

Snake venom defensin peptide mCadam10_MYO-2 attenuates collagen-driven platelet activation and arterial thrombosis via GPVI-associated signaling

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ABSTRACT

Arterial thrombosis is a principal driver of ischemic cardiovascular and cerebrovascular disease,



yet clinical antiplatelet therapy remains constrained by hemorrhagic risk. Glycoprotein VI (GPVI), a platelet collagen receptor, offers a promising hemostasis-sparing target for antithrombotic intervention. However, peptide-based scaffolds capable of modulating GPVI-dependent platelet activation remain insufficiently explored. Given the diverse hemostasis-regulatory repertoire of snake venoms, this study identified three snake venom defensin and defensin-like peptides, mCadaml0_MYO-2 (from *Crotalus adamanteus*), DACU-1 (from *Deinagkistrodon acutus*), and DACU-2 (from *Deinagkistrodon acutus*), and further examined their antiplatelet activities. Comparative sequence and structural analyses revealed a conserved cysteine framework and a β -defensin-like scaffold. Among the three peptides, mCadaml0_MYO-2 produced the most potent inhibitory effect on collagen-induced platelet aggregation. Furthermore, mCadaml0_MYO-2 reduced platelet spreading on collagen and delayed clot retraction, indicating broad suppression of collagen-driven adhesive and contractile platelet responses. Mechanistic analyses linked these effects to attenuated GPVI-associated signaling, including altered activation-related RNA signatures, reduced collagen-induced Syk and PLC γ 2 phosphorylation, and direct binding to recombinant GPVI. Additionally, mCadaml0_MYO-2 markedly prolonged carotid artery occlusion time in an electrical injury-induced thrombosis model, but did not significantly prolong tail bleeding time or alter platelet indices under the tested conditions. Collectively, these findings show that mCadaml0_MYO-2 suppresses GPVI-associated platelet activation and thrombosis, supporting its potential as a molecular scaffold for hemostasis-sparing antithrombotic development.

Keywords: Snake Venom, Defensin-like peptide, Arterial Thrombosis, Platelet, GPVI



INTRODUCTION

Arterial thrombosis is a key pathological driver of major cardiovascular diseases (CVDs) and remains a major contributor to global mortality. Among them, ischemic heart disease (IHD) and stroke currently account for approximately 13% and 10% of global deaths, respectively, placing both conditions among the leading causes of death worldwide (World Health Organization, 2024). Excessive platelet activation underlies the initiation and propagation of arterial thrombi, establishing antiplatelet therapy as a central strategy for the prevention and treatment of arterial thrombotic disease. However, the clinical efficacy of current antiplatelet regimens is limited by adverse safety profiles, most notably bleeding complications. Evidence evaluated by the US Preventive Services Task Force indicates that aspirin, one of the most widely used antiplatelet agents, is associated with 1 to 4 excess cases of intracerebral hemorrhage (ICH) per 10000 persons annually, a low but clinically relevant risk (Guirguis-Blake et al., 2022). In addition, dual antiplatelet therapy containing aspirin has been associated with an approximately 40% higher risk of ICH than aspirin monotherapy (Ha et al., 2021). These limitations underscore the need to develop novel antiplatelet agents that preserve antithrombotic efficacy while reducing bleeding liability.

Glycoprotein VI (GPVI) has emerged as one of the most compelling platelet targets for antithrombotic intervention. GPVI is a transmembrane receptor selectively expressed by platelets and megakaryocytes (Watson et al., 2010). As the dominant platelet signaling receptor for collagen exposed after endothelial injury or atherosclerotic plaque rupture, GPVI coordinates collagen-dependent platelet activation, thrombus expansion, and thrombus stabilization under flow conditions (Gawaz et al., 2023; Watson et al., 2005). Genetic evidence



supports the therapeutic relevance of this pathway, as GPVI deficiency reduces collagen-driven thrombus formation in mice without prolonging bleeding time (Nieswandt et al., 2001). Consistently, patients with GPVI deficiency exhibit impaired platelet responses to collagen but typically present with only mild bleeding manifestations (Matus et al., 2013). This apparent dissociation between antithrombotic activity and hemostatic impairment has accelerated the development of GPVI-directed therapeutics (Li et al., 2025). Two biologic agents targeting this pathway, the humanized anti-GPVI Fab glenzocimab and the soluble GPVI-Fc fusion protein revacept, have advanced to clinical evaluation (Mazighi et al., 2024; Uphaus et al., 2022). Although early-phase studies have not shown a significant increase in bleeding-related events, their clinical efficacy remains inconclusive. Glenzocimab has primarily yielded exploratory signals of benefit, whereas revacept has generated inconsistent outcomes in phase II trials (Köhrmann et al., 2026; Mayer et al., 2021). These findings support GPVI as a hemostasis-sparing antithrombotic target while emphasizing the need for new GPVI-directed molecules with greater functional potency and more consistent therapeutic efficacy.

Animal toxins are highly specialized and evolutionarily shaped molecular effectors optimized to modulate conserved physiological pathways, providing valuable templates for therapeutic lead discovery (Lai et al., 2026; Cha et al., 2024). Snake venom is a particularly rich source of bioactive proteins and peptides that regulate hemostasis by targeting coagulation factors, platelet receptors, endothelial signaling pathways, and vascular components (Green, 2025; Huang et al., 2016). Although multiple venom toxins act synergistically during envenomation, isolated venom-derived molecules can exhibit defined and potent pharmacological activities with substantially lower systemic toxicity than crude venom preparations. This translational



potential has already been demonstrated by disintegrin-derived antiplatelet drugs, including FDA-approved agents tirofiban and eptifibatide (Oliveira et al., 2022). Accumulating evidence also indicates that venom repertoires harbor molecules capable of modulating the GPVI pathway, a major platelet axis for collagen-dependent thrombus formation (Fuentes, 2022). Trowaglerix, a C-type lectin-like protein derived from *Tropidolaemus wagleri* venom, inhibits collagen-induced platelet aggregation by promoting ADAM-mediated GPVI cleavage, whereas the derived peptides Tro α 6 and Tro α 10 suppress thrombus formation through interactions with the GPVI D1/D2 domains without increasing bleeding tendency (Chang et al., 2017). Snake venom metalloproteinases, including kistomin and mutalysin-II, further demonstrate that the collagen-GPVI axis is susceptible to venom-derived modulation (Sanchez et al., 2021; Hsu et al., 2008). Current GPVI-modulating snake venom molecules are predominantly large multidomain proteins with inhibitory activity typically mediated through GPVI ectodomain shedding or proteolytic disruption of the collagen–GPVI axis. In contrast, naturally occurring small venom peptides capable of directly engaging GPVI and modulating downstream signaling remain poorly characterized.

Snake venom defensins, also known as crotamine-like peptides or myotoxins, represent a distinct class of rattlesnake-enriched toxins that adopt a conformation closely related to human β -defensins. These peptides have primarily been described in venom from *Crotalus* species and typically contain 42–45 amino acids, six conserved cysteine residues that form three disulfide bonds, and a β -defensin-like fold organized around a conserved γ -core (Porta et al., 2021). Research on this toxin family has largely focused on myotoxicity, neurotoxicity, and Kv1.3 channel blockade, with the structural diversity and biological functions of snake venom



defensin-like peptides beyond crotamine still poorly described (Coronado et al., 2013; Melendez-Martinez et al., 2025). Although defensin-like peptides constitute a characteristic scaffold in viperid venoms and have been linked to hemotoxic activity, their contributions to thrombosis, platelet regulation, and GPVI-targeted antiplatelet intervention remain largely unexplored.

This study investigated the snake venom defensin mCadam10_MYO-2 from *Crotalus adamanteus* and two homologous defensin-like toxin fragments from *Deinagkistrodon acutus*, namely DACU-1 and DACU-2, using integrated biochemical, cellular, *ex vivo*, and *in vivo* approaches. Among these candidates, mCadam10_MYO-2 preferentially inhibited collagen-induced platelet activation, reduced platelet spreading, delayed clot retraction, and suppressed thrombus formation without significantly prolonging tail bleeding time under the tested conditions. Mechanistically, mCadam10_MYO-2 directly bound to recombinant GPVI and attenuated collagen-induced phosphorylation of Syk and PLC γ 2. Together, these findings identify mCadam10_MYO-2 as a snake venom defensin with GPVI-associated antiplatelet and antithrombotic activity, supporting this peptide class as a potential scaffold for hemostasis-sparing antithrombotic development.

MATERIALS AND METHODS

Animal studies

Sprague-Dawley (SD) rats and C57BL/6 mice were purchased from Dossy Experimental Animals Co. Ltd. (Chengdu, China). Eight-week-old SD rats of both sexes (weighing approximately 220 g) were used for platelet preparation and the carotid artery thrombosis assay,



whereas eight-week-old C57BL/6 mice of both sexes (weighing approximately 25 g) were used for the tail bleeding assay. All animal experiments were conducted in accordance with the guidelines adopted by the Chengdu Institute of Biology, Chinese Academy of Sciences, and protocols approved by the Institutional Animal Care and Use Committee (Approval No. CIBDWLL2025022).

Peptide synthesis and structural prediction

Synthetic mCadaml0_MYO-2, DACU-1, and DACU-2 were commercially synthesized by GL Biochem (Shanghai, China), at a purity greater than 95%. Peptide purity and molecular mass were verified by high-performance liquid chromatography (HPLC, 4.6×250 mm C18 column with acetonitrile and water as the mobile phase) and mass spectrometry (Agilent-6125B), respectively. Peptides were dissolved in sterile phosphate-buffered saline (PBS, Beyotime, C0221A) and stored at −80 °C until use. The three-dimensional structure of mCadaml0_MYO-2 was predicted using AlphaFold Server with default parameters (Abramson et al., 2024), and the top-ranked model was used for visualization and downstream analysis.

The initial mCadaml0_MYO-2-GPVI complex was generated using AlphaFold and subjected to molecular dynamics simulations in AMBER with the ff14SB force field (Case et al., 2025).

The complex was solvated in a TIP3P water box and neutralized with counterions. After energy minimization, heating, and equilibration, a 100-ns production simulation was performed.

Binding free energy was calculated using MMPBSA.py.MPI. A total of 500 conformational frames were extracted from the production trajectory at 10-frame intervals. Protein-peptide binding free energy and per-residue energy decomposition were calculated for each extracted frame.



***In vitro* expression of recombinant GPVI**

Recombinant GPVI was expressed using a modified version of a previously described protocol (Jandrot-Perrus et al., 2000). The expression cassette encoding the extracellular domain of GPVI (GPVI-ECD) was placed under the control of the CMV enhancer/CMV promoter. A Kozak sequence was inserted immediately upstream of the coding region to facilitate translation initiation. To promote secretion, a tissue plasminogen activator (tPA) signal peptide was fused to the N terminus, followed by a FLAG tag, the GPVI-ECD sequence, and a C-terminal 8×His tag for affinity purification. Low-endotoxin plasmid DNA was prepared for mammalian cell transfection and introduced into suspension-cultured 293F cells at 2×10^6 cells/mL by PEI-mediated transient transfection. At 24 h post-transfection, the culture was supplemented with feeding medium to maintain cell growth and support continued secretion of recombinant GPVI-ECD. Culture medium was collected on day 5 after transfection and clarified to obtain the protein-containing supernatant. Recombinant GPVI-ECD was purified using a Ni Sepharose FF affinity chromatography column (Yeast Biotechnology, Shanghai, China). Eluted fractions were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and western blotting to identify fractions containing the target protein. Positive fractions were pooled and extensively dialyzed against PBS using a membrane with a ≥ 3 kDa molecular weight cutoff (Merck Millipore, UFC900396) to remove imidazole and other low-molecular-weight components and complete buffer exchange.

Preparation of platelets

Platelet-rich plasma (PRP), platelet-poor plasma (PPP), and washed platelets (WPs) were



prepared as previously described (Gibbins et al., 2004), with minor modifications. Venous blood from healthy rats was collected into 3.8% sodium citrate (D&B, K100417) at a blood-to-anticoagulant ratio of 9:1 (v/v). PRP was obtained from the supernatant after centrifugation at 220 ×g for 12 minutes at 25 °C. The remaining blood fraction was further centrifuged at 800 ×g for 12 minutes at 25 °C to prepare PPP. For WP preparation, whole blood was collected into ACD-A at a 9:1 ratio (v/v) and centrifuged at 220 ×g for 12 minutes at 25 °C to isolate PRP. Subsequently, PRP was centrifuged at 1 000 ×g for 10 minutes at 25 °C in the presence of 0.5 µg/mL prostaglandin E₁. The platelet precipitate was resuspended in modified Tyrode's buffer and subjected to a second wash under the same concentration of prostaglandin E₁ (Aladdin, P129960). Final platelet pellets were resuspended in an appropriate volume of modified Tyrode's buffer and allowed to rest at 37 °C for 30 minutes before use.

Platelet aggregation assay

Platelet aggregation was measured using an HMP660 automatic platelet aggregometer (Zhejiang Pushkang Biotechnology, China). PRP and WP suspensions (180 µL; 3×10^8 platelets/mL) were transferred to plastic sample cups and preincubated with each test sample diluted in 20 µL of PBS. Aggregation was initiated with arachidonic acid (AA, 1 mM, Zhejiang Pushkang Biotechnology, China), adenosine diphosphate (ADP, 3 µM, Zhejiang Pushkang Biotechnology, China), collagen (5 µg/mL, HYPHEN BioMed, France), or thrombin (0.5 U/mL, HYPHEN BioMed, France), respectively. After baseline recording, agonists were automatically dispensed by the aggregometer at 20 s, and platelet aggregation was monitored according to the instrument protocol.

Platelet spreading assay

Platelet spreading was assessed using a modified version of a previously reported method (Zhu et al., 2022). Round glass coverslips were placed in 24-well plates, coated overnight at 4 °C with 200 µL of collagen (20 µg/mL), washed three times with PBS, and blocked with 200 µL of 1% bovine serum albumin (BSA, Solarbio, A8020) for 1 h at room temperature. Platelet suspensions (250 µL, 2×10^7 platelets/mL) were pretreated with or without mCad10_MYO-2 (0, 12.5, 25, and 50 µM) in PBS for 5 minutes at 37 °C and then added to the collagen-coated coverslips. Plates were further incubated in a 37 °C incubator for 1 h to allow platelet adhesion and spreading. Adherent platelets were fixed with 200 µL of 4% paraformaldehyde per well for 15 minutes at room temperature, permeabilized with 200 µL of 0.1% Triton X-100 (Beyotime, ST795) for 5 minutes, and stained with 200 µL of TRITC-phalloidin (1 µg/mL, Biosharp, BL1189A) for 45 minutes in the dark. Coverslips were mounted and visualized by confocal microscopy (Leica TCS SP8, Germany).

Clot retraction assay

Clot retraction was evaluated using a previously established approach (Song et al., 2019), with some modifications. In brief, 100 µL of WP suspension (3×10^8 platelets/mL) was mixed with 200 µL of PPP and transferred to a reaction cup. mCad10_MYO-2 at 0 or 50 µM was added in PBS, and the mixture was incubated for 5 minutes at 37 °C. Clot formation was initiated by adding 10 µL of thrombin (1 U/mL), followed by gentle mixing. Reactions were maintained at 37 °C, and clot retraction was photographed every 10 minutes from 0 to 60 minutes.

Electrical injury-induced carotid artery thrombosis assay

The electrical injury-induced carotid artery thrombosis model was established using a YLS-14B thrombosis detector (Ji'nan Yiyan Technology Development Co., Ltd, China) based on a

previously reported method (Wang et al., 2020), with some modifications. SD rats were initially anesthetized with 1.5% sodium pentobarbital (30 mg/kg) via intraperitoneal injection. Then rats were treated with mCadaml0_MYO-2 (10 mg/kg), aspirin (100 mg/kg) or an equal volume of PBS solution by tail vein injection. After three minutes, the left common carotid artery was exposed by blunt dissection. Vascular injury was induced by applying a 1 mA electrical current to the carotid artery wall to promote thrombus formation and progressive blood flow obstruction. Blood flow occlusion was recorded every 4 s for 300 s using an infrared detector. The time to occlusive thrombus formation was defined as the time required for the occlusion rate to reach 90%. If the occlusion rate did not reach 90% during the recording time, the occlusion time was recorded as 300 s.

***Ex vivo* platelet aggregation assay**

Sprague-Dawley rats were randomly assigned to the vehicle and mCadaml0_MYO-2 groups. mCadaml0_MYO-2 was administered via tail-vein injection at 10 mg/kg/day for 7 consecutive days, whereas control rats received an equal volume of PBS following the same dosing schedule. The injection volume was adjusted according to the body weight of each animal. At 24 h after the final administration, the rats were anesthetized, and blood was collected into 3.8% sodium citrate at a blood-to-anticoagulant ratio of 9:1 (v/v). Platelet-rich plasma was obtained by centrifugation at $220 \times g$ for 12 min at 25 °C. PRP aliquots were transferred to aggregation cuvettes, and platelet aggregation was initiated with collagen at a final concentration of 5 µg/mL. Aggregation was monitored using an HMP660 automatic platelet aggregometer, and the maximal aggregation rate was quantified.

Tail bleeding assay in mice



The tail bleeding assay was performed based on a previously reported approach (Chang et al., 2017), with some modifications. In brief, mice received mCadam10_MYO-2 (10 mg/kg), aspirin (10 mg/kg), or PBS by tail vein injection. Three minutes after injection, mice were anesthetized, and a 3 mm segment of the tail tip was surgically transected. The tail was immediately placed on filter paper prewarmed to 37 °C, with the transected tip gently contacting the paper surface, and timing was initiated. Bleeding time was defined as the interval from tail transection to the first complete cessation of bleeding lasting at least 30 s. If bleeding did not cease within the 10-minute observation period, a bleeding time of 600 s was assigned.

Western blotting

Western blotting was conducted based on an established protocol (Chang et al., 2017), with some modifications. In brief, washed platelets (4×10^8 platelets/mL) were transferred to aggregometer cuvettes and incubated with the test compound (50 μ M) at 37 °C under stirring for 3 minutes, followed by stimulation with collagen (5 μ g/mL). Resting control samples received PBS instead of the test peptide and collagen. Platelets were lysed at the indicated time points, and protein samples were analyzed by western blotting using anti-Syk (Cell Signaling Technology, 2712S), anti-phospho-Syk (Cell Signaling Technology, 44170T), anti-PLC γ 2 (Cell Signaling Technology, 3872S), and anti-phospho-PLC γ 2 (Cell Signaling Technology, 50535S) antibodies. GAPDH (Affinity, AF7021) was used as the internal control.

Transcriptome assay

Transcriptome profiling was performed using a modified protocol based on a previously reported platelet RNA-seq method (Supernat et al., 2021). Washed platelets (4×10^8 platelets/mL) were placed in aggregometer cuvettes at 37 °C and preincubated with or without



the test peptide (50 μ M) under stirring for 3 minutes. Platelets were then stimulated with collagen (5 μ g/mL) for 2 minutes, collected immediately, flash-frozen in liquid nitrogen, and submitted for RNA extraction and sequencing. cDNA libraries were prepared and sequenced on the Illumina NovaSeq 6000 platform using paired-end 150-bp reads. After quality control, RNA sequencing (RNA-seq) reads were aligned to the Sprague-Dawley rat genome using STAR v2.7.6a with the following parameters: `--chimSegmentMin 2` and `--outFilterMismatchNmax 3`. Gene-level and transcript-level abundances were quantified using RSEM v1.2.28. Differential RNA abundance was analyzed using DESeq2, with $|\log_2 \text{fold change}| \geq 0.5$ and Benjamini–Hochberg adjusted P value (false-discovery rate, FDR) < 0.05 defined as significant.

Bio-layer interferometry (BLI) assay

The interaction between mCad10_MYO-2 and GPVI was quantified using a Gator® Plus system (GatorBio, Suzhou, China) with APS biosensors. Sensors were hydrated in PBS for at least 10 minutes before use. mCad10_MYO-2 was immobilized onto the APS sensors by immersion in peptide solution (10 μ g/mL) for 120 s, followed by equilibration in buffer for 60 s. Sensors were then blocked with 1% BSA in PBS for 60 s, and equilibrated in PBST for an additional 60 s. Association was recorded after exposure to recombinant GPVI at 500, 250, 125, 62.5, 31.25, and 15.625 nM for 150 s, followed by dissociation in buffer for 150 s. Reference sensor subtraction was performed using buffer-only sensors exposed to GPVI, and background signals were determined using peptide-loaded sensors exposed to running buffer only. Binding data were processed using Gator® Data Acquisition and Analysis software v2.18.7.0917.



Statistical analysis

Statistical analyses were performed using Origin software, and data are presented as mean $\pm$ standard error of the mean (SEM). Statistical significance between two groups was evaluated using unpaired two-tailed Student's *t*-test. Multiple-group comparisons were conducted via one-way ANOVA, with Dunnett's test for comparisons versus a single control and Tukey's test for all pairwise intergroup comparisons. $P < 0.05$ was considered statistically significant.

RESULTS

Identification and initial functional screening of three peptides

Three snake venom peptides, mCadaml0_MYO-2, DACU-1, and DACU-2, were selected for comparative structural annotation and preliminary functional screening. Among them, mCadaml0_MYO-2 represented the predicted mature peptide generated after removal of the signal peptide from the UniProt-annotated snake venom β -defensin precursor Cadaml0_MYO-2 (UniProtKB accession: A0A1W7RB98). DACU-1 and DACU-2 were peptide fragments derived from the predicted toxin sequences Dacu19417.1 and Dacu08320.1, respectively (Supplementary Figure S1). Sequence alignment revealed a highly conserved cysteine distribution pattern among the three peptides, indicating strong homology in the putative disulfide-bonding architecture (Figure 1A). Structural modeling of mCadaml0_MYO-2 placed the conserved core within a canonical $\alpha 1$ - $\beta 1$ - $\beta 2$ - $\beta 3$ β -defensin-like architecture. Visualization of the predicted structure further indicated that mCadaml0_MYO-2 adopted a compact fold conformation stabilized by three disulfide bonds, with intervening loops connecting the principal secondary structural elements (Figure 1B). Although the cysteine framework was

conserved, the three peptides differed substantially at non-cysteine residues. Variation was particularly pronounced within the intercysteine segments and the C-terminal region, suggesting potential differences in charge distribution, receptor-facing residues, and biological activity.

The antiplatelet activity of each peptide was initially evaluated in platelet-rich plasma (PRP) stimulated with arachidonic acid (AA), adenosine diphosphate (ADP), collagen (COL), or thrombin (Figure 1C). At a final concentration of 75 μ M, the peptides showed distinct inhibitory profiles. mCad10_MYO-2 exhibited preferential inhibition of collagen-induced platelet aggregation, whereas inhibition of AA-, ADP-, and thrombin-induced platelet aggregation was comparatively limited. DACU-1 produced moderate inhibition after collagen or thrombin stimulation, while DACU-2 showed weak activity under most assay conditions, although a modest effect was observed in the thrombin-induced assay.

As mCad10_MYO-2 exhibited the strongest activity against collagen-induced aggregation, this peptide was further assessed in washed platelets using collagen as the agonist. Representative aggregation traces demonstrated concentration-dependent suppression of collagen-triggered platelet aggregation by mCad10_MYO-2 (Figure 1D). Quantitative analysis confirmed dose-dependent inhibition, with clear activity at 25–50 μ M and near-complete blockade at 75 μ M (Figure 1E). Based on these data, the half-maximal inhibitory concentration (IC_{50}) of mCad10_MYO-2 against collagen-induced platelet aggregation was estimated at 37.43 μ M.

mCad10_MYO-2 affects platelet spreading and clot retraction

The effects of mCad10_MYO-2 on platelet activation-associated morphological remodeling



were examined by assessing platelet spreading on collagen-coated surfaces using confocal microscopy. Control platelets displayed extensive spreading on collagen, whereas mCadaml0_MYO-2 progressively restricted this response at 12.5, 25, and 50 μM , producing a concentration-dependent reduction in spreading morphology (Figure 2A). Quantitative analysis confirmed a corresponding decrease in mean single-platelet area, with the smallest area observed after treatment with 50 μM mCadaml0_MYO-2 ($7.73 \pm 0.36 \mu\text{m}^2$) (Figure 2B). Platelet circularity was also measured to further characterize changes in cell morphology (Figure 2C). Relative to the control group, 12.5 μM mCadaml0_MYO-2 produced a slight reduction in circularity, whereas higher concentrations, particularly 50 μM , significantly increased platelet circularity, consistent with a less spread, more rounded platelet morphology. The effect of mCadaml0_MYO-2 on platelet contractile function was then assessed using a clot retraction assay. In control samples, clots underwent progressive contraction over time, whereas mCadaml0_MYO-2 visibly delayed clot retraction (Figure 2D, Supplementary Figure S2). Time-course quantification showed a more rapid reduction in clot area in the control group, while clots exposed to mCadaml0_MYO-2 retained a larger area throughout the observation period (Figure 2E). At the final time point, the residual clot area remained significantly greater in the mCadaml0_MYO-2-treated group than in the control group, indicating that mCadaml0_MYO-2 impaired platelet-mediated clot contraction (Figure 2F).

mCadaml0_MYO-2 binds GPVI and attenuates collagen-induced downstream signaling

To explore the potential mechanism through which mCadaml0_MYO-2 suppressed collagen-induced platelet activation, transcriptome profiling was performed in collagen-stimulated platelets treated with or without the peptide. Volcano plot analysis showed that



353 mCad10_MYO-2 altered the RNA expression profile compared with collagen-stimulated
354 control platelets, with 138 transcripts decreased and 72 transcripts increased after peptide
355 treatment (Figure 3A). Functional enrichment analysis indicated that the down-regulated genes
356 were associated with platelet activation-related processes and pathways, including protein
357 biosynthesis, translation, oxidative phosphorylation, and related signaling modules (Figure 3B).
358 Representative platelet function-related down-regulated genes, including *Ccl5*, *Ppbp* and
359 *Tmsb10*, were highlighted to illustrate transcriptomic changes linked to platelet activation
360 status (Figure 3C).

361 Western blot analysis showed that pretreatment with mCad10_MYO-2 attenuated collagen-
362 induced Syk and PLC γ 2 phosphorylation (Figure 3D). Additionally, BLI was performed to
363 determine the interaction between recombinant human GPVI and mCad10_MYO-2 (Figure
364 3E). The BLI response curves demonstrated concentration-dependent binding of recombinant
365 GPVI to immobilized mCad10_MYO-2, with an equilibrium dissociation constant (K_D) of
366 71.4 ± 6.3 nM (Figure 3E). Together, the BLI and signaling data support direct GPVI
367 engagement by mCad10_MYO-2 and attenuation of collagen-induced GPVI-associated
368 platelet signaling.

369 **Molecular dynamics analysis of the mCad10_MYO-2-GPVI interaction**

370 The stability and binding architecture of the mCad10_MYO-2-GPVI complex were further
371 examined by a 100-ns molecular dynamics simulation. The backbone root-mean-square
372 deviation (RMSD) trajectory indicated that the complex underwent conformational adjustment
373 during the early and middle stages of the simulation before reaching a relatively stable plateau
374 during the final 20 ns, from 80 to 100 ns (Figure 4A). Detailed analysis of this terminal interval



showed a mean RMSD of 2.78 ± 0.27 Å, indicating minimal conformational drift after equilibration (Figure 4B). Root-mean-square fluctuation (RMSF) analysis further showed that most residues in the complex exhibited low-to-moderate fluctuations, with higher flexibility predominantly confined to localized regions likely corresponding to loop segments or termini. Notably, residues contributing to predicted hydrogen-bond contacts between the two molecules, including D85, E88, P141, K219, R206, and L225, were positioned within regions of relatively limited mobility (Figure 4C). Interface contact analysis identified recurrent hydrogen-bond interactions between D85 and K219, E88 and K219, E88 and L225, and P141 and R206 across the simulation trajectory, supporting a persistent interaction network at the predicted binding interface (Figure 4D). Molecular Mechanics/Generalized Born Surface Area (MM/GBSA) analysis estimated a total binding free energy of -21.23 ± 0.24 kcal/mol, consistent with a favorable mCad10_MYO-2-GPVI interaction under the simulated conditions (Figure 4E). A representative structure extracted from the final 50 trajectory frames further showed mCad10_MYO-2 positioned on the GPVI surface, with multiple interface residues contributing to the predicted interaction network (Figure 4F).

mCad10_MYO-2 suppresses collagen-induced platelet aggregation *ex vivo* and delays arterial thrombus formation *in vivo*

The antiplatelet activity of mCad10_MYO-2 after *in vivo* administration was evaluated by preparing PRP from rats that received mCad10_MYO-2 or vehicle, followed by *ex vivo* stimulation with collagen. Aggregation traces showed a clear reduction in collagen-induced platelet aggregation after mCad10_MYO-2 treatment compared with the control condition (Figure 5A, Supplementary Figure S3). Quantitative analysis confirmed that



mCadaml0_MYO-2 significantly inhibited collagen-stimulated platelet aggregation *ex vivo*, although residual aggregation remained detectable (Figure 5B).

The antithrombotic effect of mCadaml0_MYO-2 was then examined in an electrical injury-induced carotid artery thrombosis model. Real-time blood-flow monitoring revealed rapid arterial occlusion in control rats, whereas mCadaml0_MYO-2 delayed thrombus-mediated vessel closure. Notably, five of the six aspirin-treated animals did not reach 90% occlusion during the monitoring period, and the maximal monitoring time was therefore assigned for quantification (Figure 5C). Quantitative analysis showed that vessel occlusion occurred at 87 ± 3 s in the control group and was extended to 141 ± 15 s in the mCadaml0_MYO-2-treated group (Figure 5D).

mCadaml0_MYO-2 does not significantly prolong bleeding time or alter platelet indices under tested conditions

The effect of mCadaml0_MYO-2 on primary hemostasis was assessed *in vivo* using a mouse tail bleeding assay, with PBS as the vehicle control and aspirin as the positive control (Figure 6A). Bleeding time was comparable between the PBS-treated and mCadaml0_MYO-2-treated groups, with values of 146 ± 6 s and 145 ± 4 s, respectively (Figure 6B). In contrast, aspirin markedly prolonged bleeding time to 287 ± 15 s, significantly exceeding the values observed in both the PBS-treated and mCadaml0_MYO-2-treated groups. Coagulation parameters were also examined to determine whether mCadaml0_MYO-2 affected plasma clotting activity. Across the tested concentration range of 0–100 μ M, mCadaml0_MYO-2 did not significantly alter prothrombin time (PT), thrombin time (TT), or fibrinogen concentration. Activated partial thromboplastin time (APTT) also remained unchanged at lower concentrations, although a



clear prolongation was detected at the highest concentration tested (100 μ M) (Supplementary Figure S4). Peripheral platelet indices were then analyzed after repeated administration of PBS, mCadaml0_MYO-2, or aspirin. Mean platelet volume, platelet distribution width, platelet-large cell ratio, and plateletcrit did not differ significantly among the three groups (Figure 6C–F). These findings indicate that mCadaml0_MYO-2 did not measurably impair tail bleeding hemostasis or alter platelet volume-related hematological parameters under the tested conditions.

DISCUSSION

Platelet activation is a defining event in arterial thrombosis, and collagen-driven platelet activation occurs predominantly through the GPVI signaling axis (Li et al., 2025). Previously described venom-derived GPVI modulators have mainly comprised metalloproteinases and C-type lectin-like proteins that act through receptor cleavage or direct receptor engagement. By contrast, mCadaml0_MYO-2 is a compact, chemically synthesizable β -defensin-scaffold peptide. Its preferential inhibition of collagen-induced platelet aggregation and attenuation of GPVI-associated signaling broadens the functional repertoire of snake venom defensins beyond their established ion-channel-modulating and myotoxic activities (Melendez-Martinez et al., 2025).

Comparative sequence analysis revealed that mCadaml0_MYO-2, DACU-1, and DACU-2 retain a conserved cysteine arrangement consistent with a shared disulfide-bonding framework, despite substantial divergence at non-cysteine positions, especially charged and polar residues. This architecture suggests preservation of a common β -defensin-like core while allowing



variation in surface chemistry that may influence receptor interactions and platelet-regulatory activity. However, the initial antiplatelet screen showed that conservation of the β -defensin-like cysteine scaffold did not confer equivalent inhibition of platelet aggregation. This functional divergence implies that residues outside the conserved cysteine framework may play a decisive role in shaping platelet-directed activity. Notably, mCadaml0_MYO-2 showed inhibitory activity against collagen-induced aggregation in PRPs and retained this effect in washed platelets in a concentration-dependent manner, supporting its selection as the lead candidate for mechanistic analysis.

In addition to platelet aggregation, mCadaml0_MYO-2 inhibited platelet spreading on collagen and delayed clot retraction. Platelet spreading integrates collagen-dependent adhesion, cytoskeletal remodeling, and surface-coupled activation, whereas clot retraction represents a later platelet-driven contractile process coordinated by integrin outside-in signaling and actomyosin-mediated contractile remodeling (Song et al., 2019). The concentration-dependent reduction in single-platelet spreading area indicates that mCadaml0_MYO-2 restricted collagen-dependent adhesive activation. Changes in circularity followed a non-linear pattern, with a slight decrease at the lowest concentration and a clear increase at higher concentrations relative to collagen-stimulated control. This pattern may reflect partial interference with platelet spreading at low peptide exposure, followed by stronger suppression of platelet extension and preservation of a rounded morphology at higher concentrations. The inhibitory profile extended to clot retraction, with mCadaml0_MYO-2-treated clots retaining a larger area across the assay period. Because clot retraction requires sustained platelet activation, integrin-dependent outside-in signaling, and actomyosin-dependent contractile remodeling,



463 delayed clot retraction indicates that mCad10_MYO-2 affected both early collagen-driven
464 platelet activation and later platelet functions that contribute to thrombus consolidation (Zhu et
465 al., 2022).

466 Mechanistic evidence supports GPVI-associated signaling as a key pathway affected by
467 mCad10_MYO-2, although this pathway should be considered within the broader biological
468 network of platelet activation rather than as an isolated mechanism. Given that
469 mCad10_MYO-2 preferentially inhibited collagen-induced platelet aggregation, and that
470 GPVI is a principal receptor for collagen-mediated platelet activation, we hypothesized that
471 GPVI-associated signaling may be a plausible pathway underlying the antiplatelet effect of
472 mCad10_MYO-2 (Li et al., 2025; Nieswandt et al., 2003). RNA-seq showed that
473 mCad10_MYO-2 altered the abundance of transcripts associated with platelet activation,
474 consistent with the functional inhibition observed in aggregation assays. These transcripts
475 included *Pf4* and *Ppbp*, which encode abundant platelet and megakaryocyte α -granule
476 chemokines; *Ccl5*, which is involved in platelet-mediated inflammatory signaling; *Cd63*, a
477 granule-associated marker; and *Tmsb10* and *Myl12a*, which are associated with cytoskeletal
478 and contractile organization. However, gProfiler analysis did not reveal direct enrichment of
479 canonical platelet pathways, including the GPVI-mediated activation cascade. This may reflect
480 the anucleate nature of platelets and the fact that GPVI signaling is primarily governed by rapid
481 post-translational phosphorylation events rather than broad changes in transcript abundance.
482 Previous studies have shown that GPVI activation induces extensive phosphorylation
483 remodeling, whereas total protein abundance changes only minimally after pathway activation
484 (Babur et al., 2020; Petersen et al., 2017). Accordingly, the RNA-seq data should be interpreted

as supportive evidence that mCad10_MYO-2 changes the downstream platelet activation state, rather than as direct evidence that the canonical GPVI signaling cascade is transcriptionally suppressed. In this context, the reduction of collagen-induced Syk and PLC γ 2 phosphorylation provides a more proximal biochemical indicator of impaired GPVI/Fc γ 2 signaling, as both molecules function downstream of the GPVI/Fc γ 2 complex (Babur et al., 2020). In addition, the nanomolar-range affinity between mCad10_MYO-2 and recombinant human GPVI further supports GPVI as a likely upstream binding target.

Molecular dynamics analysis provided additional evidence for the stable interaction between mCad10_MYO-2 and GPVI. After an initial phase of structural relaxation, the mCad10_MYO-2-GPVI complex maintained a relatively stable RMSD profile, suggesting limited conformational drift during the later simulation interval. Residue-level fluctuation analysis further showed that most of the predicted interface remained structurally restrained, with higher mobility confined mainly to peripheral loops and terminal segments. Persistent interfacial contacts across the trajectory, together with a favorable MM/GBSA binding energy, supported a favorable peptide-protein interaction and aligned with the direct binding detected by BLI. Together with the biochemical and functional data, these simulations support a plausible model in which mCad10_MYO-2 limits collagen-driven platelet activation, at least partly, through engagement of GPVI-associated signaling.

Ex vivo and *in vivo* analyses extended the antiplatelet profile of mCad10_MYO-2 beyond the initial *in vitro* assays. PRP isolated from mCad10_MYO-2-treated rats showed a reduced aggregatory response to collagen, indicating that the peptide retained functional activity after systemic administration (Bao et al., 2026). In the electrical injury-induced carotid artery



thrombosis model, mCadaml0_MYO-2 significantly prolonged the time required for vessel occlusion compared with the control group, demonstrating suppression of thrombus formation under arterial injury conditions (Wang et al., 2020). Although this antithrombotic effect was less pronounced than that observed with aspirin in the current experimental setting, the delay in arterial occlusion was consistent with the inhibition of collagen-induced platelet activation and supported mCadaml0_MYO-2 as a venom-derived lead scaffold for antithrombotic development.

Hemostatic safety assays further suggested that mCadaml0_MYO-2 did not substantially compromise primary hemostasis at the tested dose. Tail bleeding time remained comparable between the mCadaml0_MYO-2-treated and PBS-treated groups, whereas aspirin markedly prolonged bleeding under the same experimental conditions. Together with the antithrombotic findings, this hemostatic profile is consistent with the concept that selective modulation of GPVI-associated platelet activation can reduce pathological thrombosis while preserving essential hemostatic function (Chang et al., 2017). Coagulation assays also indicated limited effects of mCadaml0_MYO-2 on plasma clotting parameters under the tested conditions, further supporting a restricted impact on physiological hemostasis (Favaloro et al., 2024). In addition, repeated administration of mCadaml0_MYO-2 did not significantly alter MPV, PDW, P-LCR, or PCT, suggesting no evident effects on platelet size-related hematological indices under the experimental regimen (Bao et al., 2026).

In this broader therapeutic landscape, mCadaml0_MYO-2 can be viewed as an early-stage peptide candidate within the emerging class of GPVI-directed antiplatelet strategies, alongside glenzocimab and revacept. Glenzocimab is an antibody-based GPVI antagonist designed to



inhibit GPVI-dependent platelet activation, whereas revacept is a soluble GPVI-Fc fusion protein that interferes with collagen-mediated platelet recruitment at sites of vascular injury. Distinct from these biologics, mCadaml0_MYO-2 represents a venom-derived peptide scaffold associated with GPVI-related inhibition of collagen-induced platelet activation. However, as a venom-derived peptide, mCadaml0_MYO-2 should not yet be directly compared with clinically evaluated GPVI-targeted biologics in terms of efficacy or safety. Further studies defining the binding epitope, GPVI selectivity, pharmacokinetic properties, proteolytic stability, immunogenicity, and therapeutic window will be required to determine whether this peptide scaffold has translational potential.

CONCLUSION

This study identified snake venom defensins as a previously underexplored class of platelet-regulatory molecules and established mCadaml0_MYO-2 as a promising candidate molecule against collagen-induced platelet activation. Notably, mCadaml0_MYO-2 suppressed platelet aggregation, restricted collagen-dependent spreading, delayed clot retraction, and reduced arterial thrombus formation, without significantly prolonging tail bleeding time or altering platelet indices under the tested conditions. Mechanistic evidence linked these effects to GPVI-associated signaling, supported by direct binding to GPVI and attenuation of collagen-induced Syk and PLC γ 2 phosphorylation. These findings expand the functional scope of defensin-related venom peptides and suggest that mCadaml0_MYO-2 may serve as a natural GPVI-associated antithrombotic scaffold for future therapeutic development.

DATA AVAILABILITY

The raw RNA-sequencing data reported in this study have been deposited in the National Genomics Data Center (NGDC, <https://ngdc.cncb.ac.cn/>) under accession code PRJCA065951. All other data generated during the current study are included in the article.

SUPPLEMENTARY DATA

Supplementary figures and tables to this article can be found online.

COMPETING INTERESTS

The authors declare that a patent application related to this work has been submitted under application number 202610874393.1.

AUTHOR CONTRIBUTIONS

Y. Z. L.: Original draft writing, Methodology, Validation & Investigation. D. D. Z.: Data curation & Validation. C. C. Y.: Data curation & Validation. N. Q. H.: Data curation. Y. Q. Z.: Investigation & Data curation. Z. J. G.: Conceptualization, Writing-review & editing. J. T. L.: Conceptualization, Supervision, Writing-review & editing, Funding acquisition. All authors read and approved the final version of the manuscript.

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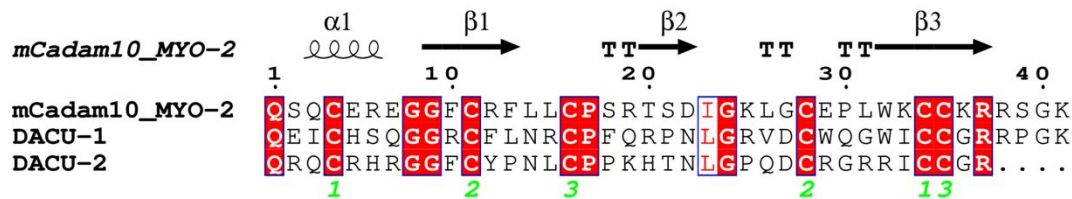
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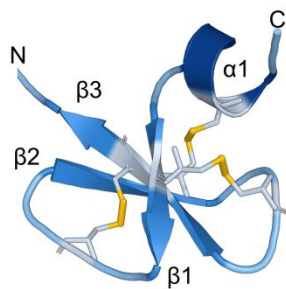
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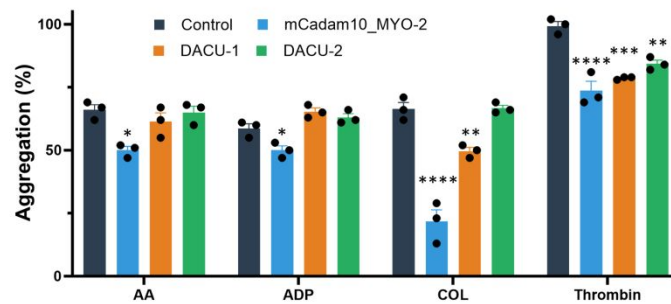
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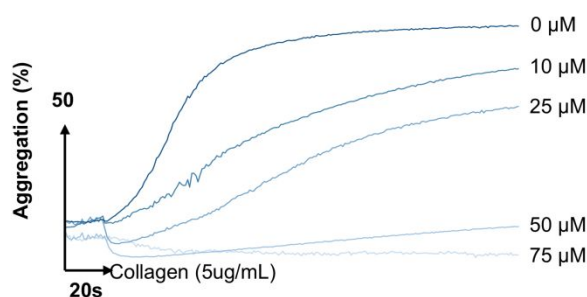
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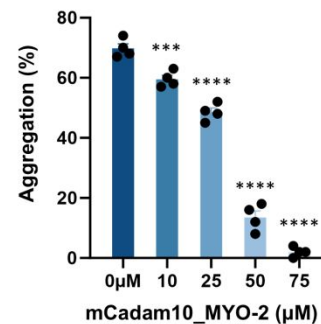


Figure 1 Sequence characteristics, predicted structure, and antiplatelet activity of mCad10_MYO-2, DACU-1, and DACU-2

A: Sequence alignment of mCad10_MYO-2 with the homologous peptides DACU-1 and DACU-2. Conserved residues are shaded red, black dots denote omitted residues, green numbers indicate predicted disulfide bond pairings, and secondary structural elements of

693 mCadam10_MYO-2 are shown above the alignment. B: Predicted three-dimensional structure
694 of mCadam10_MYO-2, with disulfide bonds shown in yellow (pTM = 0.7; mean pLDDT =
695 92.07). C: Platelet aggregation in PRP induced by arachidonic acid (AA), adenosine
696 diphosphate (ADP), collagen (COL) or thrombin after preincubation with mCadam10_MYO-
697 2, DACU-1, or DACU-2 at a final concentration of 75 μ M for 3 minutes at 37 °C ($n = 3$). D:
698 Representative aggregation traces of collagen-induced aggregation in washed platelets
699 preincubated with mCadam10_MYO-2 at 0, 10, 25, 50, or 75 μ M for 3 minutes at 37 °C.
700 Collagen was used at 5 μ g/mL. E: Quantification of collagen-induced aggregation in washed
701 platelets treated with increasing concentrations of mCadam10_MYO-2 ($n = 4$). Data are
702 presented as mean $\pm$ SEM. ns, not significant, $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, $****P <$
703 0.0001 versus the control (model) group.

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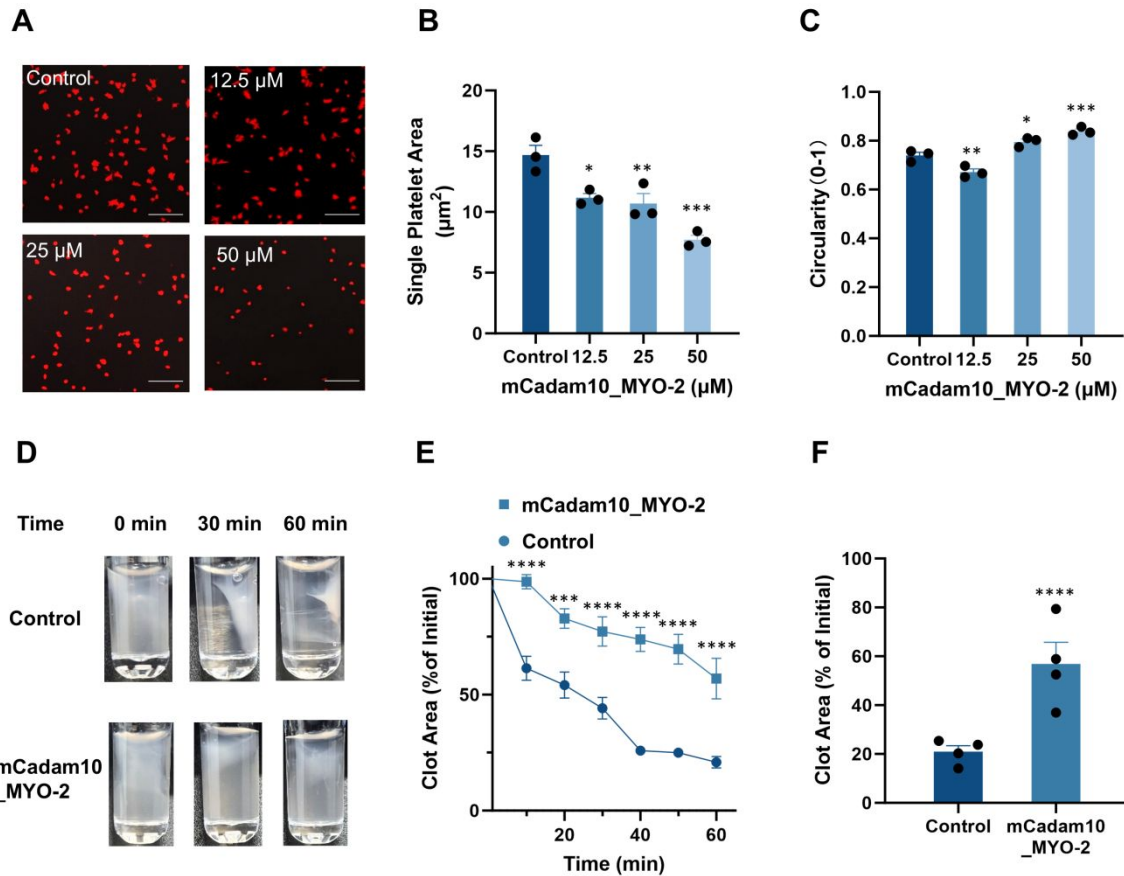
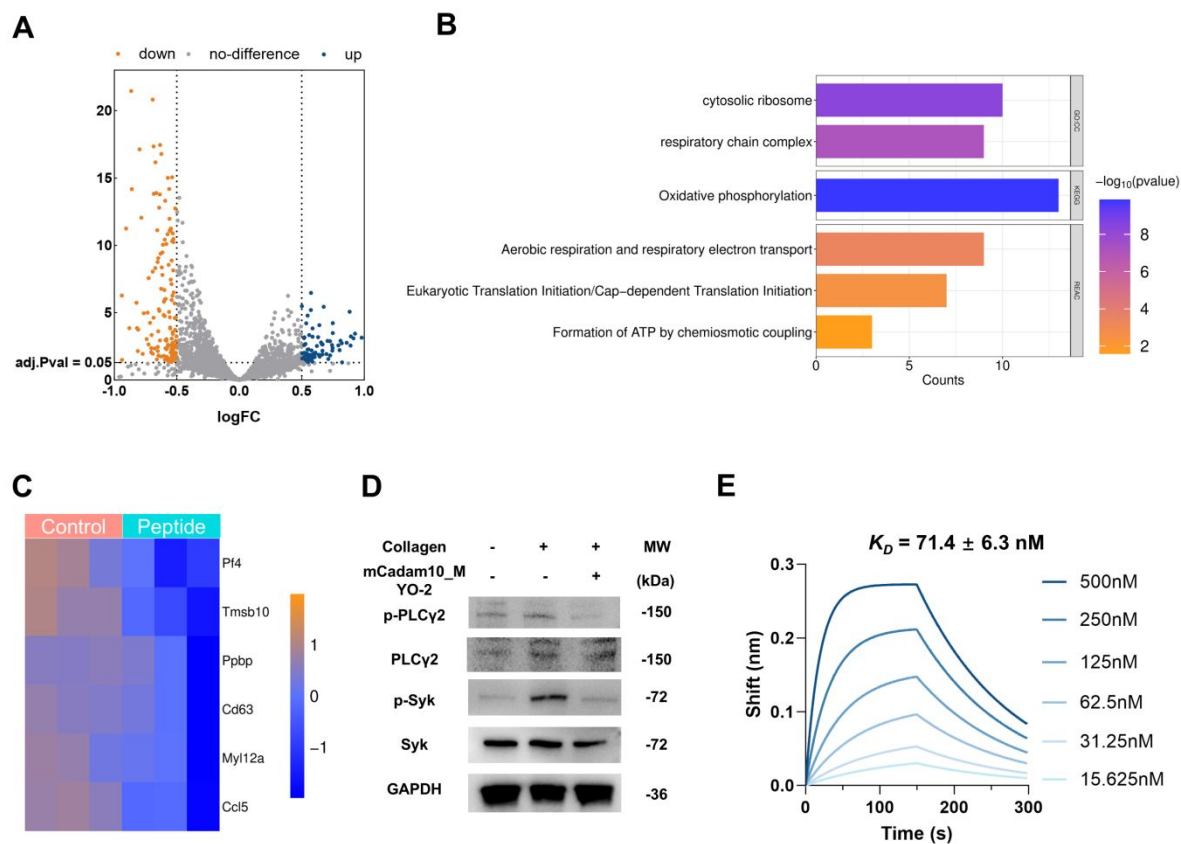


Figure 2 mCad10_MYO-2 attenuates platelet spreading and clot retraction

A: Representative microscopy images of platelet spreading on collagen after pretreatment with mCad10_MYO-2 at 0, 12.5, 25, or 50 μ M for 5 minutes. Scale bar, 25 μ m ($n = 3$). B: Quantification of single-platelet spreading area under indicated concentrations of mCad10_MYO-2 (μm^2 , $n = 3$). C: Quantification of platelet circularity at the indicated concentrations of mCad10_MYO-2, with values ranging from 0 to 1 ($n = 3$). D: Representative images of clot retraction at the indicated time points in control and mCad10_MYO-2-treated samples (50 μ M). E: Time-course quantification of clot area, expressed as a percentage of the initial clot area ($n = 4$). F: Quantification of clot area at the final time point (time = 60 minutes, $n = 4$). Data are presented as the mean $\pm$ SEM, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$ versus the control group.



718

719 **Figure 3 mCadam10_MYO-2 alters platelet RNA expression and binds directly to GPVI**

720 A: Volcano plot showing differentially expressed genes (DEGs) between collagen-stimulated

721 control platelets and collagen-stimulated platelets treated with mCadam10_MYO-2. Up-

722 regulated, down-regulated, and unchanged genes are shown in different colors, with selected

723 platelet function-related genes labeled. B: Functional enrichment analysis of down-regulated

724 genes, showing selected enriched terms from GO Biological Process (GO:BP), GO Cellular

725 Component (GO:CC), KEGG, and Reactome (REAC). Bar length indicates the number of

726 intersecting genes in each term, and color intensity represents enrichment significance as

727 $-\log_{10}(P\text{-value})$. C: Heatmap of selected down-regulated genes involved in platelet activation,

728 grouped by functional category. Yellow indicates relatively higher expression, whereas blue

729 indicates relatively lower expression. RNA-seq analysis was performed using three

730 independent biological replicates per group. D: Western blot analysis of washed platelets
731 pretreated with mCad10_MYO-2 and then stimulated with collagen. Representative blots
732 from three independent experiments are shown. E: Representative BLI sensorgrams showing
733 binding of recombinant GPVI to immobilized mCad10_MYO-2. GPVI analyte
734 concentrations from top to bottom: 500, 250, 125, 62.5, 31.25, 15.625 nM.

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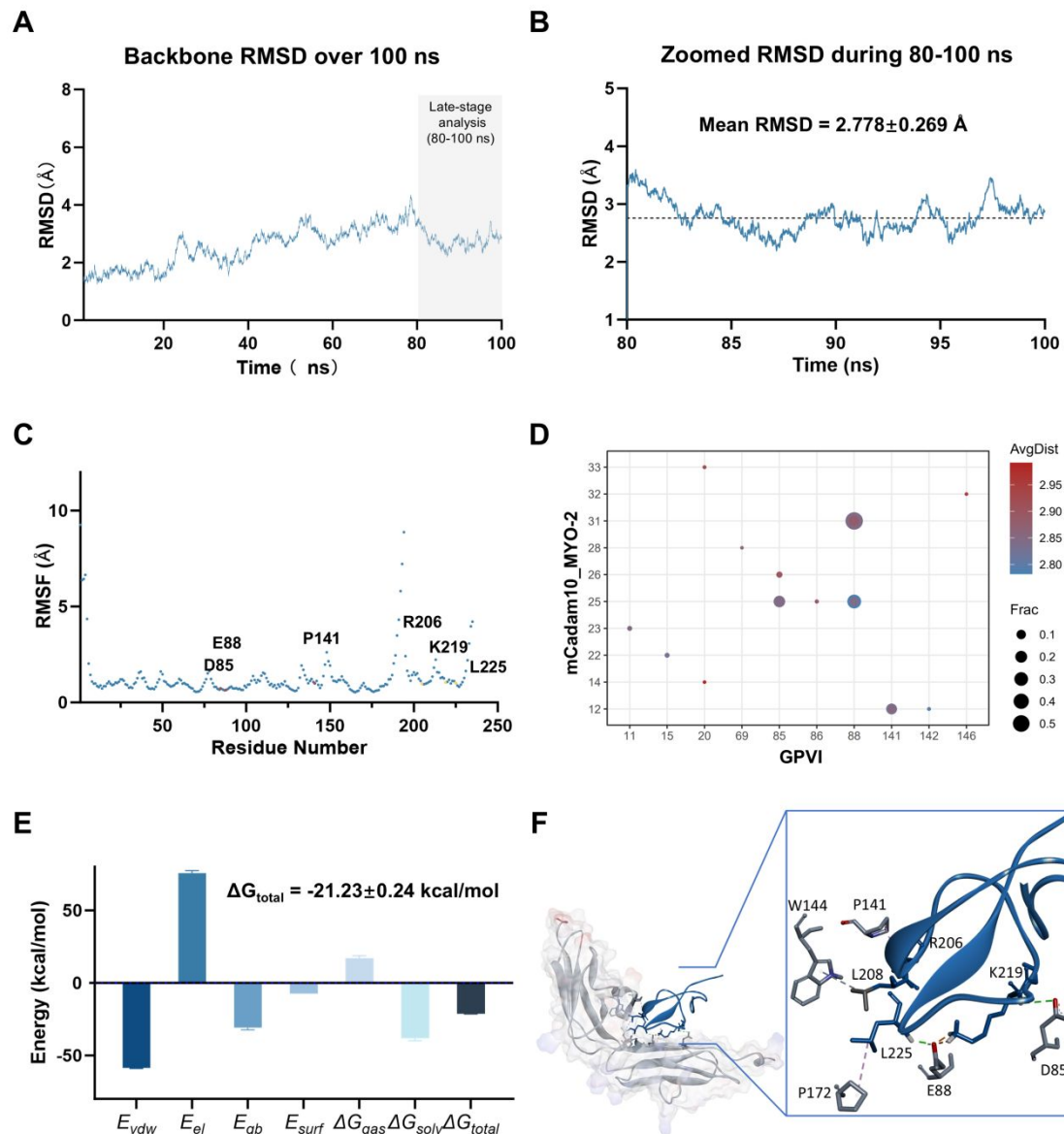


Figure 4 Molecular dynamics analysis of the interaction between mCad10_MYO-2 and GPVI

A: Backbone RMSD profile of the mCad10_MYO-2-GPVI complex during the 100-ns molecular dynamics simulation. Shaded region indicates the late-stage trajectory used for further analysis. B: Enlarged RMSD profile of the 80–100 ns interval, with the mean RMSD value shown for the late-stage simulation. C: Per-residue RMSF profile of the mCad10_MYO-2-GPVI complex, with selected residues contributing to relatively stable hydrogen-bond contacts indicated. D: Hydrogen-bond contact map between GPVI and

745 mCadam10_MYO-2 during the simulation trajectory. Dot size represents contact fraction, and
746 color indicates average interaction distance. E: MM/GBSA-based binding energy
747 decomposition of the mCadam10_MYO-2-GPVI complex, with calculated total binding free
748 energy shown. F: Representative binding mode of mCadam10_MYO-2 on the GPVI surface,
749 with interacting residues at the predicted binding interface indicated; GPVI is shown in grey
750 and mCadam10_MYO-2 is in dark blue; key residues involved in weak intermolecular
751 interactions, including hydrogen bonds (distance for D85-K219 is 2.59 Å, E88-L225 is 2.37 Å,
752 P141-R206 is 2.85 Å) and other weak non-covalent contacts (distance for W144-L208 is 3.96
753 Å, P172-L225 is 4.15 Å), are shown as sticks and labeled.

754



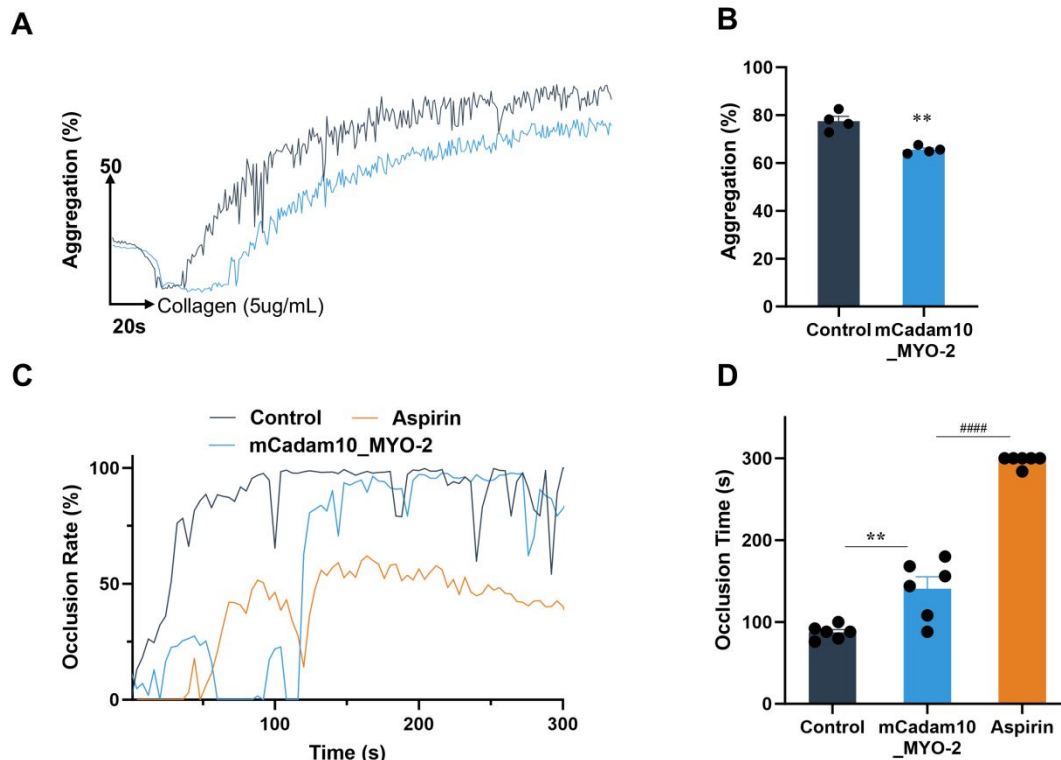


Figure 5 *Ex vivo* antiplatelet activity and *in vivo* antithrombotic effect of mCadam10_MYO-2

A: Representative aggregation traces of PRP prepared from rats treated with PBS or mCadam10_MYO-2. Platelet aggregation was induced with collagen at 5 µg/mL. B: Quantification of collagen-induced PRP aggregation in rats treated with PBS or mCadam10_MYO-2 (10 mg/kg, $n = 4$). C: Representative blood-flow occlusion curves in the rat carotid artery thrombosis model after treatment with PBS, mCadam10_MYO-2 (10 mg/kg), or aspirin (100 mg/kg). Thrombus formation was induced by electrical stimulation. D: Quantification of carotid artery occlusion time after treatment with PBS, mCadam10_MYO-2, or aspirin ($n = 6$). If occlusion was not reached within the 300 s monitoring period, 300 s was assigned as the maximal observation time. Data are presented as mean $\pm$ SEM. $**P < 0.01$ versus the control group; $####P < 0.0001$ versus the aspirin group.

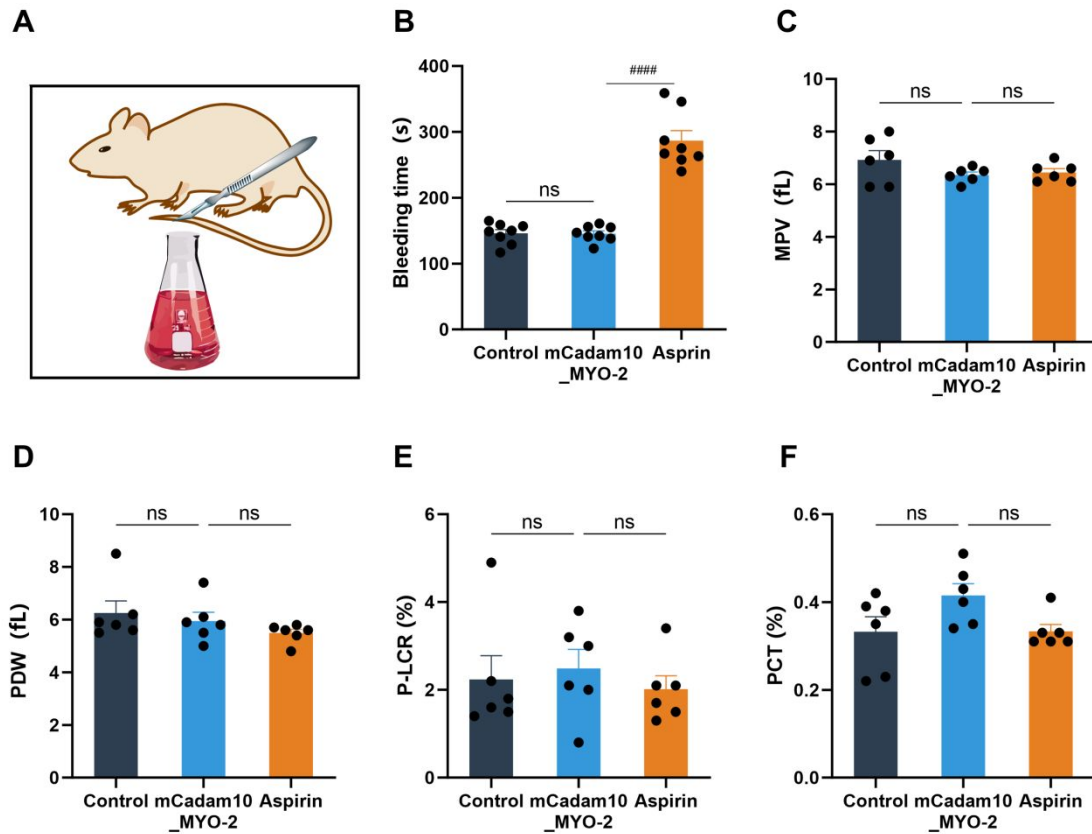


Figure 6 Effects of mCad10_MYO-2 on hemostasis and platelet indices

A: Schematic of tail transection bleeding assay. B: Bleeding time in mice administered vehicle, mCad10_MYO-2 (10 mg/kg), or aspirin (10 mg/kg) 3 minutes before surgery ($n = 8$). C–F: Platelet indices after daily administration of vehicle, mCad10_MYO-2, or aspirin for 7 days: mean platelet volume (MPV, C), platelet distribution width (PDW, D), platelet-large cell ratio (P-LCR, E), and plateletcrit (PCT, F). For platelet index analysis, both mCad10_MYO-2 and aspirin were administered at 10 mg/kg ($n = 6$). Data are presented as mean $\pm$ SEM. ns, not significant. #### $P < 0.0001$ versus the aspirin group, as indicated.

779 **Supplementary Data:**

780

781 1. Supplementary Figure S1-S4

782 2. Raw BLI sensorgrams of the interaction between mCad10_MYO-2 and recombinant

783 GPVI

784 3. Extended molecular dynamics analyses of the mCad10_MYO-2–GPVI complex

785 4. DNA map and sequence of GPVI

786 5. HPLC and MS spectrometry of three peptides

787 6. Original data of mCad10_MYO-2 inhibiting the collagen-induced platelet aggregation

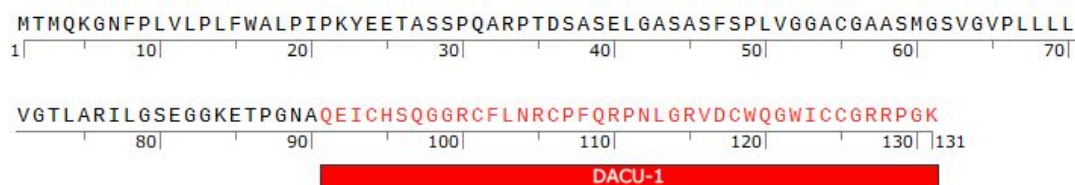
788 7. Original data of blood flow occlusion rate in the electrical injury-induced carotid artery
789 thrombosis assay

790



791 **Supplementary Figure**

792 A.



793

794 B.



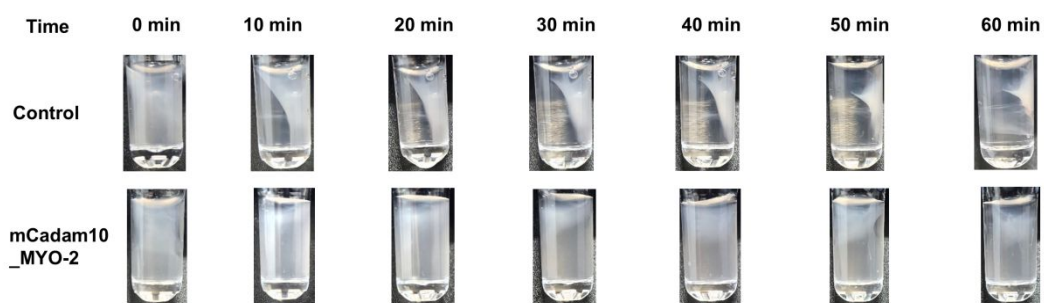
795

796 **Supplementary Figure S1.** Sequence of Dacu19417.1 (A) and Dacu08320.1 (B). DACU-1

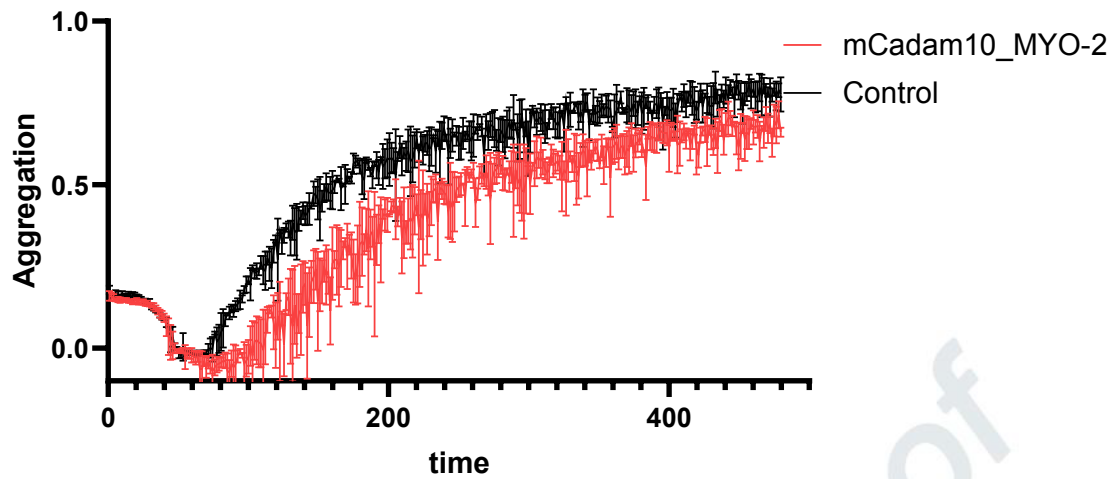
797 and DACU-2 fragments are highlighted in red. Both toxin sequences are predicted from the

798 genome of *Deinagkistrodon acutus*.

799



Supplementary Figure S2. Representative images of clot retraction at the indicated time points from 0 to 60 minutes in control and mCadaml0_MYO-2 (50 μ M) treated samples;

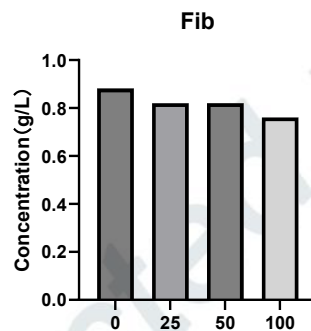
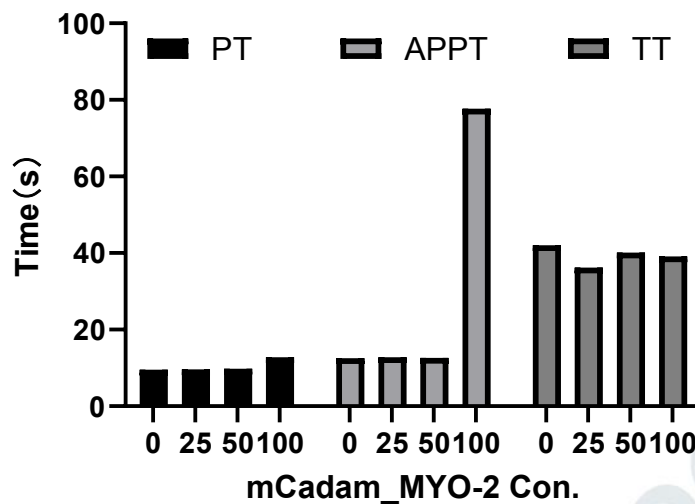


805

806 **Supplementary Figure S3** Aggregation traces of PRP prepared from with PBS or

807 mCad10_MYO-2 treated rat. Platelet aggregation was induced by collagen at 5 $\mu\text{g/mL}$.

808



Supplementary Figure S4. Effects of mCadamy10_MYO-2 on plasma coagulation parameters. Plasma samples were incubated with increasing concentrations of mCadamy10_MYO-2 peptide (0, 25, 50, and 100 μ M), followed by measurement of prothrombin time (PT), activated partial thromboplastin time (APTT), thrombin time (TT), and fibrinogen concentration (FIB).

819 蛇毒防御素多肽 mCadam10_MYO-2 通过 GPVI 相关信号通路抑制胶原驱动的
820 血小板活化和动脉血栓形成

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829

830 **摘要:** 动脉血栓是缺血性心脑血管疾病的主要诱因, 临床治疗多受到出血风险的限制。
831 血小板胶原受体糖蛋白 VI (Glycoprotein VI, GPVI) 可作为抗血栓干预中极具前景的低
832 出血风险作用靶点。然而, 可调控 GPVI 依赖性血小板活化多肽类分子的研究仍较为缺
833 乏。鉴于蛇毒中含有丰富多样的止血调控活性成分, 本研究鉴定了 3 条来源于东部菱斑
834 响尾蛇 (*Crotalus adamanteus*) 的 mCadam10_MYO-2, 以及来源于尖吻蝾
835 (*Deinagkistrodon acutus*) 的 DACU-1 和 DACU-2 的蛇毒防御素及防御素样多肽, 并进
836 一步评价其抗血小板活性。序列与结构比较分析显示, 3 条多肽具有保守的半胱氨酸框
837 架和 β -防御素样结构。其中, mCadam10_MYO-2 对胶原诱导的血小板聚集表现出最强
838 的抑制作用, 并可减少血小板在胶原表面的铺展、延缓血凝块收缩。机制研究表明, 上
839 述作用与 GPVI 相关信号通路减弱有关, 具体表现为血小板活化相关 RNA 表达特征改
840 变、胶原诱导的 Syk 和 PLC γ 2 磷酸化水平降低, 以及 mCadam10_MYO-2 与重组 GPVI



841 直接结合。此外，mCadaml0_MYO-2 可显著延长电损伤诱导血栓模型中的颈动脉闭塞
842 时间，且在本研究条件下未显著延长断尾出血时间或改变血小板相关指标。综上，上述
843 研究表明 mCadaml0_MYO-2 可抑制 GPVI 介导的血小板活化和血栓形成，为兼顾止血
844 安全性的抗血栓药物研发提供潜在的分子骨架。

845

846 **关键词：**蛇毒，防御素样多肽，动脉血栓，血小板，糖蛋白 VI

uncorrected proof

