

**ASK1 inhibition triggers platelet apoptosis via p38-MAPK-mediated mitochondrial dysfunction**

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**ASK1 inhibition triggers platelet apoptosis *via* p38-MAPK-mediated mitochondrial dysfunction**

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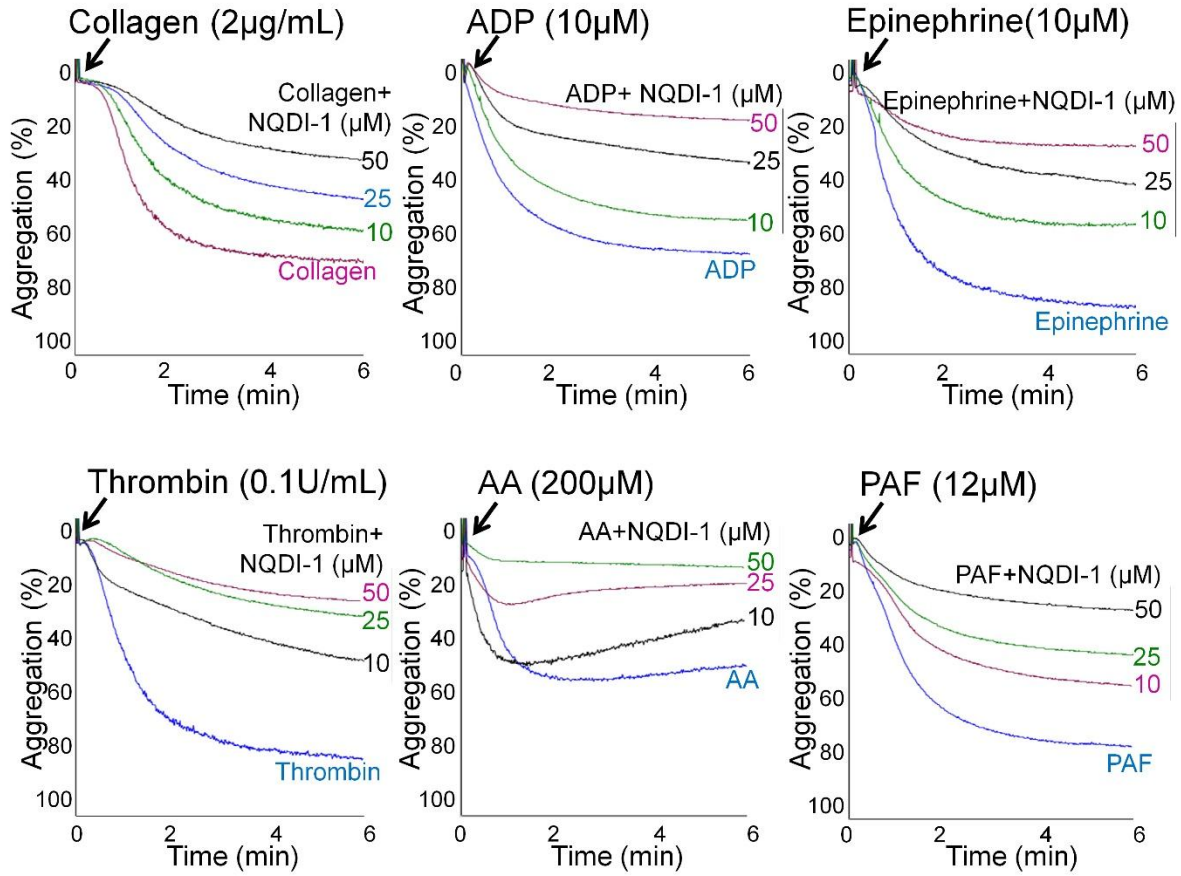
# MK and NSK contributed equally to this work.

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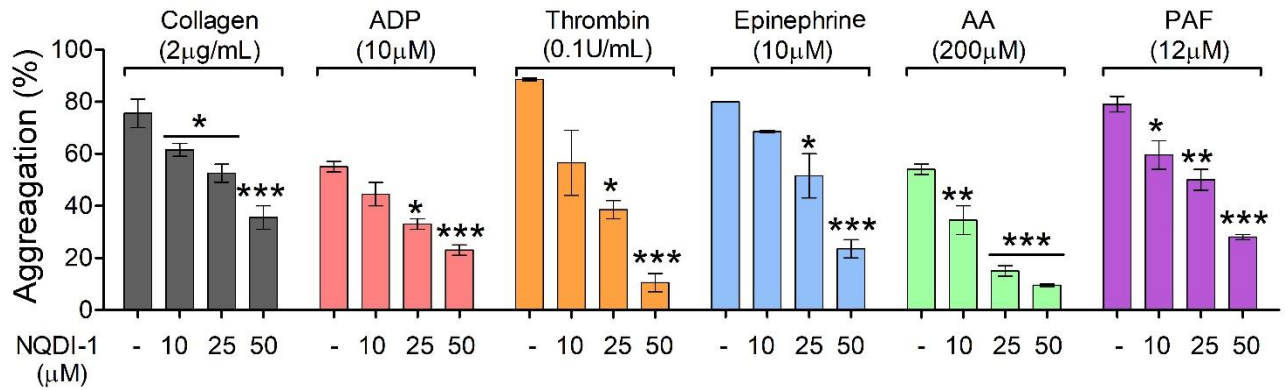
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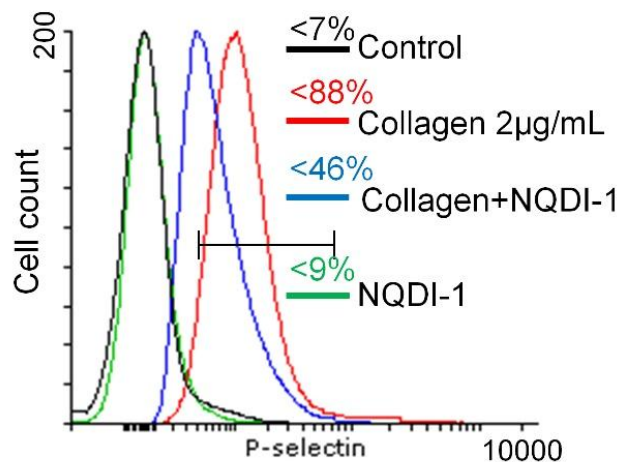
**Supplementary information:**



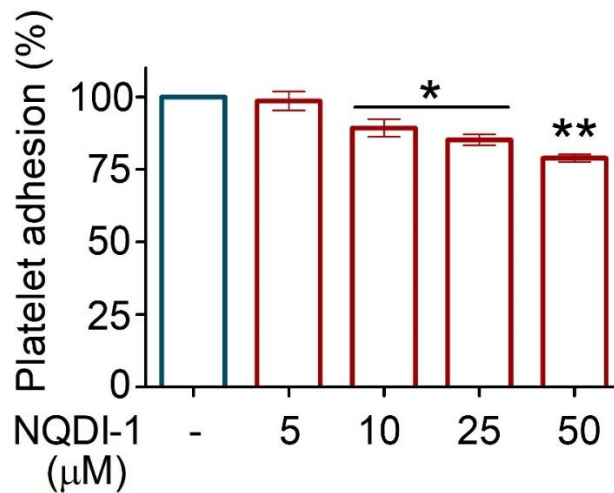
**Figure S1:** Aggregation of washed platelet in various platelet aggregation inducers [Collagen, ADP, Epinephrine, Thrombin, Arachidonic acid, PAF] in presence or absence of various concentration of NQDI-1.



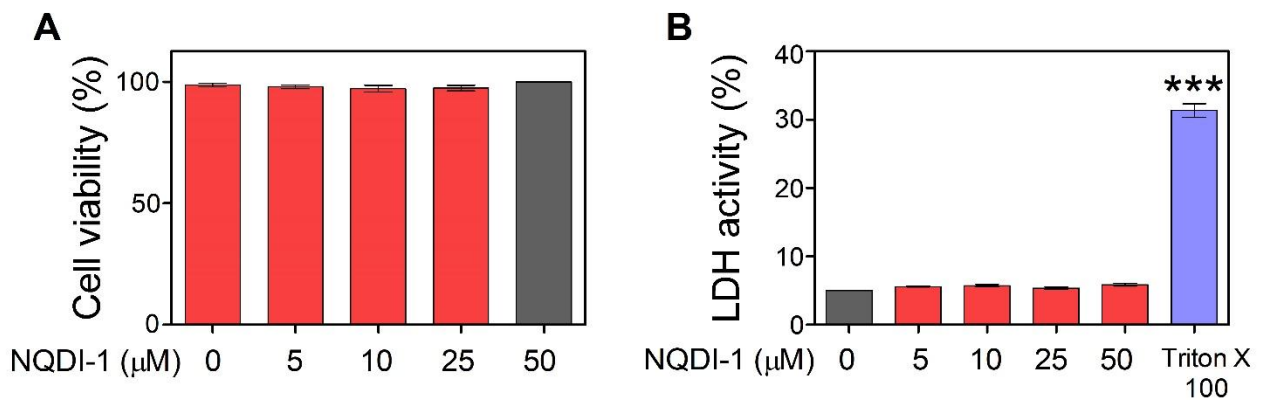
**Figure S2:** Representative bar diagram of platelet aggregation induced by various agonists [Collagen, ADP, Epinephrine, Thrombin, Arachidonic acid, PAF] in presence or absence of various concentration of NQDI-1. Data represented are presented as mean  $\pm$  SEM (n=5), One-way ANOVA followed by Bonferroni *post-hoc* test was used for statistical analysis;  $p^* < 0.05$ ,  $p^{**} < 0.01$ ,  $p^{***} < 0.001$ .



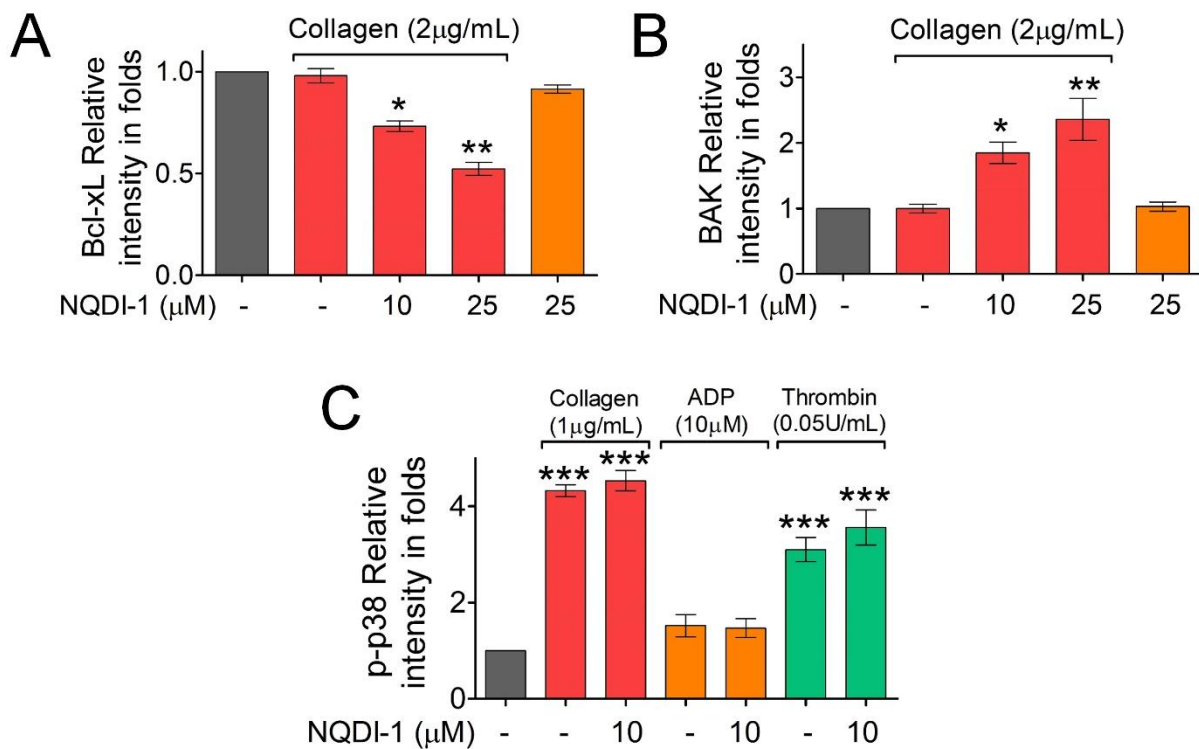
**Figure S3:** Determination of P-selectin levels in collagen-treated platelets pretreated with NQDI-1 (25 μM).



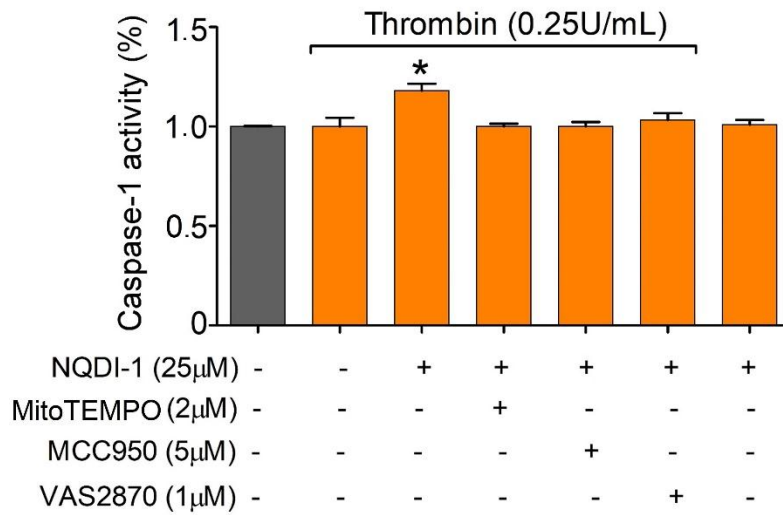
**Figure S4:** Collagen adhesion analysis of platelets treated with various doses of NQDI-1 on immobilized collagen coated wells. Data represented are presented as mean  $\pm$  SEM (n=5). One-way ANOVA followed by Bonferroni *post-hoc* test was used for statistical analysis;  $p^* < 0.05$ ,  $p^{**} < 0.01$ ; \* is significant compared to collagen alone control sample.



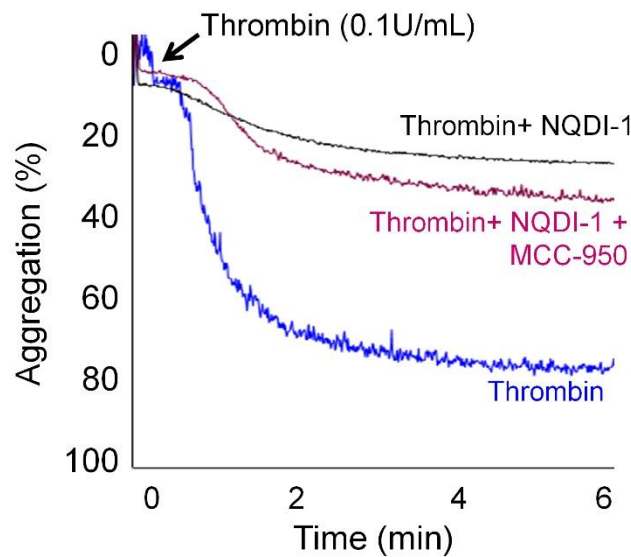
**Figure S5:** Platelet viability assay in various doses of NQDI-1 treated platelets. A) MTT B) LDH activity. Data represented are presented as mean  $\pm$  SEM (n=5). One-way ANOVA followed by Bonferroni *post-hoc* test was used for statistical analysis;  $p^{***} < 0.001$ .



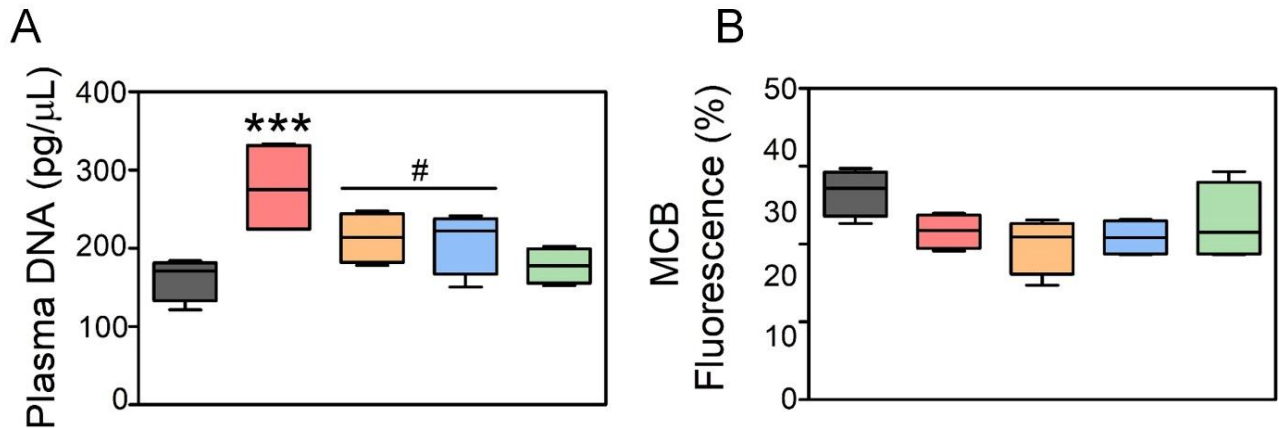
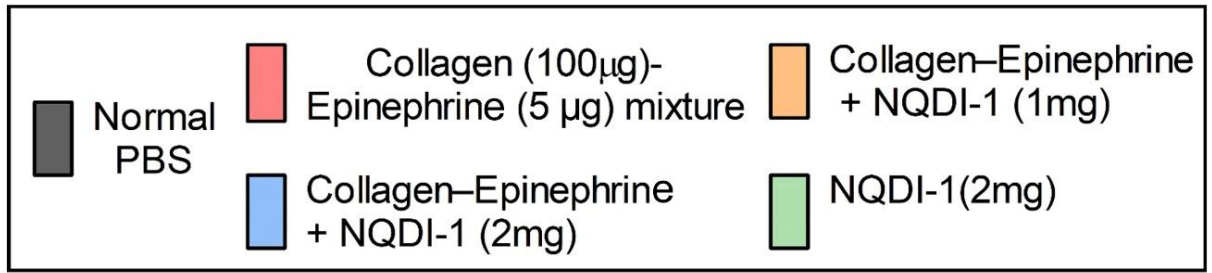
**Figure S6:** Densitometry of immunoblots. A) Densitometry of Bcl-xL, B) BAK (as presented in Figure 2E) measured after normalization with the respective loading controls. C) Densitometry of phospho- p38 (as presented in Figure 2F) levels in treatment with collagen, ADP and thrombin in presence or absence of NQDI-1, measured after normalization with the respective loading controls. Data represented are presented as mean  $\pm$  SEM (n=3). One-way ANOVA followed by Bonferroni *post-hoc* test was used for statistical analysis;  $p^* < 0.05$ ,  $p^{**} < 0.01$ ,  $p^{***} < 0.001$ ; \* is significant compared to untreated cells.



**Figure S7:** Fluorometric analysis of caspase-1 activity using fluorogenic caspase-1 substrate (Ac-YEVD-AMC) in thrombin and NQDI-1 treated platelets pre-treated with MitoTEMPO (Mitochondrial ROS quencher), MCC950 (NLRP3 inhibitor) and VAS2870 (NADPH oxidase inhibitor). Data represented are presented as mean  $\pm$  SEM (n=3). One-way ANOVA followed by Bonferroni *post-hoc* test was used for statistical analysis;  $p < 0.05$ ; \* is significant compared to untreated cells.

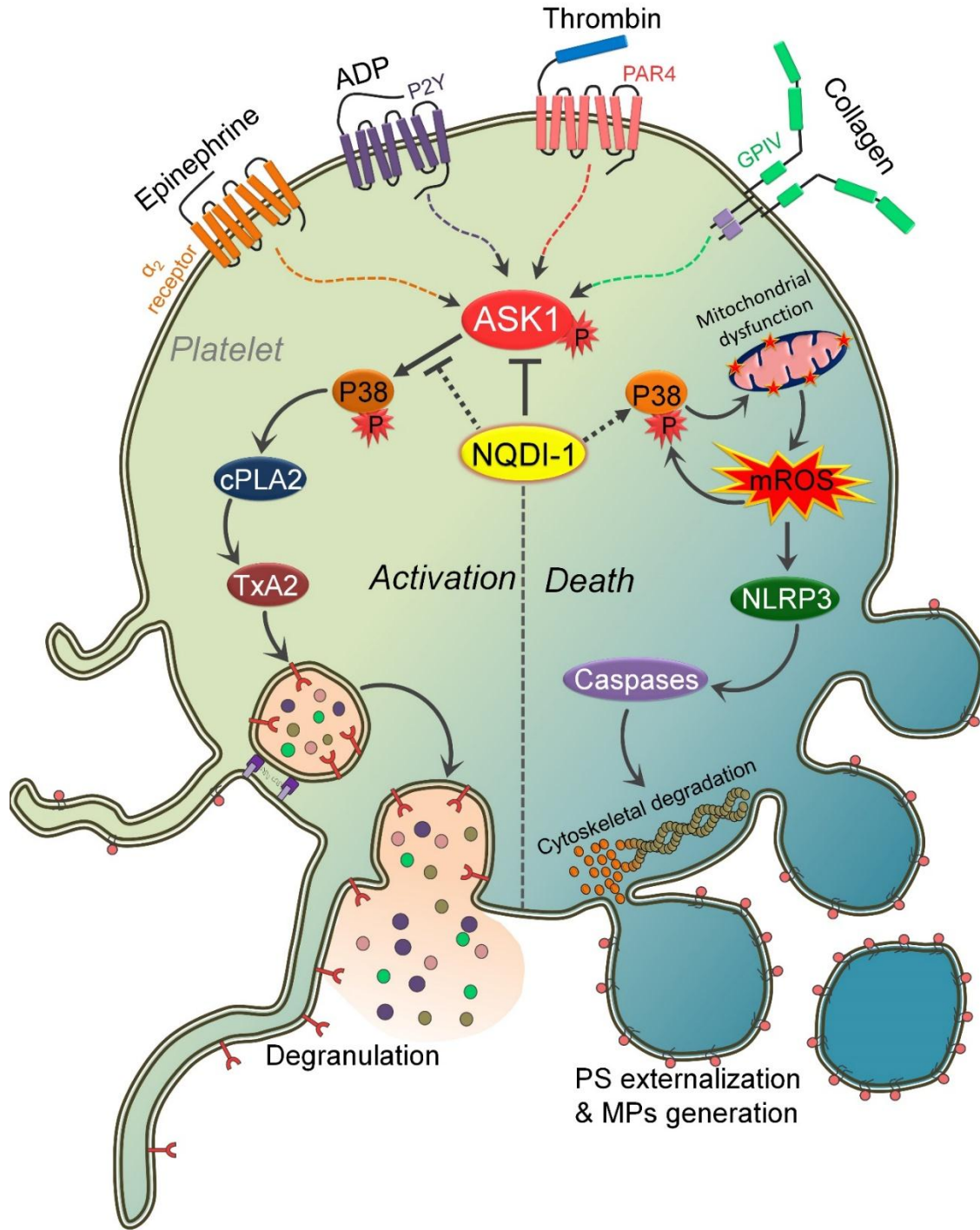


**Figure S8:** Assessment of platelet aggregation in thrombin-treated washed platelets pre-treated with NQDI-1 and MCC- 950 (NLRP3 inhibitor).



**Figure S9: Estimation of plasma DNA and intracellular glutathione levels:** (A) Quantification of cell free DNA in plasma obtained from experimental mice using Pico green fluorophore and (B) Fluorometric analysis of reduced cellular GSH in platelets obtained from experimental mice using MCB. Data represented are presented as mean  $\pm$  SEM (n=5). One-way analysis of variance, followed by Dunnett's post-hoc test was used for statistical analysis;  $p^*/\# < 0.05$ ,  $p^{***}/#### < 0.001$ ; \* is significant compared to control PBS group; # is significant compared to collagen and epinephrine treated group.





**Figure S10:** Schematic representation of proposed mechanism. Role of ASK1 in platelet activation and death in presence of ASK1 inhibitor (NQDI-1).