

RAP1-RHO small GTPase cross-talk mediates integrin-dependent and -independent platelet procoagulant response

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Received: July 8, 2025.

Accepted: February 25, 2026.

Early view: March 5, 2026.

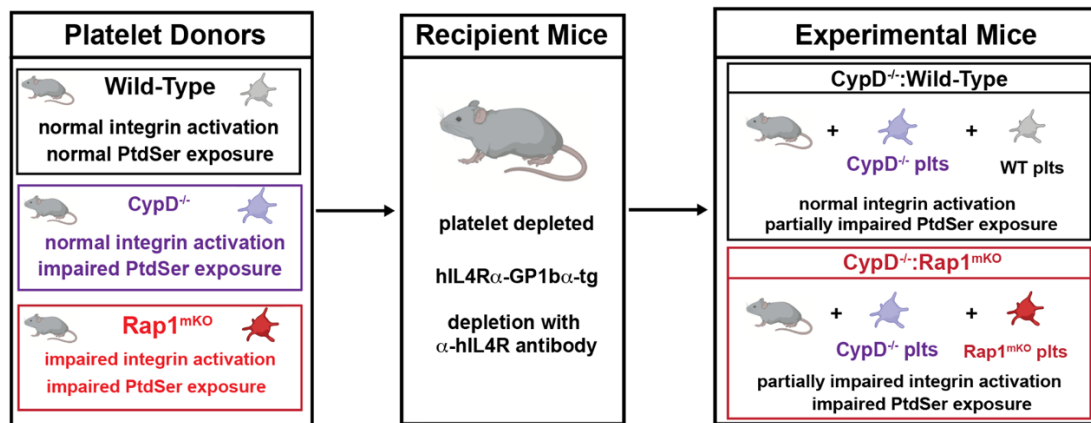
<https://doi.org/10.3324/haematol.2025.288459>

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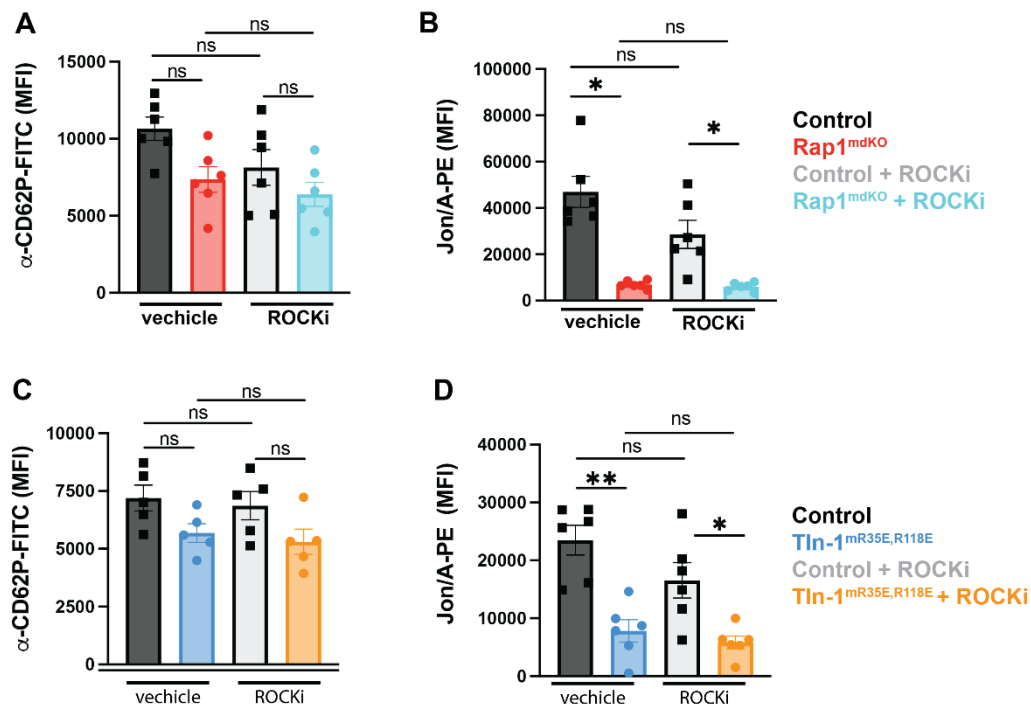


Supplemental Material



Supplemental figure 1. Schematic representation for adoptive transfer experiment.

Platelet adoptive transfer approach. Platelets from *CypD*^{-/-} and wild-type or *CypD*^{-/-} and *Rap1*^{mKO} mice were transfused into a thrombocytopenic recipient mouse.



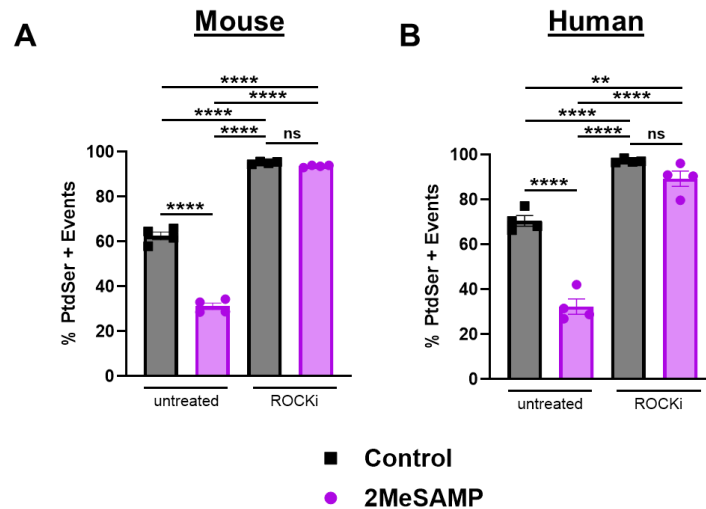
Supplemental figure 2. ROCK inhibition does not affect granule secretion or α IIb β 3 activation.

(A) Flow cytometry analysis of granule secretion (α -CD62P-FITC MFI normalized to platelet size) in control (black/grey bars) or *Rap1^{mdKO}* platelets (red/cyan bars) stimulated with 50 ng/ml CVX + 250 μ M Par4p in the presence or absence of ROCK inhibitor (n=6).

(B) Flow cytometry analysis of α IIb β 3 integrin activation (JON/A-PE MFI normalized to platelet size) in control or *Rap1^{mdKO}* platelets stimulated with 50 ng/ml CVX + 250 μ M Par4p in the presence or absence of ROCK inhibitor (n=6).

(C) Flow cytometry analysis of granule secretion (α -CD62P-FITC MFI) in control (black/grey bars) or *Tln1^{mR35/118E}* platelets (blue/yellow bars) stimulated with 50 ng/ml CVX + 250 μ M Par4p in the presence or absence of ROCK inhibitor (n=6).

(D) Flow cytometry analysis of α IIb β 3 integrin activation (JON/A-PE MFI) in control or *Tln-1^{mR35/118E}* platelets stimulated with 50 ng/ml CVX + 250 μ M Par4p in the presence or absence of ROCK inhibitor (n=6).



Supplemental figure 3. Impaired PtdSer exposure due to inhibition of P2Y12 is recovered with ROCK inhibitor.

Flow cytometry analysis of Annexin V binding (% PtdSer + events) to mouse (**A**) or human (**B**) platelets stimulated with 50 ng/ml convulxin + 250 μ M Par4p in the presence or absence of ROCK inhibitor (20 μ M Y-27632) and/or P2Y12 inhibitor (100 μ M 2MeSAMP; purple bars) (n=4). **<0.01, ****<0.0001, ns: not significant.