SUPPLEMENTAL MATERIALS

Manuscript title: Homophilic interaction between Transmembrane-JAM-A and soluble JAM-A regulates thrombo-inflammation: Implications for Coronary Artery Disease

Author's names: Dominik Rath[a] M.D, Vera Rapp[a] M.D, Jessica Schwartz[a] M.D, Stefan Winter[b] Ph.D, Frederic Emschermann[a] M.Sc, Daniel Arnold[c] M.Sc, Johannes Rheinlaender[c] Ph.D, Manuela Büttcher[a] Vet. Med., Michael Strebl[d] M.Sc, Michael B. Braun[d] M.Sc, Konstanze Altgelt[a] M.D, Álvaro Petersen Uribe[a] M.D, Christoph Schories[a] Cand. Med., Denis Canjuga[a] M.Sc, Elke Schaeffeler[b] Ph.D, Oliver Borst[a,e] M.D, Tilman E. Schäffer[c] Ph.D, Harald Langer[a] M.D, Thilo Stehle[d] Ph.D, Matthias Schwab[b,f,g] M.D, Tobias Geisler[a] M.D, Meinrad Gawaz[a] M.D, Madhumita Chatterjee[a] Ph.D

Author's affiliation: [a]Department of Cardiology and Angiology, University Hospital Tübingen, [b]Dr. Margarete Fischer-Bosch Institute of Clinical Pharmacology, Stuttgart, and University of Tübingen, [c]Institute of Applied Physics, [d]Interfaculty Institute of Biochemistry, [e]DFG Heisenberg Group Thrombocardiology, Department of Cardiology and Angiology, University Hospital Tübingen, [f]Department of Pharmacy and Biochemistry, University of Tübingen, [g]Department of Clinical Pharmacology, University Hospital Tübingen, Germany.

Address for correspondence: Madhumita Chatterjee, Dominik Rath (responsible for clinical data), Department of Cardiology and Angiology, University Hospital Tübingen. Address: Otfried Müller Straße 10, 72076, Tübingen; Germany. Phone: +49-7071-2982888, Fax: +49-7071-294474, Email: madhumita.chatterjee@med.uni-tuebingen.de; dominik.rath@med.uni-tuebingen.de

Running title: Pro-thrombotic and thrombo-inflammatory JAM-A may affect prognosis in CAD

1 **Materials and methods:**

Reagents, antibodies	Manufacturer	Experimental purpose
Mouse anti-human JAM-A-FITC	Biolegend	Surface association of JAM-A on platelets by flowcytometry
Goat polyclonal anti-human JAM-A unconjugated antibody	R&D systems	Blocking transmembrane-JAM-A
Goat control IgG	Santa Cruz Biotechnologies	Control IgG
Human JAM-A ELISA kit	Cloud clone Inc.	Serum sJAM-A estimation
L-selectin Duo set ELISA kit	R&D systems	Serum sL-selectin estimation
P-selectin Duo set ELISA kit	R&D systems	Serum sP-selectin estimation
Legendplex human proinflammatory chemokine panel	Biolegend	Cytometric bead array (CBA) for chemokines
Legendplex human inflammation panel-1	Biolegend	CBA for cytokines
Anti-human CD42b-PE	BD Biosciences	Platelet marker, flowcytometry
Anti-human CD42b-FITC	Beckman Coulter	Platelet marker, flowcytometry
PAC-1-FITC	BD Biosciences	GPIIbIIIa activation, flowcytometry
Anti-human CD62P-FITC	Beckman Coulter	Degranulation, flowcytometry
GPIbβ-X-488 antibody	Emfret analytics	*In vivo* platelet labelling for intravital microscopy (IVM)
Rabbit-anti-mouse Alexa Fluor-647 secondary antibody	Invitrogen	Immunofluorescence confocal microscopy
Donkey anti-goat Alexa Fluor-568 secondary antibody	Invitrogen	Immunofluorescence confocal microscopy
Trypan blue	Sigma Aldrich	Surface fluorescence quenching
PP2	Sigma Aldrich	Src inhibitor
Ly29004	Cell signaling	PI3K inhibitor
SH-6	Cell signaling	Akt inhibitor
Gö6976	Cayman chemicals	PKC inhibitor
Antibodies against phosphorylated Src, PKC, PI3K, Akt	Cell Signaling	Phosphoblots, immunoblots
Anti-β3-integrin Y^{733} phosphorylated	Abcam	Phosphoblot, immunoblot
Anti-α-tubulin, anti-β-actin, anti-GAPDH antibodies	Cell Signaling	Loading control for Immunoblots
Protease-phosphatase inhibitor cocktail	Cell Signaling	Component of lysis buffer
Fibrinogen	Merck Millipore	Coating adhesion surface
Bovine serum albumin fraction V molecular grade	Sigma Aldrich	Control coating substance and buffer component
Horm Collagen	Takeda Austria GmbH	Coating pro-thrombotic surface
Reagents for thromboelastography	Haemonetics	Clot formation
Reagents for thrombinoscopy	Stago	Thrombin generation
Anti-human CD14-APC,	R&D systems	Monocyte marker, surface expression by flowcytometry

Anti-CD86-FITC, Anti-CD209-PE	R&D systems	Macrophage markers, surface expression by flowcytometry
Anti-CD11c-APC, Anti-CD11b-FITC	Biolegend	Monocyte/macrophage marker, surface expression by flowcytometry
Anti-CD36 FITC	Beckman Coulter	Surface expression by flowcytometry
Anti-CD68-FITC	Dako	Surface expression by flowcytometry
Annexin V-FITC	Immunotools	PS exposure
Tetramethylrhodamine ethyl ester (TMRE)	Invitrogen	Mitochondrial transmembrane potential loss ($\Delta\psi m$)
RPMI 1640 medium	Gibco	Platelet-monocyte co-incubation or co-culture
Fetal bovine serum	Fischer Scientific	Platelet-monocyte co-incubation or co-culture
Penicillin-Streptomycin solution	Sigma-Aldrich	Platelet-monocyte co-incubation or co-culture
L-Glutamine	Sigma-Aldrich	Platelet-monocyte co-incubation or co-culture
Trypsin	Gibco	Harvest of monocytes
Ficoll	Merk	Differential gradient centrifugation for PBMC isolation
0.3µmand 1µm beads	Thermo Fisher	Gating strategy for pMPs

Animal experimentation: Platelet-megakaryocyte lineage specific *F11R* deficient *Pf4-Cre^{+}jama$^{fl/fl}$* and *Pf4-Cre^{-}jama$^{fl/fl}$* littermates (C57BL/6J-Tg(Pf4-cre)^{Q3Rsko}/J x C57BL/6J-F11r^{tm1Dej}) were used for *ex vivo* thrombus formation assay. C57BL/6 mice (The Jackson Laboratories, Bar Harbor, Maine, USA) were used to explore arterial (carotid) thrombosis *in vivo*, by intravital microscopy (IVM). 7-10 weeks old mice of either sex were used in the study. Randomization was done for the treatments specified and the experimenter was blinded to the treatments during the course of the experiments until final data analysis and statistical evaluation. For surgical procedures mice were anesthetized by injection of midazolam (5 mg/kg body weight), medetomidine (0.5 mg/kg body weight) and fentanyl (0.05 mg/kg body weight). Blood collection from retro-orbital plexus of mice was done under Isoflurane. All animal experimentations were conducted according to German law for the welfare of animals, ARRIVE guidelines and approved by local authorities (M18/14).

Platelet surface-associated JAM-A by flowcytometry: Blood from CAD patients were analyzed gating for the platelet-specific marker CD42b(GPIbα). Blood collected in CPDA was diluted 1:50 with PBS and incubated with anti-human-JAM-A-FITC and anti-human CD42b/GPIbα-PE. Samples were incubated for 30 minutes at room temperature in the dark, fixed with 0.5% paraformaldehyde and analyzed by flowcytometry (FACS Calibur, BD Biosciences)(1). Threshold cytometer settings were ascertained using respective isotype controls.

To detect surface association of JAM-A following *in vitro* activation, platelets in platelet-rich plasma (PRP) from healthy donors was used. CPDA-anticoagulated blood was centrifuged at 190xg for 20 min to collect PRP. PRP platelets (10^6/sample) were stimulated with ADP (5, 25,100μM), CRP (5μg/mL), and TRAP (5, 10, 25μM) for 30 minutes at room temperature in the presence of anti-human-JAM-A-FITC, parallel to anti-human-CD62P-FITC as platelet activation marker, fixed in 0.5% paraformaldehyde and analyzed by flowcytometry (1).

Platelet derived microparticle (pMP) surface-associated JAM-A by flowcytometry: Washed platelets were either kept under resting condition or treated with 0.1U/ml of Thrombin (Roche) and incubated at 37ºC under constant shaking at 800 r.p.m. for 30 min in presence of anti-human-JAM-A/JAM-1/F11R-FITC and platelet specific marker anti-human CD41-BV421. At the end of incubation period samples were fixed in 0.5% paraformaldehyde, 0.3μm and 1μm latex beads were added to the samples and acquired by flowcytometry (BD FACSLyric). Surface associated JAM-A on platelets and pMPs were evaluated by gating for platelets specific marker CD41 (**Fig. 1h**).

Platelet isolation: Washed platelets were isolated from healthy subjects as previously described, and used for Western Blot analysis, platelet spreading and adhesion experiments (2). Briefly, blood was collected in acid-citrate-dextrose (ACD)-buffer and centrifuged at 190xg for 20 min. Platelet-rich plasma (PRP) thus obtained was added to Tyrodes-HEPES buffer (HEPES-2.5mM; NaCl-150mM; KCl-1mM; $NaHCO_3$-2.5mM; NaH_2PO_4-0.36mM; glucose-

5.5mM; BSA-1mg/ml; pH 6.5) and centrifuged at 800xg for 10 min. The platelet pellet was suspended in PBS (supplemented with $CaCl_2$, $MgCl_2$).

Immunoblotting: Detection of JAM-A in platelets, sJAM-A shed into the activated platelet supernatant (APS) was performed by Western blot analysis (2). Washed human platelets were kept under resting condition or treated with ADP-(100µM), or TRAP-(25µM). Supernatants from activated platelets were collected after 60 mins by centrifugation, and the platelet pellet was lysed at 4°C for 5 min with lysis buffer (155mM NaCl+15mM Tris+1mM EDTA+0.005 % NaN3+1 % NP-40+protease inhibitor cocktail), to perform simultaneous Western blot analysis of platelets lysates and activated platelet supernatant (APS). Samples were prepared with reducing Laemmli buffer with 10% β-mercaptoethanol, denatured at 95°C for 5 min, separated on an SDS-polyacrylamide gel and transferred onto polyvinylidene fluoride membrane (Millipore) using a SemiDry Transfer Cell System (Peqlab). An equal volume of APS were loaded per well for each experimental setting (resting, ADP/TRAP-activated) (3). For the platelet lysates, equal amounts of protein were loaded, and α-tubulin was used as loading control. Subsequently, the membrane was blocked using 5% powdered skimmed milk in PBST (PBS with 0.1 % Tween 20) and incubated with primary anti-JAM-A and α-tubulin for overnight at 4°C.

To explore the impact of sJAM-A on activatory signaling cascade, washed platelets were either kept under resting condition or treated with full-length sJAM-A-D1D2 (30µg/mL) for 5, 15, 30 minutes, lysed and processed as mentioned. Following immunoblot, membranes were incubated with respective primary antibodies against phospho-Src (1:1000), phospho-PKC (1:200), phospho-PI3K (1:500), phospho-Akt (1:200), phospho-β_3-integrin (1:500), α-tubulin (1:1000), GAPDH (1:500), β-actin (1:500) for overnight at 4°C. For all immunoblot detection, corresponding secondary fluorochrome-labelled antibodies and the Odyssey infrared imaging system (LI-COR, Bad Homburg, Germany) were used (2,3).

Generation of soluble human (sJAMA-D1, sJAM-AD1D2) and murine smJAM-A(D1D2) proteins: Generation of soluble human (sJAMA-D1, sJAM-A-D1D2) and murine smJAM-A(D1D2) proteins:

The recombinant JAM-A proteins were produced as described in detail and published previously(4) The extracellular domains D1 (residues 27-129) or D1D2 (27-233) of JAM-A were cloned into a pGEX-4T-3 expression vector, containing an N-terminal GST affinity tag and a Thrombin cleavage site. The recombinant protein was produced in BL21(DE3) *E. coli* cells after induction with 0.2 mM IPTG at 20°C for 16 h. Cells were harvested by centrifugation, resuspended in lysis buffer (50 mM TRIs pH 7.8, 3 mM EDTA, 2 mM β-mercaptoethanol, 1 mM PMSF, 1 % Triton X-100) and lyzed by sonication. GST-JAM-A was purified from the cleared lysate by glutathione affinity chromatography using a 5 ml GSTrap HP column (Cytiva). The GST-tag was removed by proteolytic cleavage on the column with Thrombin at 20°C overnight. For final polishing, a size exclusion chromatography was performed using a buffer containing 10 mM HEPES pH 7.5 and 150 mM NaCl with a Superdex 200 Increase 10/300 column (Cytiva).

Degranulation (CD62P surface expression), $\alpha_{IIb}\beta_3$-integrin activation (PAC-1 binding): PRP platelets(10^6/sample) from healthy subjects (n=5) were either kept under resting condition or treated with sJAM-A (30µg/mL sJAM-AD1D2 or 10µg/mL sJAM-AD1) for 30 minutes at room temperature in the presence of anti-human CD62P-FITC or PAC-1-FITC, and subsequently incubated with platelet agonists ADP (10µM), TRAP (25µM) for 30 minutes at room temperature. Kinase inhibitors Ly29004 (PI3K-25µM), SH-6 (Akt-20µM), PP2 (Src-10µM), PKC inhibitor (Gö6976-1µM) and respective vehicle controls, anti-JAM-A-antibody (10µg/mL) or control IgG (10µg/mL) were given as a pretreatment for 15 minutes before sJAM-A. Samples were fixed in 0.5% paraformaldehyde and analyzed by flowcytometry (1).

Light transmission aggregometry: PRP platelets (200×10^3 platelets/µL) were either kept untreated or pre-treated with sJAM-D1 (10µg/ml), sJAMA-D1D2 (30µg/ml) for 30 minutes at

room temperature. Aggregation was carried out in response to ADP (10µM) for 8-10 min at 37°C under stirring condition using AGGROLINK software (ChronoLog) (5).

Impedance aggregometry: 600µL blood (diluted 1:1 in PBS supplemented with $CaCl_2$+$MgCl_2$) from healthy subjects acquired in hirudinized tubes (Sarstedt) was used. Whole blood was kept untreated or pre-treated with sJAM-A (30µg/mL sJAM-AD1D2, or 10µg/mL sJAM-AD1) for 30 minutes at room temperature. Aggregation was initiated by adding TRAP (10µM). Where specified, blood was incubated with anti-JAM-A-antibody (10µg/mL) or control IgG (10µg/mL) for 15 minutes before adding sJAM-AD1D2 or sJAM-AD1 (6).

Platelet spreading on fibrinogen: Washed platelets isolated from healthy subjects were treated with 30µg/mL sJAM-AD1D2 or 10µg/mL sJAM-AD1 for 30 minutes at room temperature and then adhered over fibrinogen (100µg/mL) coated surface. After 15 and 30 minutes, non-adherent platelets were washed off with PBS and samples were fixed with 1% paraformaldehyde and observed under the microscope for platelets at difference stages of adhesion employing Axiovert 200, Carl Zeiss microscope. Images were acquired with AxioVision software (Carl Zeiss) and analyzed for fully spread (with lamellipodia), spread, dendritic (with filopodia) and round (resting) platelets.

Live imaging of platelet spreading by Scanning Ion Conductance Microscopy (SICM): SICM topography images of spreading platelets were recorded with a custom-built SICM[9]. Platelets were isolated from human blood as described previously and incubated with sJAM-AD1D2 (30µg/mL) for 30 min; thereafter allowed to adhere to culture dishes (CELLSTAR 627160, Greiner Bio-One, Kremsmünster, Austria) coated overnight with fibrinogen (100µg/mL). After initial contact with the culture dish, platelet spreading was imaged with typical 20s per frame until no further increase in spreading area was observed. Typical image resolution was 80x80 pixels within 10x10µm^2 scan range. Spreading rate for each platelet was derived using a custom-written analysis in Igor Pro (Wavemetrics, Lake Oswego, Oregon). Briefly, the topography images were binarized with a height threshold of 50nm. Spreading was

quantified by the final spreading area, spreading duration until 90% of the final spreading area, and initial spreading rate normalized by final spreading area determined by a linear fit up to 60% of the relative area (7,8).

***Ex vivo* thrombus formation in flow chamber assay:** Blood from healthy donors (n=5) was collected in CPDA anticoagulant. Heparinized murine blood was collected from the retro-orbital plexus of *F11R* deficient *PF4-Cre*$^{+}$*-Jama*$^{fl/fl}$ mice or wild type littermates *PF4-Cre*$^{-}$ *Jama*$^{fl/fl}$ (n=6 mice/group) and diluted 1:3 in modified Tyrode buffer supplemented with 2mM $CaCl_2$. Blood samples were kept untreated or treated with sJAM-A (30µg/mL sJAM-AD1D2, 10µg/mL sJAM-AD1 or 10µg/mL murine smJAM-A) for 30 min at room temperature and perfused through a transparent flow chamber (slit depth 50µm) over collagen (100µg/mL)-coated surface at 1700 $s^{-}1$ (human), or 1000 s^{-1} (murine) shear rates employing Axiovert 200, Carl Zeiss microscope, optical objective $20x^2$. Where specified, human blood samples were pre-incubated with anti-JAM-A-antibody (10µg/mL) or respective IgG control (10µg/mL) for 15 minutes before adding sJAM-AD1D2 or sJAM-AD1. Thrombus area was analyzed offline using the captured images with AxioVision software (Carl Zeiss) (5).

Arterial thrombosis *in vivo* and intravital microscopy (IVM): 7-12 weeks old C57Bl6 mice (n=5 mice/group) were administered with soluble murine smJAM-A (50µg/mouse i.v.) or BSA control 30 min prior to surgical procedure under anesthesia. Circulating platelets were stained *in vivo* with GPIbβ-X488-(0.1µg/gm b.wt) administered i.v. *In vivo* thrombus formation in the carotid artery was induced by topical application of 10% $FeCl_3$ for 1 minute and monitored by IVM for 20 minutes or until complete occlusion (blood flow stopped for > 1 min) with NIS-Elements (Nikon) Microscope using a 10x objective. Digital images/video were recorded and analyzed off-line (1,2).

Confocal microscopic analysis of platelet spread on collagen and immobilized sJAM-A: Washed platelets were adhered over immobilized s-JAM-AD1D2 (30µg/mL) or s-JAM-AD1 (10µg/mL), or collagen (100µg/mL)-coated surface. After 1hr, non-adherent platelets were

washed off with PBS and samples were fixed with 1% paraformaldehyde, permeabilized with 0.3% Triton-X-100 and stained first for actin with Phalloidin-Alexa Fluor-488 (1:400) for 2hrs at room temperature. Then the samples were washed with washing buffer (PBS+0.3% Triton-X-100+0.1% Tween-20) and stained with mouse anti-human α-tubulin (1:200) for overnight at 4ºC, and counter-stained with secondary rabbit anti-mouse Alexa Fluor-647 (1:200) for 2hrs at room temperature. Samples were washed and mounted with anti-fade mounting medium (Dako). Images were acquired by confocal microscopy as mentioned (2).

Platelet adhesion over immobilized sJAM-A: 5-(and-6)-Carboxyfluorescein Diacetate fluorescently labelled washed human platelets were adhered over s-JAM-A- (sJAM-AD1D2-30μg/mL or sJAM-AD1-10μg/mL) coated surface (coated overnight at 4ºC), collagen-(as positive control 100μg/mL) or BSA-(1% solution as negative control) coated surface. Platelets were pre-treated with blocking antibody against JAM-A (10μg/mL) or control IgG (10μg/mL) for 30 minutes at room temperature where specified. After 1hr of adhesion, non-adherent platelets were washed off with PBS, and fluorescence signal from adherent platelets was captured with a fluorescence plate reader (GloMax™-Promega) (9).

Immunofluorescence confocal microscopic analysis of JAM-A expression on inflamed endothelial cells: Confluent monolayer of HUVEC were activated with TNFα (50ng/mL) +IFNγ (20ng/mL), fixed in 2% paraformaldehyde, permeabilized in 0.3% Triton-X-100, blocked with 1% BSA and stained overnight with primary anti-JAM-A (1:250) at 4ºC. Samples were washed with washing buffer (PBS+0.3% Triton-X-100+0.1% Tween-20), and counter-stained with anti-goat Alexa Fluor-568 conjugated secondary antibody-(1:200) for 2hrs at room temperature. After that, samples were thoroughly washed, mounted with fluorescent mounting medium (Dako) and analyzed using Zeiss LSM 510 Meta, Axioplan 2 Imaging Confocal Laser Scanning Microscope with a 100x ocular at lower (1x) and higher magnifications (digital zoom 2x).

Platelet adhesion on activated endothelium: Confluent monolayer of HUVECs were treated with TNFα (50ng/mL) +IFNγ (20ng/mL) for 4hrs at 37°C, 5% CO_2, humidified atmosphere to trigger JAM-A surface (apical) exposure. Platelets were treated with sJAM-AD1D2 (30µg/mL). Where specified, anti-JAM-A-antibody (10µg/mL) or respective control IgG (10µg/mL) was employed as pre-treatment for 15 minutes before sJAM-AD1D2. Thereafter platelets were perfused over HUVECs at arterial shear rates (2000 s^{-1}). Experiments were recorded in real-time and evaluated off-line (3).

Thrombin generation: Thrombin generation in response to tissue factor in platelet-rich plasma (PRP) from 4 healthy subjects was measured by calibrated automated thrombinoscopy. Platelet count of PRP was adjusted with platelet poor plasma (PPP) to a final count of $150x10^3$/µl. PRP samples were pre-treated with full-length sJAM-AD1D2 (30µg/mL) for 30 minutes at room temperature and added to tissue factor (5pM) filled wells. By adding the fluorescent thrombin substrate (Z-Gly-Gly-Arg aminomethyl coumarin, 2.5mM), coagulation reaction was started and monitored under shaking at 37°C. Calibration was done using a thrombin calibrator. Peak thrombin generation was measured using the specified Thrombinoscope software (Stago)(5,6).

Thromboelastography: TEG analysis-(CK reaction) was performed to estimate the rate of blood clot formation in presence of full-length sJAM-AD1D2 (30µg/ml) according to manufacturer's instructions (5,6).

Detection of platelet apoptosis by Annexin V exposure and mitochondrial membrane depolarization (Δψm) by TMRE: PRP platelets ($1x10^6$/sample) from healthy donors were kept under resting condition or pre-treated with sJAM-AD1D2 (30µg/mL) for 30 minutes at room temperature and subsequently incubated with TRAP (25µM) for 1 hr at room temperature, then analyzed for apoptosis by flowcytometry as described (2).

Isolation of peripheral blood monocytes: Peripheral blood monocytes were isolated using leukocyte buffy coat preparations from healthy donors, by differential centrifugation through Ficoll gradient as described previously (9).

Platelet-monocyte aggregate formation: Monocyte and platelets were co-incubated with sJAM-AD1D2 (30µg/mL) or sJAM-AD1 (10µg/mL) for 1hr at room temperature, labelled with platelet-specific CD42b-FITC and monocyte-specific CD14-APC, fixed in 1% paraformaldehyde and analyzed by flowcytometry assessing $CD14^{+}CD42b^{+}$ platelet-monocyte aggregates. Where specified, platelets were pre-incubated with anti-JAM-A-antibody (10µg/mL) or control IgG (10µg/mL) before adding sJAM-AD1D2 or sJAM-AD1(9).

Phagocytic uptake of platelets by monocytes: Washed platelets were treated with sJAM-AD1D2 (30µg/mL) or sJAM-AD1 (10µg/mL) for 1hr and labelled with CD42b-FITC, then added to monocytes, and incubated for 4hrs in RPMI-1640 medium (supplemented with 10% FCS+100U/mL penicillin+100µg/mL streptomycin+2mM L-glutamine) at 37°C, 5% CO_2, humidified atmosphere. Where specified, platelets were pre-incubated with anti-JAM-A-antibody (10µg/mL) or control IgG (10µg/mL) for 15 minutes. Samples were fixed in 1% paraformaldehyde and analyzed by flowcytometry. Trypan blue (10%) was used to quench surface fluorescence coming from adherent (but not phagocytosed) platelets. $CD42b^{+}$ monocyte population was analyzed (9).

Monocyte-platelet co-culture: Monocytes and platelets were co-cultured (in RPMI-1640 medium+10% FCS +100U/mL penicillin+ 100µg/mL streptomycin +2mM L-glutamine) at 37°C, 5%CO_2 humidified atmosphere for 10 or 15 days (for foam cells) in the presence/absence of sJAM-A-D1D2 (30µg/mL) and/or anti-JAM-A (10µg/mL) or control IgG (10µg/mL) as specified. Differentiated macrophages/foam cells (Oil-red stained) were visualized under the microscope, their numbers counted during post-acquisition image analysis using ImageJ software(9). Surface expression of CD86, CD11c, CD11b, CD209, CD68 and CD36 were evaluated by flowcytometry using respective fluorochrome conjugated antibody for each marker. Culture supernatants were evaluated for pro-inflammatory mediators using Legendplex (Biolegend) human inflammation panel 1 as per manufacturer's instructions and analyzed by flowcytometry.

References for supplementary material:

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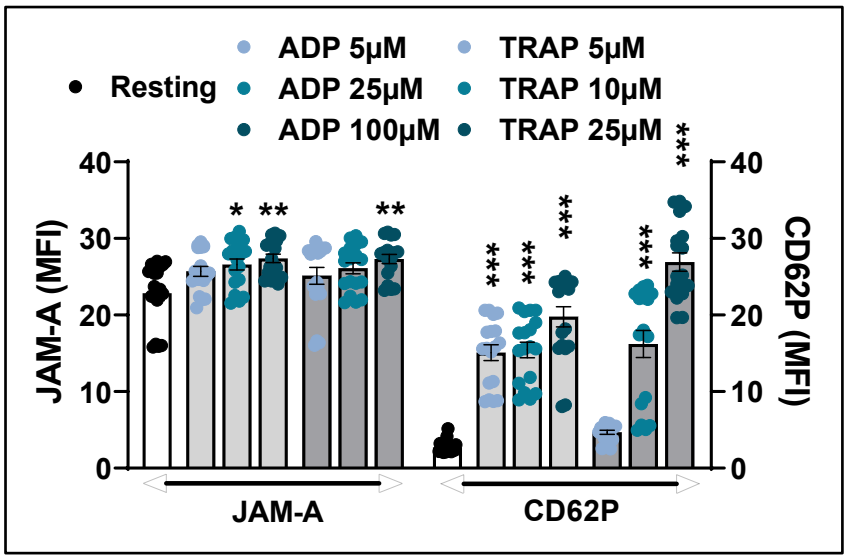
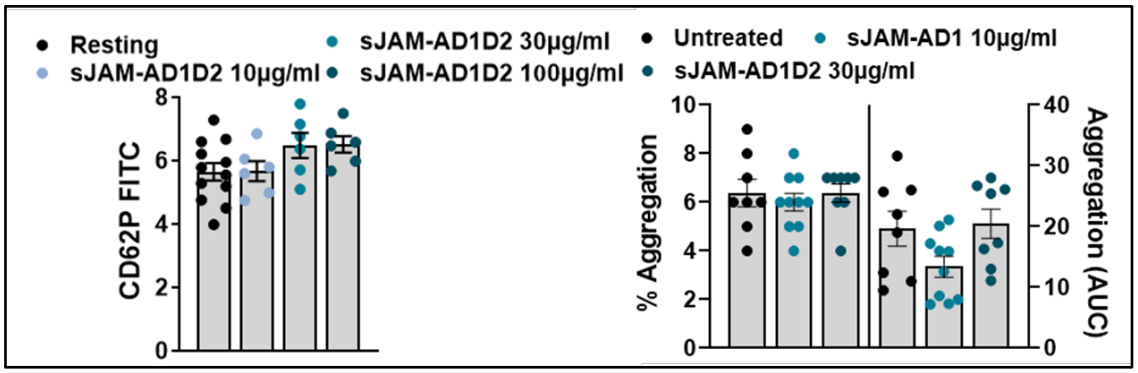
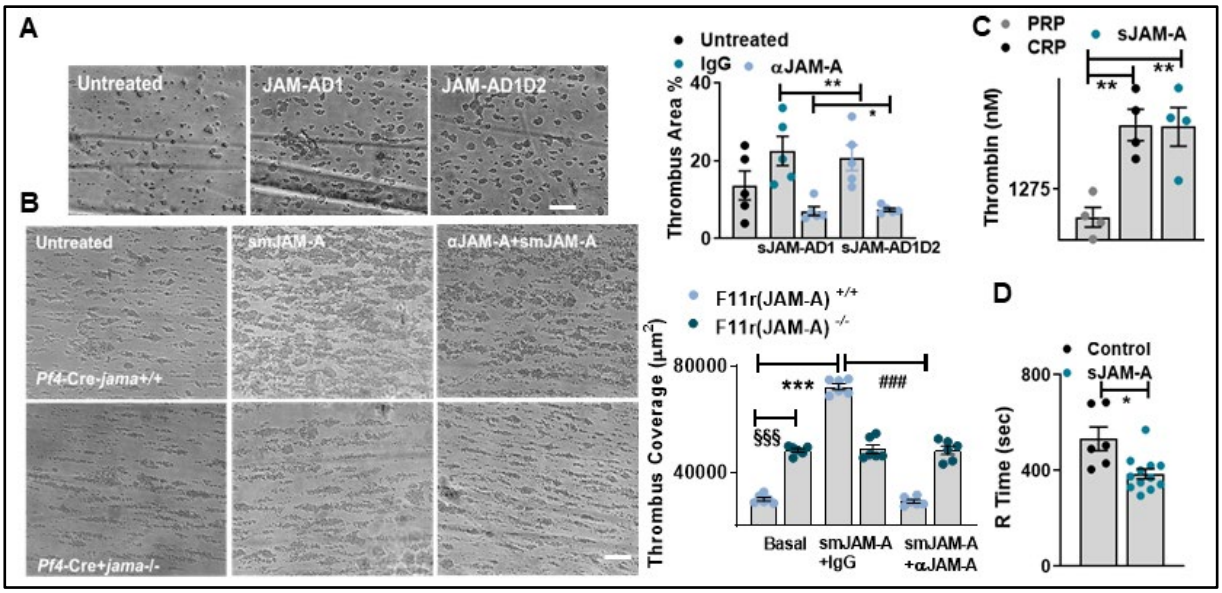
<u>Supplemental Figures 1 – 3 and figure legends</u>



Supplemental figure 1: Bar diagram showing significantly enhanced platelet membrane-associated JAM-A upon activation, as compared to platelet degranulation response denoted by CD62P surface expression. *p<0.05, **p<0.01, ***p<0.0001 vs resting platelets**.** Data are mean±SEM of 9 independent experiments with technical replicates.



Supplemental figure 2: Bar diagram showing degranulation (CD62P surface expression on platelets) and aggregatory response of platelets in response to sJAM-A in absence of additional platelet agonist. Data are mean±SEM of 5 independent experiments with technical replicates.



Supplemental figure 3: sJAM-A exerts thrombotic response in a homophilic interaction with platelet transmembrane-JAM-A. (A) Phase-contrast images of thrombus coverage (Bar=50µm), and bar diagram showing thrombus coverage ±sJAM-AD1D2-(30µg/mL) or sJAM-AD1-(10µg/mL) and ±anti-JAM-A-antibody(αJAM-A) (10µg/mL) or IgG control-(10µg/mL). *p<0.05,**p<0.01. **(B)** Phase-contrast images of thrombus coverage (Bar=50µm),

while blood±smJAM-A-(10µg/mL) from *Pf4-Cre*$^{+}$*Jama*$^{-/-}$ mice and control (*Pf4-Cre*$^{-}$*Jama*$^{fl/fl}$) littermates (n=6 mice/group) were perfused over collagen *ex vivo*. ***p<0.001;###p<0.001;§§§p<0.001. Data are mean±SEM. **(C)** Thrombin generation (n=4) in response to tissue factor ±sJAM-AD1D2-(30µg/mL) and CRP-(5µg/mL). **p<0.01.**(D)** Thromboelastographic analysis of clot formation (n=3 donors) in presence and absence of sJAM-AD1D2-(30µg/mL). Data are mean±SEM.

Supplemental tables: 1-3

Supplemental table 1: Results of multivariable Cox PH regression analyses as well as Fine and Gray's 'proportional sub-distribution hazards' regression model for myocardial infarction (MI) with different treatments at hospital discharge as covariates.

	Cox PH model		Fine-Gray model	
Variable	**HR (95% CI)**	**p**	**HR (95% CI)**	**p**
CABG/PCI	3.98 (0.51-31.10)	0.188	3.04 (0.32-29.00)	0.330
No intervention/PCI	1.68 (0.63-4.49)	0.304	1.70 (0.71-4.07)	0.240
ASA (yes/no)	1.24 (0.34-4.57)	0.745	1.25 (0.38-4.19)	0.710
Clopidogrel (yes/no P2Y12 inhibitor)	2.66 (0.81-8.73)	0.107	2.60 (0.88-7.72)	0.085
Prasugrel or ticagrelor (yes/no P2Y12 inhibitor)	3.22 (0.94-10.99)	0.062	3.18 (1.00-10.08)	0.050
Simvastatin (yes/no statin)	1.20 (0.53-2.72)	0.663	1.26 (0.56-2.84)	0.580

Atorvastatin (yes/no statin)	1.59 (0.53-4.74)	0.409	1.74 (0.59-5.15)	0.320
Other statins* (yes/no statin)	0.64 (0.18-2.26)	0.489	0.68 (0.20-2.34)	0.540
***F11R*-SNP-rs2774276** (recessive genetic model)	2.27 (1.28-4.02)	Unadjusted-**0.005** Holm-adjusted-**0.020**	2.36 (1.30-4.28)	Unadjusted-**0.005** Holm-adjusted-**0.018**
Variable	**HR (95% CI)**	**p**	**HR (95% CI)**	**p**
CABG/PCI	3.86 (0.49-30.16)	0.198	2.93 (0.31-28.03)	0.350
No intervention/PCI	1.62 (0.60-4.35)	0.343	1.64 (0.67-3.97)	0.280
ASA (yes/no)	1.18 (0.32-4.39)	0.808	1.19 (0.35-4.00)	0.780
Clopidogrel (yes/no P2Y12 inhibitor)	2.63 (0.80-8.65)	0.111	2.58 (0.86-7.68)	0.090
Prasugrel or ticagrelor (yes/no P2Y12 inhibitor)	3.27 (0.96-11.15)	0.058	3.23 (1.02-10.26)	**0.047**
Simvastatin (yes/no statin)	1.21 (0.53-2.75)	0.646	1.27 (0.57-2.87)	0.560
Atorvastatin (yes/no statin)	1.50 (0.50-4.49)	0.470	1.64 (0.55-4.92)	0.380
Other statins* (yes/no statin)	0.65 (0.18-2.29)	0.502	0.69 (0.20-2.40)	0.560
***F11R*-SNP- rs790056** (recessive genetic model)	2.15 (1.11-4.18)	Unadjusted-**0.023** Holm-adjusted-0.069	2.23 (1.13-4.39)	Unadjusted-**0.021** Holm-adjusted-0.063

Variable	HR (95% CI)	p	HR (95% CI)	p
Intervention (yes/no)†	0.82 (0.27-2.48)	0.721	0.79 (0.25-2.44)	0.680
ASA (yes/no)	5.11 (0.69-37.66)	0.109	5.08 (0.70-34.64)	0.110
Clopidogrel (yes/no P2Y12 inhibitor)	0.63 (0.17-2.30)	0.486	0.67 (0.18-2.47)	0.540
Prasugrel or ticagrelor (yes/no P2Y12 inhibitor)	0.65 (0.17-2.46)	0.522	0.68 (0.18-2.62)	0.570
Simvastatin (yes/no statin)	1.83 (0.57-5.86)	0.307	1.71 (0.54-5.41)	0.360
Atorvastatin (yes/no statin)	1.38 (0.35-5.49)	0.644	1.35 (0.34-5.29)	0.670
Other statins* (yes/no statin)	0.84 (0.14-5.10)	0.846	0.80 (0.13-4.86)	0.810
sJAM-A ≤median vs >median (median =1.71ng/mL)	3.21 (1.71-6.00)	Unadjusted-**<0.001** Holm-adjusted-**0.001**	3.09 (1.65-5.78)	Unadjusted-**<0.001** Holm-adjusted-**0.002**

*Rosuvastatin, Pravastatin, Fluvastatin, Lovastatin

† Only n=2 CABG cases. CABG and PCI cases were therefore merged and compared to patients w/o intervention.

Supplemental Table 2: Correlations between serum sJAM-A levels and inflammatory mediators in CAD patients.

Inflammatory mediators or markers	Spearman correlation coefficient (rho)	p value	n= CAD patients with serum sJAM-A values
hsCRP	0.175	**0.003**	n=282 of 401

Serum sL-selectin	0.138	**0.097**	n=152 (matched with SNP cohort)
Serum sP-selectin	0.143	**0.086**	n=152 (matched with SNP cohort)
Serum MCP-1 (CCL2)	0.200	**0.007**	n=152 (matched with SNP cohort)
Serum TARC (CCL17)	0.245	**0.002**	n=152 (matched with SNP cohort)

Supplemental table 3: Baseline characteristics of non-CAD patients (sJAM-A median = 1.52ng/mL)

Baseline characteristics	**sJAM-A ≤ median (n=36)**	**sJAM-A > median (n=36)**	**p**
Age (mean ± SD)	70.0 (±14.3)	61.8 (±15.4)	**0.021**
Gender (male %)	23 (63.9 %)	28 (77.8 %)	0.195
LVEF% (mean ± SD)	48.5 (±12.6)	43.3 (±16.3)	0.172
Risk factors			
Arterial hypertension (%)	27 (75.0 %)	24 (66.7 %)	0.437
Hyperlipidemia (%)	9 (25.0 %)	8 (22.2 %)	0.781
Diabetes mellitus type II (%)	10 (27.8 %)	9 (25.0 %)	0.789
Smoking (%)	8 (22.2 %)	12 (33.3 %)	0.327
Medication on admission			
ASA (%)	13 (36.1 %)	7 (19.4 %)	0.173
Clopidogrel (%)	0 (0.0 %)	1 (2.8 %)	0.292
Prasugrel (%)	0 (0.0 %)	1 (2.8 %)	0.292
Ticagrelor (%)	0 (0.0 %)	0 (0.0 %)	-

ACE inhibitors (%)	15 (41.7 %)	13 (36.1 %)	0.853
AT-1 antagonists (%)	7 (19.4 %)	5 (13.9 %)	0.641
Calcium channel blockers (%)	12 (33.3 %)	5 (13.9 %)	0.080
Beta blockers (%)	18 (50.0 %)	20 (55.6 %)	0.361
Statins (%)	16 (44.4 %)	6 (16.7% %)	**0.019**

Supplemental video files 1-2 (video file and legends)

Supplemental movie 1 (video file): IVM analysis of thrombus formation in $FeCl_3$ injured carotid artery; mice administered with control BSA (50µg/mouse i.v.).

Supplemental movie 2 (video file): IVM analysis of thrombus formation in $FeCl_3$ injured carotid artery; mice administered with smJAM-A (50µg/mouse i.v.).