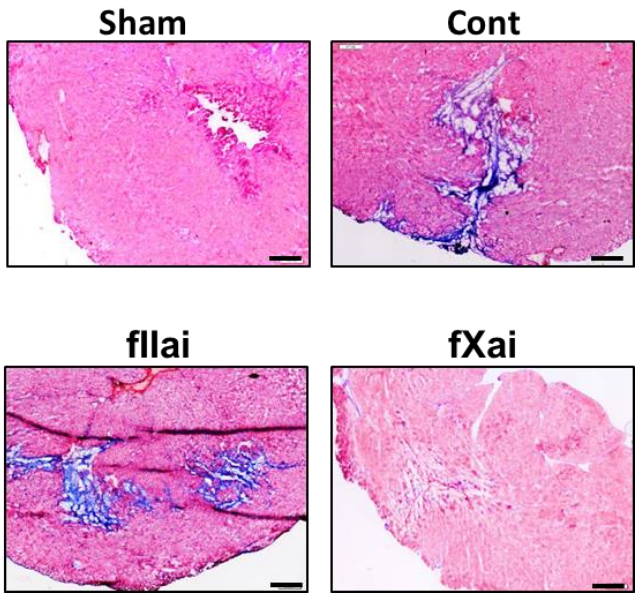
Mice without (Cont) or with fIIai pretreatment alone (fIIai) or 3K3A-aPC on the top fIIai pretreatment (fIIai+3K3A-aPC). a, b: n=6; c: n=7 for each group); ***P*<0.01 (ANOVA).

Online Fig. XIII



Online Fig. XIII (corresponding to Fig. 8): Effect of anticoagulation by fXai and fIIai during the 28-day period post myocardial IRI.
Prothrombin time (PT) and thrombin anti-thrombin complex (TAT) and platelet factor 4 (PF4) levels in plasma samples in fIIai and fXai treated mice compared to non-treated controls (Cont) 28-days post myocardial IRI; boxplot summarizing data (a-c: n= 6 for each group); *P<0.05, **P<0.01 (ANOVA).

Online Fig. XIV



Online Fig. XIV (corresponding to Fig. 8): fXai, but not fIIai, ameliorates cardiac fibrosis following myocardial IRI.
Representative overview images of Masson's trichrome stained mouse heart sections 4 weeks after myocardial IRI; size bar: 10 µm. Mice without (Cont) or with fIIai or fXai pretreatment.