Received: 9 February 2024 | Revised: 11 June 2024 | Accepted: 16 July 2024

https://doi.org/10.1016/j.rpth.2024.102524

rpth research & practice in thrombosis & haemostasis

BRIEF REPORT

Transmembrane thiol isomerase TMX1 counterbalances the effect of ERp46 to inhibit platelet activation and integrin αIIbβ3 function

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Handling Editor: Dr Carsten Depperman

Abstract

Background: Previous studies have shown that thiol isomerases such as ERp46 positively regulate platelet function by reducing integrin αIIbβ3 disulfides, and the transmembrane thiol isomerase TMX1 negatively regulates integrin αIIbβ3 activation. However, whether and how the positive and negative thiol isomerases interact with each other and their interactions participate in platelet activation remain unknown.

Objectives: To investigate whether and how TMX1 regulates the effect of ERp46 on platelet function.

Methods: Using ERp46- and TMX1-deficient platelets, anti-TMX1 antibody, and wild-type TMX1 (TMX1-CPAC, TMX1-SS) and inactive TMX1 (TMX1-SPAS, TMX1-OO) proteins, we studied the antagonistic effect of TMX1 on ERp46 in platelet aggregation, clot retraction, and integrin αIIbβ3 signaling. The underlying mechanisms were further determined using thiol labeling, reductase activity, and other assays.

Results: Anti-TMX1 antibody and TMX1-OO reversed the decreased aggregation of ERp46-deficient platelets induced by thrombin, convulxin, and U46619. Anti-TMX1 antibody reversed the attenuated integrin αIIbβ3 function of ERp46-deficient platelets. TMX1 inhibited ERp46 reductase activity in a concentration-dependent manner. TMX1 oxidized thiols of ERp46 and those of integrin αIIbβ3 generated by ERp46. Moreover, TMX1 deficiency increased free thiols of ERp46 in platelets, which was reversed by the addition of wild-type TMX1 protein. Besides, anti-TMX1 antibody increased free thiols of ERp46 in wild-type activated platelets.

Conclusion: TMX1 not only oxidizes integrin αIIbβ3 disulfides that are reduced by ERp46 but also directly oxidizes ERp46 to suppress its reduction of integrin αIIbβ3. Thus, TMX1 is critical for maintaining platelets in a quiescent state and counterbalancing the effect of ERp46 to prevent platelet overactivation.

KEYWORDS
integrin αIIbβ3, platelet, redox, thiol isomerase, TMX1

Essentials

- Thiol isomerase ERp46 enhances platelet function and thrombosis, and TMX1 has a negative role.
- We investigated whether and how TMX1 regulates the effect of ERp46 on platelet function.
- TMX1 inhibition reversed ERp46-deficient platelet function defects; TMX1 oxidized ERp46.
- TMX1 counterbalances the effect of ERp46 to prevent platelet overactivation.

1 | INTRODUCTION

Thiol isomerases contain a thioredoxin-like domain with active motif Cys-X-X-Cys and play a pivotal role in catalyzing thiol-disulfide exchange reactions [1]. The formation of free thiols in platelet integrin αIIbβ3 is required for conformational change for higher affinity binding of fibrinogen [2,3]. Using genetically modified mouse models, we and others found that thiol isomerases such as ERp46, PDI, ERp57, and ERp72 enhance platelet function by reducing integrin αIIbβ3 disulfide bonds [4–14]. ERp46, PDI, ERp57, and ERp72 are secreted from activated platelets and bind to platelet surface integrin αIIbβ3. Subsequently, they reduce integrin αIIbβ3 disulfides resulting in its conformational change, supporting platelet aggregation and platelet thrombus formation [3,4,6–14]. Moreover, we also found that in contrast to these positive thiol isomerases, the transmembrane member TMX1 with an active-site CPAC motif oxidizes integrin αIIbβ3 disulfides and inhibits platelet function [15,16]. Thus, we propose that TMX1 maintains integrin αIIbβ3 in an oxidized form and prevents its activation in resting platelets [1]; when platelets become activated by receptor agonists, the positive thiol isomerases are released and counteract the effect of TMX1 to reduce integrin αIIbβ3 disulfides [1].

Although the previous studies suggest that TMX1 and positive thiol isomerases constitute "off-on" redox switches regulating integrin αIIbβ3 function [1], whether and how TMX1 counteracts positive thiol isomerases on platelet integrin αIIbβ3 activation remain unknown. Notably, ERp46 with 3 CGHC redox-active sites was found to be the most efficient in catalyzing disulfide reaction [17] and exhibited higher reductase activity than PDI and ERp57 [18]. Therefore, this study aimed to explore whether TMX1 regulates the role of ERp46 in platelet activation and integrin αIIbβ3 function. We employed ERp46-deficient platelets, TMX1-deficient platelets, and TMX1 inhibitors in conjunction with thiol-labeling approaches. We found that TMX1 oxidizes both ERp46-reduced integrin αIIbβ3 and ERp46 itself, thereby antagonizing its role of ERp46 in platelet activation and integrin αIIbβ3 function.

2 | METHODS

Materials and methods are detailed in Supplementary Materials and Methods.

3 | RESULTS AND DISCUSSION

3.1 | Generation and characterization of ERp46-knockout mice

We generated ERp46-knockout (KO; $ERp46^{-/-}$) mice using the KO-first conditional-ready strategy (Supplementary Figure S1A). The germline-transmitted targeted allele was confirmed by genotyping (Supplementary Figure S1B). In ERp46-KO platelets, the absence of ERp46 mRNA was shown by reverse transcription PCR, and the expression of other thiol isomerases PDI, ERp5, ERp57, and ERp72 remained comparable with those of wild-type (WT) mouse platelets (Supplementary Figure S1C). As detected by immunoblotting, ERp46 protein was not expressed in platelets and white blood cells of $ERp46^{-/-}$ mice (Supplementary Figure S1D). These data indicate the successful deletion of the ERp46 gene in mice.

3.2 | Inhibition of TMX1 reverses the decreased platelet function caused by ERp46 deficiency

Using the specific inhibitory anti-TMX1 antibody (Ab; B01P), recombinant WT extracellular domain of WT TMX1 (CPAC, TMX1-SS), and inactive extracellular domain of mutant TMX1 (SPAS, TMX1-OO) [15], we investigated whether TMX1 regulates platelet function by interacting with ERp46. First, we found that compared with $ERp46^{+/+}$ platelets, $ERp46^{-/-}$ platelets had decreased aggregation induced by convulxin, thrombin, and U46619 (Figure 1A–D). As we reported previously [15], the anti-TMX1 Ab B01P (15 μg/mL) enhanced convulxin-induced WT mouse platelet aggregation, and we used this Ab for activity and characterization assays. Inhibition of TMX1 by this Ab reversed the decreased aggregation of $ERp46^{-/-}$ platelets in response to 16 and 21.5 ng/mL of convulxin (Figure 1A, B). When platelets were stimulated with a higher concentration of convulxin (40 ng/mL), the anti-TMX1 Ab did not have a significant effect (Supplementary Figure S2A), suggesting that overactivated platelets secrete high levels of prothrombotic thiol isomerases including ERp46, the effect of which could not be reversed by TMX1 inhibition. Similarly, 15 μg/mL of anti-TMX1 Ab also significantly reversed the decreased ERp46-deficient aggregation in response to thrombin and U46619 (Figure 1C, D), while the low concentration of this Ab at

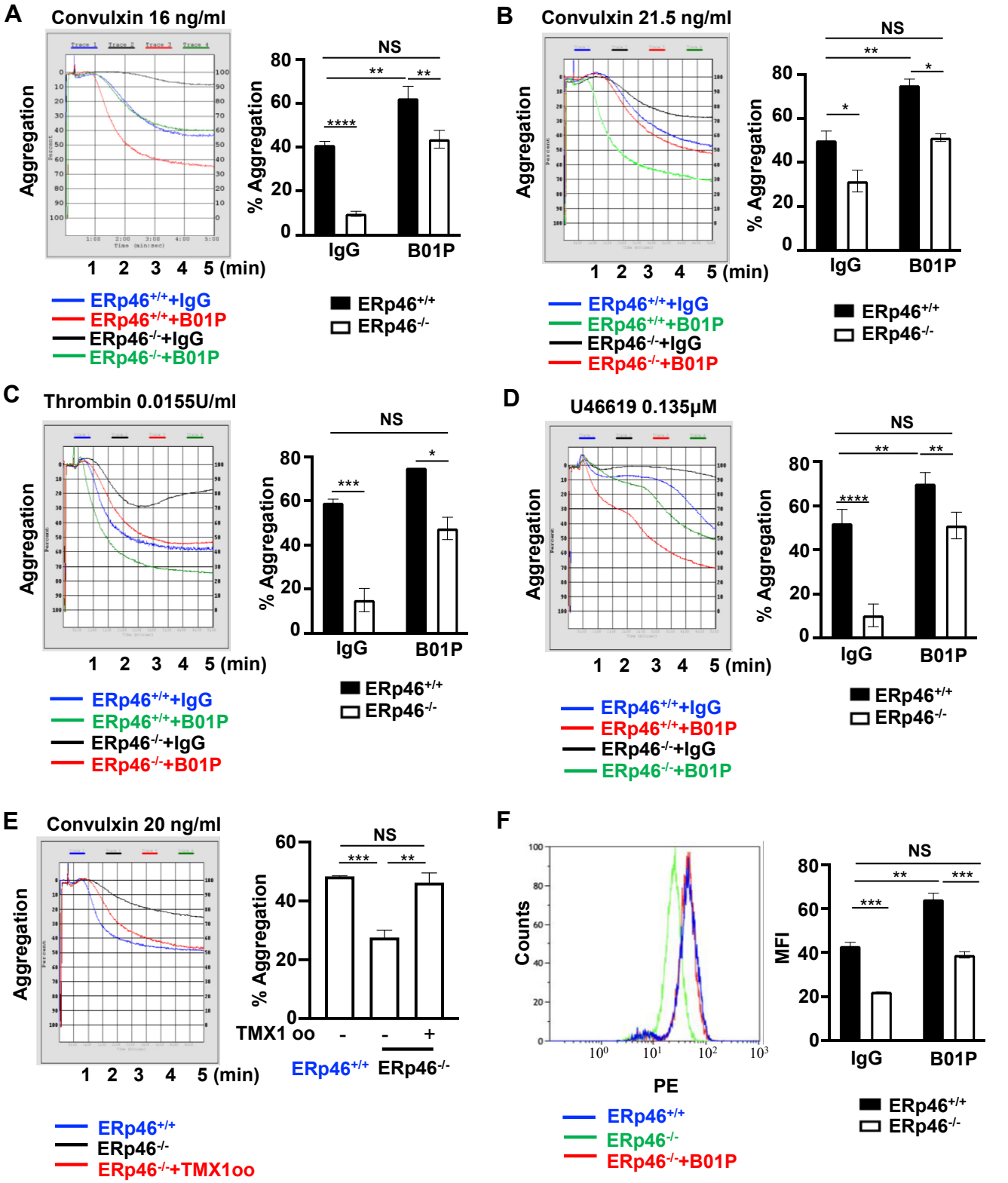

FIGURE 1 Inhibition of TMX1 reverses the decreased activation of ERp46-deficient platelets in response to various stimuli. (A–D) The effect of a monoclonal anti-TMX1 antibody B01P on aggregation of ERp46$^{+/+}$ platelets and ERp46$^{-/-}$ platelets stimulated by different concentrations of (A, B) convulxin, (C) thrombin, and (D) U46619. B01P (15 μg/mL) was added 5 minutes prior to stimulation. Isotype immunoglobulin G (IgG; 15 μg/mL) was used as a control. Representative aggregation curves (left) and combined data (right) are shown. Data are shown as mean ± SEM, n = 3. (E) The effect of recombinant protein expressing the inactive TMX1 extracellular domain (TMX1-OO) on aggregation of ERp46$^{+/+}$ platelets and ERp46$^{-/-}$ platelets. TMX1-OO (2 μM) was added 5 minutes prior to stimulation. Representative aggregation curves (left) and combined data (right) are shown. Data are shown as mean ± SEM, n = 4. (F) ERp46$^{+/+}$ platelets and ERp46$^{-/-}$ platelets were pretreated with 15 μg/mL of control IgG or B01P for 5 minutes, followed by stimulation with 100 ng/mL of convulxin. JON/A binding was measured by flow cytometry. Left panel shows a representative histogram; right panel shows combined results. Data are shown as mean ± SEM, n = 4. MFI, mean fluorescence intensity; NS, not significant. *P < .05; **P < .01; ***P < .001; ****P < .0001, analysis of variance.

5 μg/mL had no effect (Supplementary Figure S2B). These data indicate that TMX1 inhibition–mediated reversal of ERp46-deficient platelet aggregation defect is independent of primary receptor stimulation. TMX1-OO protein had a similar augmentation effect with anti-TMX1 Ab on decreased aggregation of ERp46-deficient platelets (Figure 1E), suggesting that the absence of ERp46 renders TMX1 with stronger inhibition of integrin αIIbβ3, and the inactive TMX1-OO protein competitively inhibits endogenous TMX1. Besides, the anti-TMX1 Ab also reverses the decreased integrin αIIbβ3 activation (JON/A binding) of ERp46-deficient platelets (Figure 1F). Together, TMX1 counterbalances the function of ERp46 in platelet aggregation and integrin αIIbβ3 activation.

3.3 | Anti-TMX1 Ab accelerates the delayed clot retraction of ERp46-deficient platelets

When platelets become activated, the binding of fibrinogen to activated integrin αIIbβ3 triggers outside-in signaling of integrin αIIbβ3 leading to intracellular activation events, which mediates irreversible cytoskeletal reorganization and clot retraction [19]. Thus, we evaluated whether inhibition of TMX1 by anti-TMX1 Ab also affects clot retraction of ERp46-deficient platelets. As shown in Figure 2, the anti-TMX1 Ab enhanced clot retraction of WT platelets; ERp46 deficiency increased clot size and delayed clot retraction, but anti-TMX1 Ab accelerated the delayed clot retraction of ERp46-deficient platelets, indicating that TMX1 has an antagonistic effect on ERp46-enhanced integrin αIIbβ3 outside-in signaling.

3.4 | TMX1 inhibits ERp46 reductase activity and oxidizes its disulfide formation

To explore the mechanism underlying the antagonizing effect of TMX1, we evaluated the impact of TMX1 on the reductase activity of ERp46. As shown in Figure 3A, ERp46 protein had strong reductase activity assessed by cleavage of Di-E-GSSG substrate. In a concentration-dependent fashion, TMX1 markedly decreased ERp46 cleavage of Di-E-GSSG in a cell-free system (Figure 3A), indicating that TMX1 directly inhibits the reductase activity of ERp46. Since TMX1 is a thiol oxidase [15], we next tested whether TMX1 inhibition of ERp46 activity is associated with its oxidation of ERp46 disulfide. In a purified system, TMX1 strongly oxidized free thiols of ERp46 (Figure 3B), whereas it had no effect on albumin thiols (Figure 3C). Moreover, compared with WT platelets, the TMX1-null platelets had increased thiols of ERp46 secreted from activated platelets and the addition of TMX1-SS abolished the increase (Figure 3D), indicating that TMX1 oxidizes ERp46 in platelets, which is consistent with its inhibition of ERp46 reductase activity (Figure 3A). More interestingly, compared with control immunoglobulin G, B01P increased free thiols of ERp46 in WT activated platelets (Figure 3E), which accounts for B01P reversing the decreased platelet function of ERp46-deficient platelets (Figures 1 and 2).

3.5 | TMX1 oxidizes integrin αIIbβ3 disulfides that are reduced by ERp46

As TMX1 oxidizes integrin αIIbβ3 [15], we evaluated whether TMX1 oxidizes integrin αIIbβ3 disulfides that are reduced by ERp46. Shown by 3-(N-Maleimidylpropionyl) biocytin labeling, ERp46 reduced integrin αIIbβ3 disulfides and the addition of TMX1 completely oxidized integrin αIIbβ3 disulfides reduced by ERp46 (Figure 4A), suggesting that ERp46 and TMX1 target the same integrin αIIbβ3 disulfides but have opposite reduction and oxidation effects, respectively. Similarly, when ERp46 was oxidized by TMX1, the oxidized ERp46 lost its activity to reduce integrin αIIbβ3 (Figure 4B). Moreover, free integrin αIIbβ3 thiols were observed in resting platelets as previously reported [20–22]. When ERp46 was incubated with platelets, it reduced integrin αIIbβ3; however, the addition of TMX1 not only oxidized this integrin but also prevented the effect of ERp46 on the reduction of integrin αIIbβ3 (Figure 4C). Possibly, TMX1 oxidizes integrin αIIbβ3 through 2 mechanisms: direct oxidation of integrin αIIbβ3 by itself and indirect oxidation by converting reduced ERp46 to oxidized ERp46 (Supplementary Figure S3) thereby counteracting the effect of ERp46 on reduction of integrin αIIbβ3 disulfides (Supplementary Figure S4).

We and others have previously shown that prothrombotic thiol isomerases exhibit reductase activity toward integrin αIIbβ3, facilitating the formation of a high fibrinogen binding affinity conformation [10–14,23]. ERp46 processes 3 catalytically active motifs [18,24,25] and exhibits higher disulfide reductase activity compared with other thiol isomerases [18]. ERp46 deficiency attenuated platelet aggregation and integrin αIIbβ3 activation [14] (Figure 1), supporting that ERp46 potentiates platelet aggregation via reduction of integrin αIIbβ3 disulfides [14]. In this study, we found that TMX1 antagonizes the role of ERp46 in both inside-out and outside-in signaling of integrin αIIbβ3, evident by the observations that the anti-TMX1 Ab B01P and TMX1-OO reversed the decreased aggregation, integrin αIIbβ3 activation, and clot retraction of ERp46-deficient platelets. In a reductase assay, TMX1 directly diminishes the reductase activity of ERp46 in a concentration-dependent fashion (Figure 3), consistent with the fact that disulfide exchange exists between 2 thiol isomerases [17,26,27]. TMX1 oxidized thiols of ERp46 at the purified protein level and activated platelets (Figures 3 and 4), implying that TMX1 oxidizes ERp46 disulfides, which accounts for its suppression of ERp46 reductase activity and the positive role of ERp46 in platelet function.

Our findings demonstrate that TMX1 inhibits platelet activation by antagonizing the effects of ERp46, providing the first evidence that the interaction between positive and negative thiol isomerases offers fine control of integrin αIIbβ3 activation. Previous studies have shown that integrin αIIbβ3 is the main substrate for both positive and negative thiol isomerases [1]. Our current new observations, together with our previous report [15], raise a possibility that negative thiol isomerase TMX1 and positive thiol isomerase ERp46 regulate the same functional disulfides with opposite effects. Likely, TMX1 regulates integrin activation through 2 mechanisms: (1) direct oxidation of

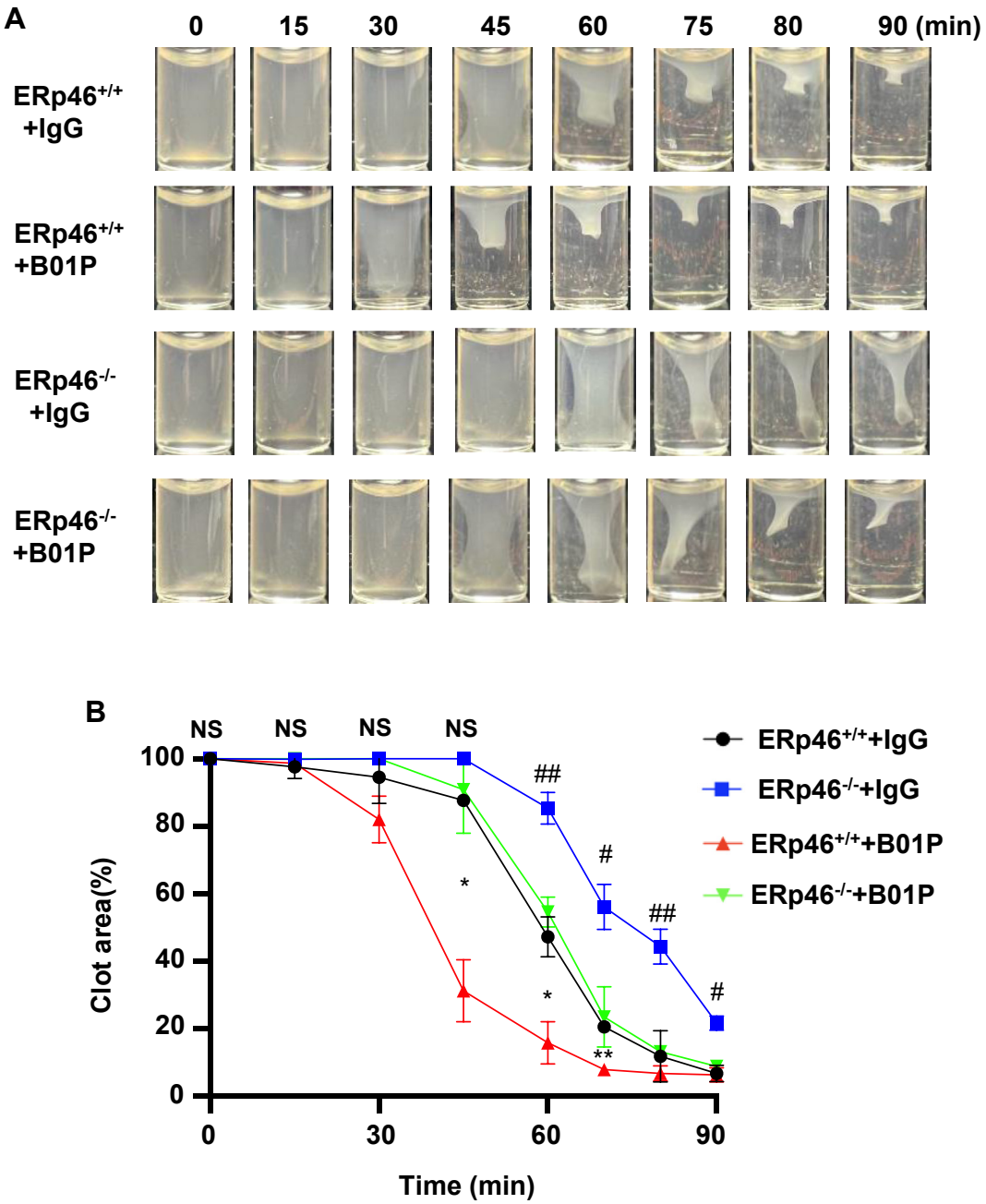

FIGURE 2 Effect of anti-TMX1 antibody on clot retraction of ERp46-deficient platelets. (A) Washed ERp46$^{+/+}$ platelets and ERp46$^{-/-}$ platelets (3×10^8/mL) suspended with 50 μL of normal mouse plasma in Tyrode's buffer were incubated with control immunoglobulin G (IgG) or B01P (15 μg/mL) for 10 minutes at 37 °C. Clot retraction was initiated with thrombin (0.05 U/mL), and images were taken at the indicated times. (B) Clot retraction was assessed by measurement of clot area over the time points. Data are shown as mean ± SEM, n = 3. NS, not significant. *$P < .05$; **$P < .01$; $^{\#}P < .05$; $^{\#\#}P < .01$; *between groups of ERp46$^{+/+}$ + IgG and ERp46$^{+/+}$ + B01P; $^{\#}$between groups of ERp46$^{-/-}$ + IgG and ERp46$^{-/-}$ + B01P, analysis of variance

integrin αIIbβ3 (ERp46-independent) and (2) indirect regulation of integrin αIIbβ3 through oxidation of ERp46, thus decreasing ERp46 reduction of integrin αIIbβ3 disulfides (ERp46-dependent). Thus, inhibition of TMX1 may have 2 effects: reducing integrin αIIbβ3 disulfides (ERp46-independent) and keeping ERp46 in reduced form (ERp46-dependent). The former possibility may explain why inhibition of TMX1 augments platelet aggregation in the absence of ERp46 and the absence of ERp46 enables TMX1 to have stronger inhibition of integrin αIIbβ3, which, however, was reversed by TMX1 inhibitors.

The cross talk between TMX1 and ERp46 supports our hypothesis on the "yin and yang" balance, formed by positive and negative thiol isomerases controlling platelet activation [1]. On the surface of quiescent platelets, TMX1 maintains integrin αIIbβ3 at a low ligand binding affinity state by oxidizing its thiols when there is no vascular

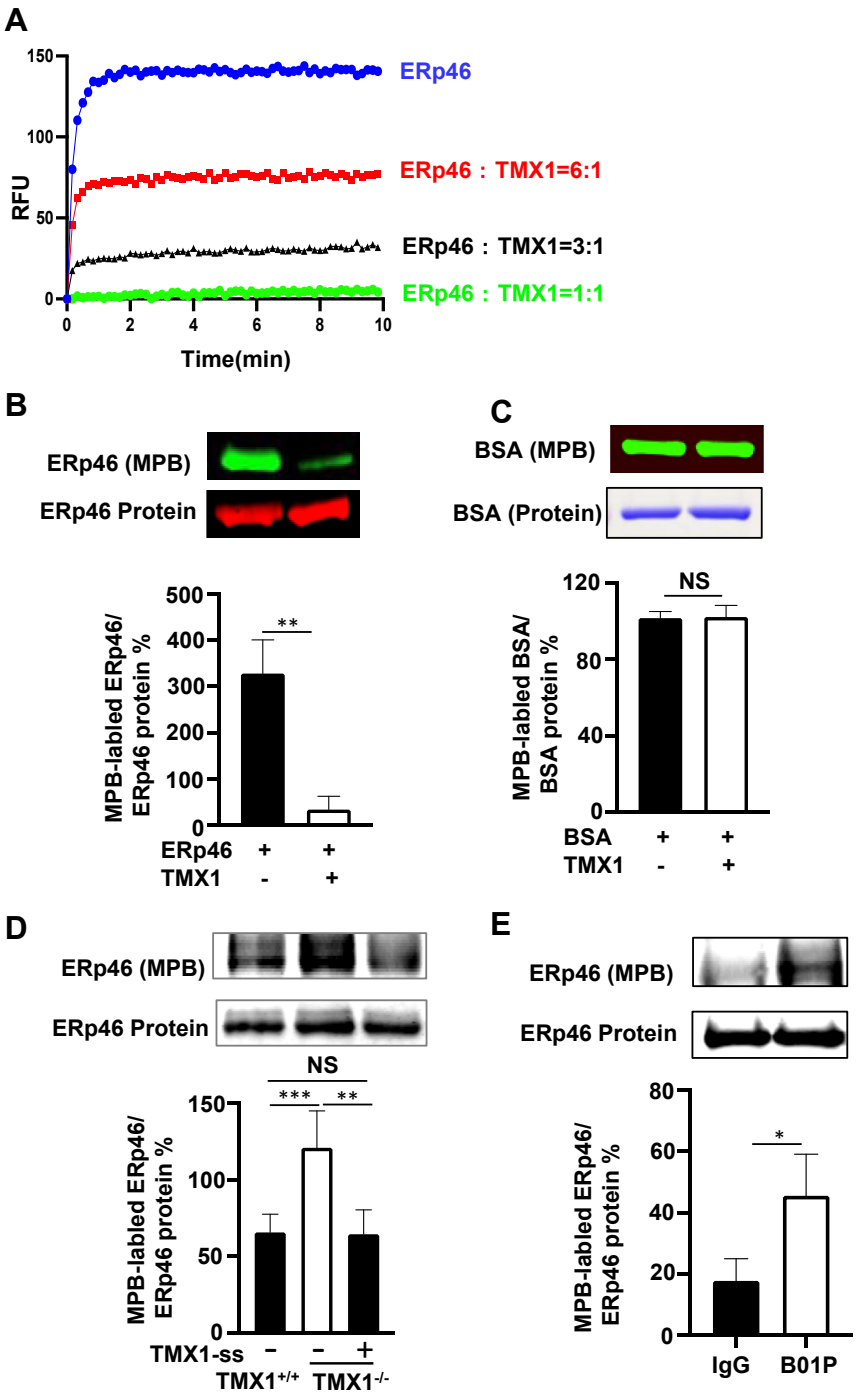

FIGURE 3 TMX1 inhibits the reductase activity of ERp46 and oxidizes ERp46 disulfides. (A) The effect of TMX1 on reductase activity of ERp46 in Di-E-GSSG assay. TMX1 at various concentrations was incubated with ERp46 for 10 minutes at 37 °C before the addition of Di-E-GSSG (150 nM). Phosphate Buffered Solution (PBS) was used as the control. (B, C) The effect of TMX1 on thiols of (B) ERp46 and (C) Bovine Serum Albumin (BSA). ERp46 or BSA (1 μM) was incubated with or without 1 μM of TMX1 at 37 °C for 30 minutes, followed by thiol labeling with 20 μM 3-(N-Maleimidylpropionyl) biocytin (MPB). Labeled thiols in ERp46 and BSA were detected by blotting with fluorescence-conjugated streptavidin. (B) The membranes were also blotted for total ERp46 protein as loading control. (C) The loading of BSA was verified by Coomassie blue staining. Representative blot (upper) and cumulative data (lower). Data are shown as mean ± SEM, (B) $n = 4$ and (C) $n = 3$. $^{**}P < .01$, *t*-test. (D) TMX1 decreases the thiol labeling of ERp46 released from activated platelets. Washed platelets (10^9) from $TMX1^{+/+}$ and $TMX1^{-/-}$ mice were incubated with 2 μM of TMX1-ss or not, followed by stimulation with 0.05 U/mL thrombin. After platelets were lysed, 100 μM MPB was added to label thiols in ERp46. ERp46 was immunoprecipitated, and the labeled thiols were detected by blotting with fluorescence-conjugated streptavidin. Isotype immunoglobulin G (IgG) was used as the nonimmune control in immunoprecipitation. The membranes were reprobed with anti-ERp46 antibody, and the MPB label was normalized to the protein density. Representative blot (upper) and cumulative data (lower). Data are shown as mean ± SEM, $n = 4$. $^{**}P < .01$; $^{***}P < .001$, 1-way analysis of variance. (E) B01P increased the thiol labeling of ERp46 in activated platelets. Wild-type platelets (10^9) were incubated with 30 μg/mL of B01P or isotype IgG for 10 minutes. After stimulation with 0.05 U/mL of thrombin, MPB labeling was performed as described above. The intensity of each band was calculated using the Image J program, and the ratio of MPB label to protein intensity was compared. Data are shown as mean ± SEM. NS, not significant. $^{*}P < .05$, *t*-test. RFU, relative fluorescence unit.

injury. When platelets are activated, positive thiol isomerases like ERp46 are secreted and bind to integrin αIIbβ3, subsequently cleaving its disulfides, causing the conformational change with high ligand binding affinity. Meanwhile, the expression and oxidase activity of TMX1 increase on activated platelets [15], and TMX1 directly oxidizes integrin αIIbβ3 disulfides that are reduced by ERp46; on the other hand, TMX1 oxidizes ERp46 to suppress its reduction activity on integrin αIIbβ3, which may prevent platelet overactivation and

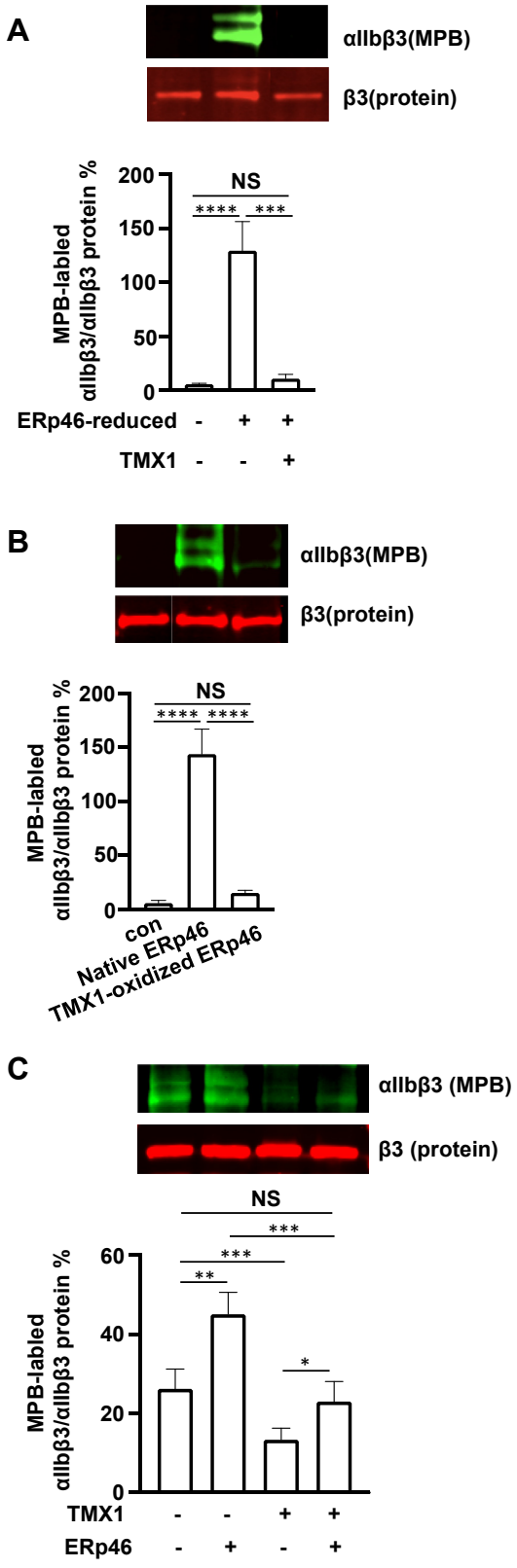

FIGURE 4 TMX1 inhibits ERp46 reduction of integrin αIIbβ3 disulfides. (A) One microgram of purified integrin αIIbβ3 protein was incubated with ERp46-conjugated Ni Sepharose beads (GE healthcare) or control empty beads at 37 °C for 30 minutes. After removal of the beads, ERp46-reduced integrin αIIbβ3 was incubated with 2.5 μM TMX1 at 37 °C for 30 minutes. The samples were labeled by 3-(N-Maleimidylpropionyl) biocytin (MPB) and analyzed as described above. The membranes were reprobed with the anti-β3 antibody and the MPB-labeled integrin αIIbβ3 was normalized to total protein. Representative blot (upper) and cumulative data (lower). Data are shown as mean ± SEM, $n = 4$. ***$P < .001$; ****$P < .0001$, 1-way analysis of variance (ANOVA). (B) TMX1 oxidation of ERp46 in regulation of αIIbβ3 thiol-disulfide exchange. ERp46 (2.5 μM) was incubated with TMX1 fused with Glutathione (GST) in Sepharose beads or control beads at 37 °C for 30 minutes. After removal of the beads, the TMX1-oxidized ERp46 was incubated with 1 μg purified αIIbβ3 protein at 37 °C for 30 minutes. Integrin αIIbβ3 thiols were labeled with MPB and analyzed as described above. Representative blot (upper) and cumulative data (lower). Data are shown as mean ± SEM, $n = 4$. ****$P < .0001$, 1-way ANOVA. (C) Effect of ERp46 and TMX1 on the redox state of αIIbβ3 in human platelets. Human platelets were incubated with PBS (lane 1), 2 μM ERp46 (lane 2), 2 μM TMX1 (lane 3), and ERp46 followed by incubation of TMX1 (lane 4) at 37 °C for 30 minutes, followed by labeling with 100 μM MPB. After platelet lysis, integrin αIIbβ3 was immunoprecipitated. Labeled integrin αIIbβ3 thiols were detected by blotting with fluorescence-conjugated streptavidin. The membranes were reprobed for total β3 protein. The band intensity in the blots was calculated using the ImageJ software (NIH). Representative blot (upper) and cumulative data (lower). Data are shown as mean ± SEM. NS, not significant. *$P < .05$; **$P < .01$; ***$P < .001$; ****$P < .0001$, 1-way ANOVA

subsequent unnecessary platelet thrombus formation (Supplementary Figure S4).

Although our data demonstrate a critical role for TMX1 regulation of ERp46 in platelet activation, we need to resolve several remaining outstanding issues. First, our further investigations identifying specific disulfides of both αIIb and β3 subunits that are targeted by TMX1 and ERp46, and the ERp46 CXXC motifs that are targeted by TMX1 are underway. Second, more effects are needed to characterize how TMX1 interacts with ERp46 platelet surface, which seems to be a transient and complex process. Third, although TMX1 and ERp46 interaction is shown at protein and platelet levels, the biological importance of their interaction needs to be demonstrated using *in vivo* studies. Moreover, besides ERp46, other thiol isomerases such as ERp57, ERp72, and PDI also contribute to integrin αIIbβ3 activation via similar reduction activity on its disulfides. Our current study has motivated us to further investigate whether TMX1 has a general role in the regulation of these enzymes in another study.

In conclusion, this study demonstrates that TMX1 counterbalances the effect of ERp46, providing the first evidence that TMX1 and ERp46 form the "yin-yang" redox balance controlling integrin αIIbβ3 activation [1]. This new finding not only provides new insights into the crucial redox network of thiol isomerases in platelet activation but will also promote further investigation of contribution of other thiol isomerases to the complex network formation as well as their cross-talk mechanism. Better knowledge of the role of thiol isomerases in platelet activation will facilitate our understanding of the contribution of the disordered redox network in thrombotic and hemostatic disease states.

FUNDING

This work was supported by grants from the National Natural Science Foundation of China (81970128, 82200147, 82170129, 81770138, 82270136, 31970890, 82020108003, and 8217011021), the China Postdoctoral Science Foundation (2021TQ0231 and 2022M722322), the Jiangsu Funding Program for Excellent Postdoctoral Talent, the Jiangsu Provincial Medical Innovation Center (CXZX202201), the Translational Research Grant of National Clinical Research Center for Hematologic Dieseases (2020ZKPA02 and 2020WSA04), the collaboration fund from State Key Laboratory of Radiation Medicine and Protection (GZN1201802), and the Priority Academic Program Development of Jiangsu Higher Education Institutions (PAPD).

AUTHOR CONTRIBUTIONS

Z.Z., Y.C., A.Y., Y.Z., and M.P. performed the experiments. Y.H. and D.W. assisted with experimental design and critical reagents. Z.Z., A.Y., and Y.W. conceived and designed the study and wrote the manuscript.

RELATIONSHIP DISCLOSURE

There are no competing interests to disclose.

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REFERENCES

[1] Wu Y, Essex DW. Vascular thiol isomerases in thrombosis: the yin and yang. *J Thromb Haemost*. 2020;18:2790–800.
[2] Yan B, Smith JW. A redox site involved in integrin activation. *J Biol Chem*. 2000;275:39964–72.
[3] Essex DW, Li M, Miller A, Feinman RD. Protein disulfide isomerase and sulfhydryl-dependent pathways in platelet activation. *Biochemistry*. 2001;40:6070–5.
[4] Jordan PA, Stevens JM, Hubbard GP, Barrett NE, Sage T, Authi KS, et al. A role for the thiol isomerase protein ERP5 in platelet function. *Blood*. 2005;105:1500–7.
[5] Cho J, Furie BC, Coughlin SR, Furie B. A critical role for extracellular protein disulfide isomerase during thrombus formation in mice. *J Clin Invest*. 2008;118:1123–31.
[6] Reinhardt C, von Brühl ML, Manukyan D, Grahl L, Lorenz M, Altmann B, et al. Protein disulfide isomerase acts as an injury response signal that enhances fibrin generation via tissue factor activation. *J Clin Invest*. 2008;118:1110–22.
[7] Holbrook LM, Sasikumar P, Stanley RG, Simmonds AD, Bicknell AB, Gibbins JM. The platelet-surface thiol isomerase enzyme ERp57 modulates platelet function. *J Thromb Haemost*. 2012;10:278–88.
[8] Passam FH, Lin L, Gopal S, Stopa JD, Bellido-Martin L, Huang M, et al. Both platelet- and endothelial cell-derived ERp5 support thrombus formation in a laser-induced mouse model of thrombosis. *Blood*. 2015;125:2276–85.
[9] Holbrook LM, Sandhar GK, Sasikumar P, Schenk MP, Stainer AR, Sahli KA, et al. A humanized monoclonal antibody that inhibits platelet-surface ERp72 reveals a role for ERp72 in thrombosis. *J Thromb Haemost*. 2018;16:367–77.
[10] Wu Y, Ahmad SS, Zhou J, Wang L, Cully MP, Essex DW. The disulfide isomerase ERp57 mediates platelet aggregation, hemostasis, and thrombosis. *Blood*. 2012;119:1737–46.
[11] Zhou J, Wu Y, Wang L, Rauova L, Hayes VM, Poncz M, et al. The disulfide isomerase ERp57 is required for fibrin deposition in vivo. *J Thromb Haemost*. 2014;12:1890–7.
[12] Zhou J, Wu Y, Wang L, Rauova L, Hayes VM, Poncz M, et al. The C-terminal CGHC motif of protein disulfide isomerase supports thrombosis. *J Clin Invest*. 2015;125:4391–406.
[13] Zhou J, Wu Y, Chen F, Wang L, Rauova L, Hayes VM, et al. The disulfide isomerase ERp72 supports arterial thrombosis in mice. *Blood*. 2017;130:817–28.
[14] Zhou J, Wu Y, Rauova L, Koma G, Wang L, Poncz M, et al. A novel role for endoplasmic reticulum protein 46 (ERp46) in platelet function and arterial thrombosis in mice. *Blood*. 2022;139:2050–65.
[15] Zhao Z, Wu Y, Zhou J, Chen F, Yang A, Essex DW. The transmembrane protein disulfide isomerase TMX1 negatively regulates platelet responses. *Blood*. 2019;133:246–51.
[16] Hogg PJ. TMX1: a new vascular thiol isomerase. *Blood*. 2019;133:188–90.
[17] Oka OBV, Yeoh HY, Bulleid NJ. Thiol-disulfide exchange between the PDI family of oxidoreductases negates the requirement for an oxidase or reductase for each enzyme. *Biochem J*. 2015;469:279–88.
[18] Funkner A, Parthier C, Schutkowski M, Zerweck J, Lilie H, Gyrych N, et al. Peptide binding by catalytic domains of the protein disulfide isomerase-related protein ERp46. *J Mol Biol*. 2013;425:1340–62.
[19] Tucker KL, Sage T, Gibbins JM. Clot retraction. *Methods Mol Biol*. 2012;788:101–7.
[20] Passam F, Chiu J, Ju L, Pijning A, Jahan Z, Mor-Cohen R, et al. Mechano-redox control of integrin de-adhesion. *eLife*. 2018;7:e151–68.
[21] Pijning AE, Blyth MT, Coote ML, Passam F, Chiu J, Hogg PJ. An alternate covalent form of platelet αIIbβ3 integrin that resides in focal adhesions and has altered function. *Blood*. 2021;138:1359–72.
[22] Wang L, Zhou J, Wang L, Wang CC, Essex DW. The b′ domain of protein disulfide isomerase cooperates with the a and a′ domains to functionally interact with platelets. *J Thromb Haemost*. 2019;17:371–82.
[23] Wang L, Wu Y, Zhou J, Ahmad SS, Mutus B, Garbi N, et al. Platelet-derived ERp57 mediates platelet incorporation into a growing thrombus by regulation of the αIIbβ3 integrin. *Blood*. 2013;122:3642–50.
[24] Sullivan DC, Huminiecki L, Moore JW, Boyle JJ, Poulsom R, Creamer D, et al. EndoPDI, a novel protein-disulfide isomerase-like protein that is preferentially expressed in endothelial cells acts as a stress survival factor. *J Biol Chem*. 2003;278:47079–88.
[25] Knoblach B, Keller BO, Groenendyk J, Aldred S, Zheng J, Lemire BD, et al. ERp19 and ERp46, new members of the thioredoxin family of endoplasmic reticulum proteins. *Mol Cell Proteomics*. 2003;2:1104–19.
[26] Araki K, Iemura S, Kamiya Y, Ron D, Kato K, Natsume T, et al. Ero1-alpha and PDIs constitute a hierarchical electron transfer network of endoplasmic reticulum oxidoreductases. *J Cell Biol*. 2013;202:861–74.
[27] Rowe ML, Ruddock LW, Kelly G, Schmidt JM, Williamson RA, Howard MJ. Solution structure and dynamics of ERp18, a small endoplasmic reticulum resident oxidoreductase. *Biochemistry*. 2009;48:4596–606.

SUPPLEMENTARY MATERIAL

The online version contains supplementary material available at https://doi.org/10.1016/j.rpth.2024.102524