University of Reading

Intracellular trafficking, localization, and mobilization of platelet-borne thiol isomerases

Article

Supplemental Material

Crescente, M., Pluthero, F. G., Li, L., Lo, R. W., Walsh, T. G., Schenk, M. P., Holbrook, L. M., Loureiro, S. ORCID: https://orcid.org/0009-0000-8886-6893, Ali, M. S., Vaiyapuri, S. ORCID: https://orcid.org/0000-0002-6006-6517, Falet, H., Jones, I. M. ORCID: https://orcid.org/0000-0002-7738-2516, Poole, A. W., Kahr, W. H. A. and Gibbins, J. M. ORCID: https://orcid.org/0000-0002-0372-5352 (2016) Intracellular trafficking, localization, and mobilization of platelet-borne thiol isomerases. Arteriosclerosis Thrombosis and Vascular Biology, 36 (6). pp. 1164-1173. ISSN 1079-5642 doi: https://doi.org/10.1161/ATVBAHA.116.307461 Available at https://centaur.reading.ac.uk/64077/

It is advisable to refer to the publisher's version if you intend to cite from the work. See Guidance on citing.

To link to this article DOI: http://dx.doi.org/10.1161/ATVBAHA.116.307461

Publisher: Lippincott, Williams & Wilkins

All outputs in CentAUR are protected by Intellectual Property Rights law,

University of Reading

including copyright law. Copyright and IPR is retained by the creators or other copyright holders. Terms and conditions for use of this material are defined in the End User Agreement.

www.reading.ac.uk/centaur

CentAUR

Central Archive at the University of Reading

Reading's research outputs online

Online supplementary material

Videos show 3D volume renders (maximum intensity) of laser confocal immunofluorescence image sequences of representative megakaryocytes depicted in the main text figures.

Videos 1-4 PDI and ERp57 in developing MKs. 3D volume renders of images shown in Figure 1A (Video 1), 1B (Video 2), 1C (Video 3) and 1D (Video 4).

Video 5 ERp57 is not associated with the trans-Golgi or endocytic vesicles in human MKs. 3D render showing the distribution of ERp57 (green), TGN46 (red), CD71/TF receptor (magenta) and DNA (light blue) in a human MK imaged via structured illumination immunofluorescence microscopy.

Video 6 PDI shows a different pattern of distribution from α-granule cargo proteins during MK development. 3D render of image shown in Figure S1A.

Video 7 Calnexin and ERp57 in human MK. 3D render of image shown in Figure S2A.

Figure S.I.

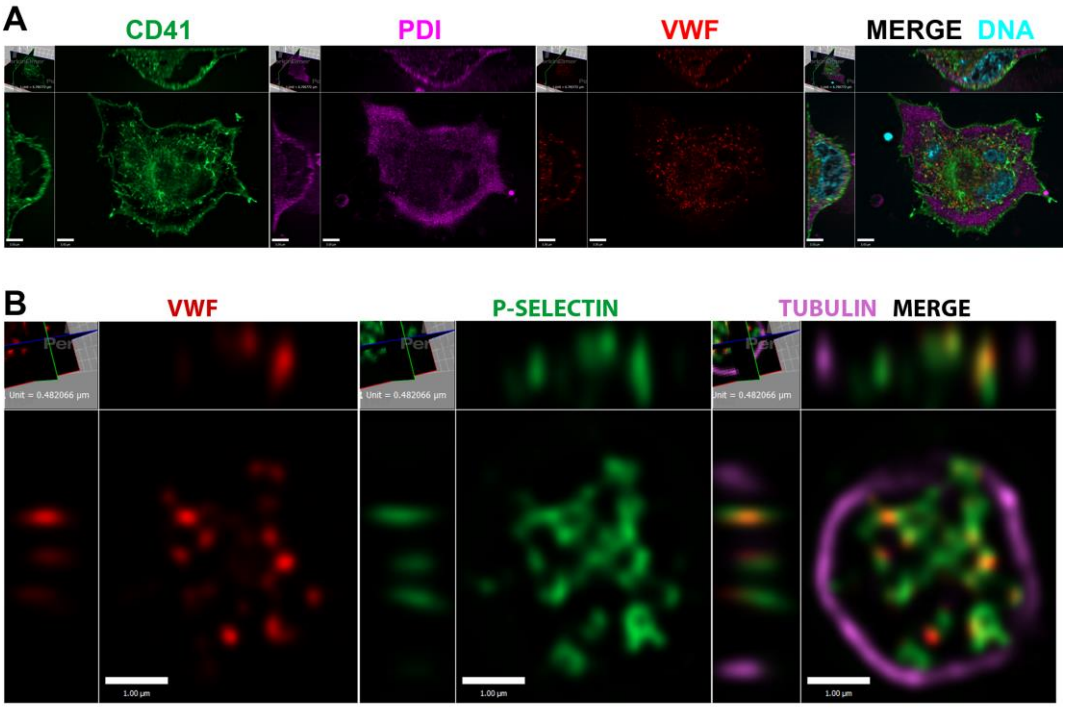

Figure S1 – Comparison of the distribution of thiol isomerases and platelet granule cargo. A) Confocal immunofluorescence images (Z-sections with YZ, XY and XZ profiles; bar = 5μm) of a representative mature cultured mouse MK stained for CD41/integrin αIIb (green), PDI (magenta) and Von Willebrand Factor (VWF; red) shows a punctate distribution of VWF being packaged into α-granules, while the thiol isomerase distribution remains more diffuse but possibly associated with a membrane system (see also Video 6) . **B)** Representative image of a human platelet stained for VWF (red), P-selectin (green) and α-tubulin (magenta; bar = 1 μm) present in the circumferential cytoskeletal ring. While α-granule-borne proteins like VWF often show little colocalization with each other, they do show consistent association with P-selectin.

Figure S.II.

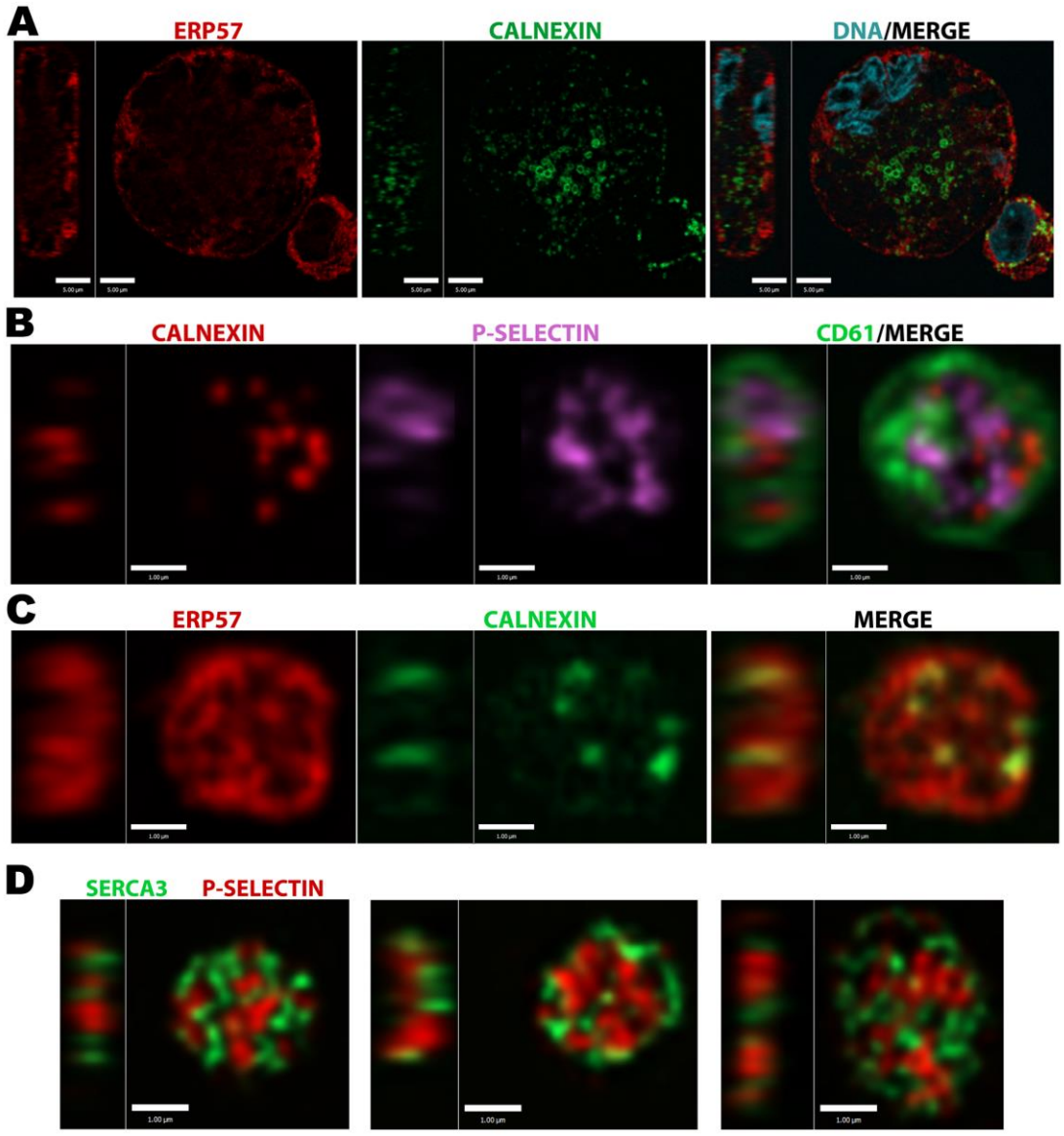

Figure S2 – Endoplasmic/sarcoplasmic reticulum proteins in MKs and platelets. A) IFM imaging of a mature human MK shows extensive ER stained with calnexin (green) while most ERp57 (red) is in the periphery (bars = 5μm; see Video 5). **B**) Representative image of a platelet stained for calnexin (red), P-selectin (magenta) and CD61 (green) shows little overlap between these proteins, which appear to define distinct intracellular membrane systems. **C)** In a resting platelet, calnexin is concentrated in what are likely DTS-associated structures that show overlap with ERp57, also present in other parts of the cell. **D)** IFM imaging of SERCA3 (green), which has been shown to be localized to the inner surface of the platelet outer membrane by immuno-EM, shows a similar distribution with little overlap with P-selectin (red). B-D bars = 1μm; all images are confocal mid-cell YZ and XY sections.

Figure S.III.

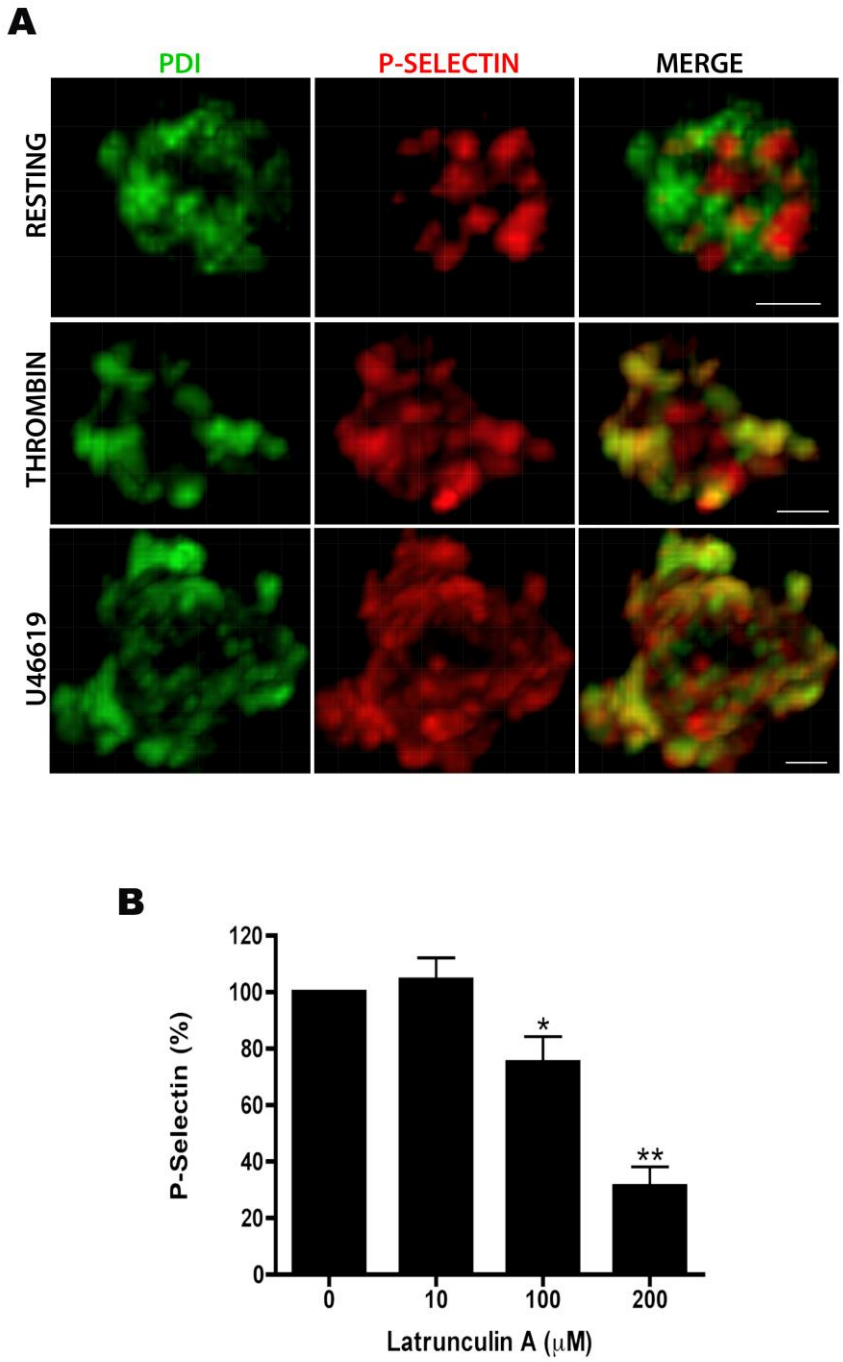

Figure S3 – Thiol isomerase mobilization in activated platelets and concentration-response effect of latrunculin A on P-selectin surface exposure. **A)** IFM imaging of a representative resting platelet and cells activated with thrombin or U46619 immunostained for PDI (green) and P-selectin (red) shows both proteins concentrated at the platelet surface after activation (maximum intensity renders; bars = 1 μM), consistent with flow cytometry observations (see Table 1 and Fig 5B). **B)** The effect of varying concentrations of latrunculin A on U46619-induced platelet P-

selectin surface mobilization was measured by flow cytometry. Graphs show mean percentages compared with controls, error bars indicate SEM (n=3-5); asterisks represent significant differences: *p≤.05, ** p≤.01, Student's *t* test. Results indicate that P-selectin mobilization was >50% inhibited by 200μM latrunculin A.