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.

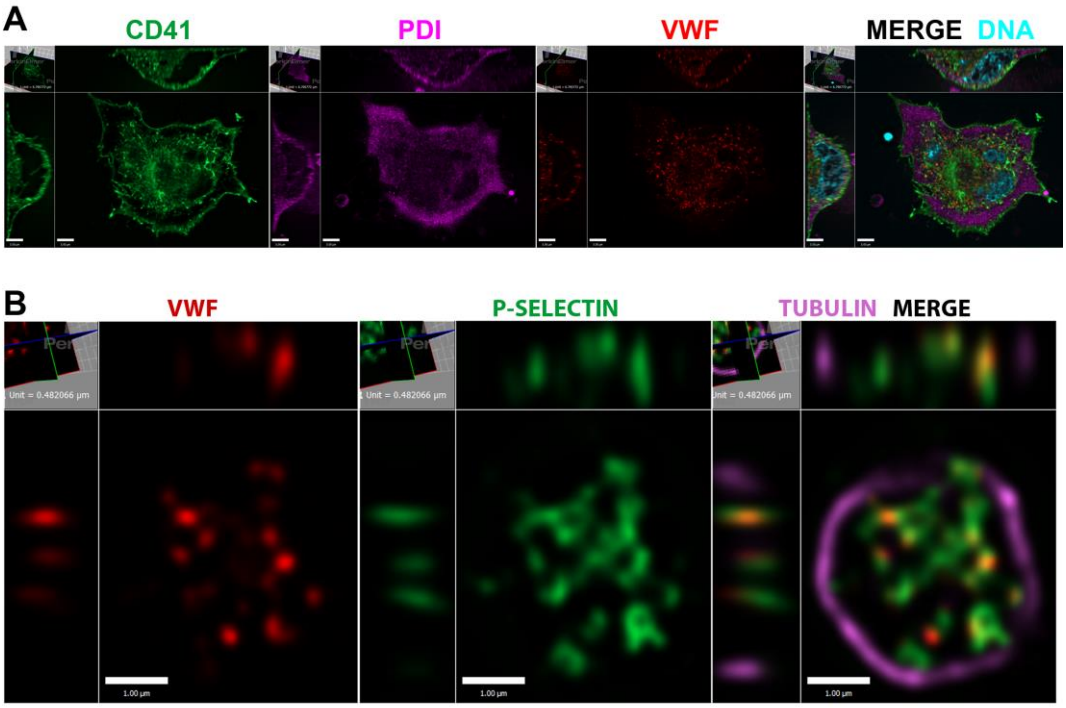

Figure S1 – Comparison of the distribution of thiol isomerases and platelet granule cargo. A) Confocal immunofluorescence images (Z-sections with YZ, XY and XZ profiles; bar = 5μm) of a representative mature cultured mouse MK stained for CD41/integrin αIIb (green), PDI (magenta) and Von Willebrand Factor (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.

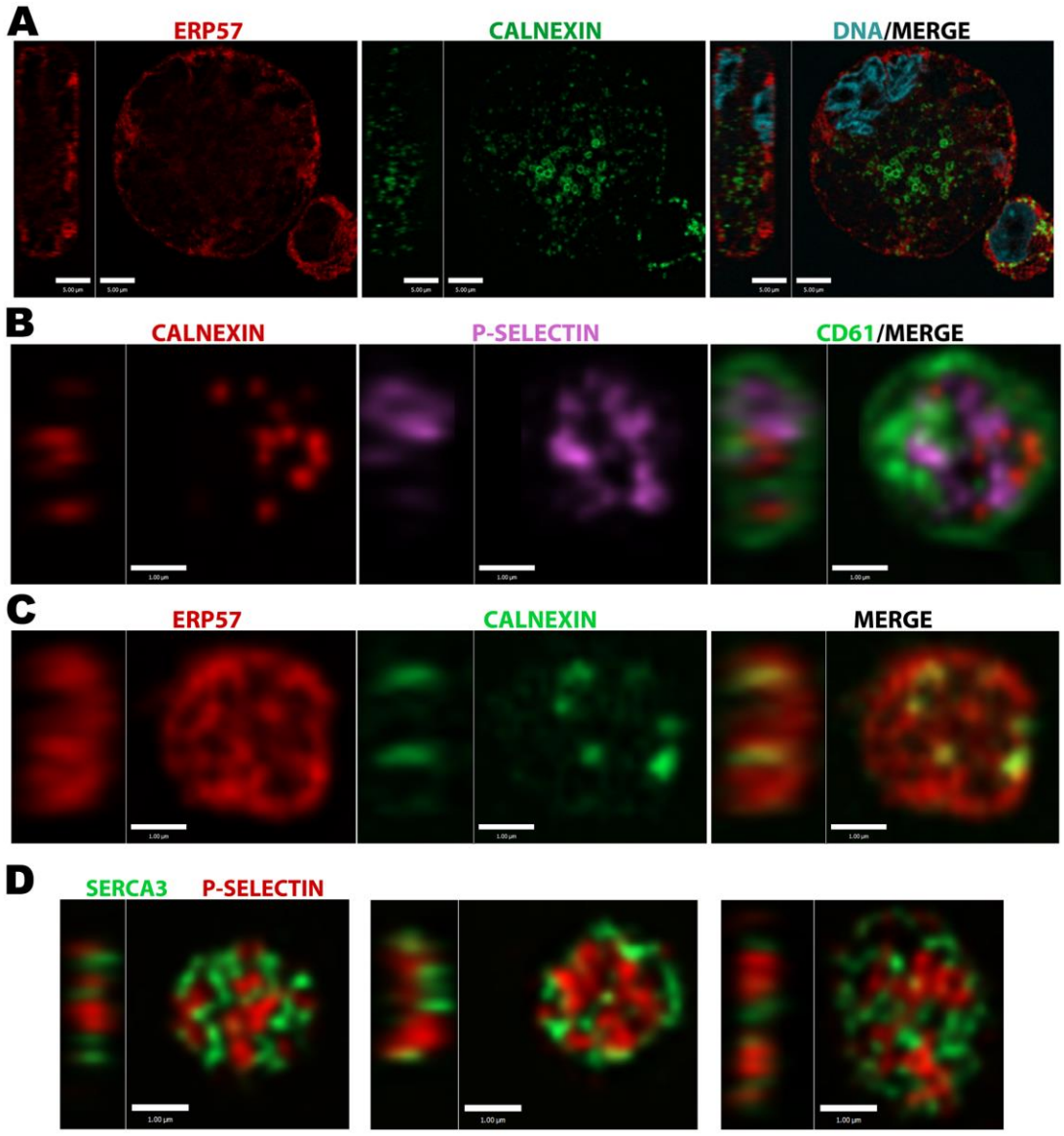

Figure S2 – Endoplasmic/sarcoplasmic reticulum proteins in MKs and platelets. A) IFM imaging of a mature human MK shows extensive ER stained with calnexin (green) while most ERp57 (red) is in the periphery (bars = 5μm; see Video 5). **B**) Representative image of a platelet stained for calnexin (red), 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.

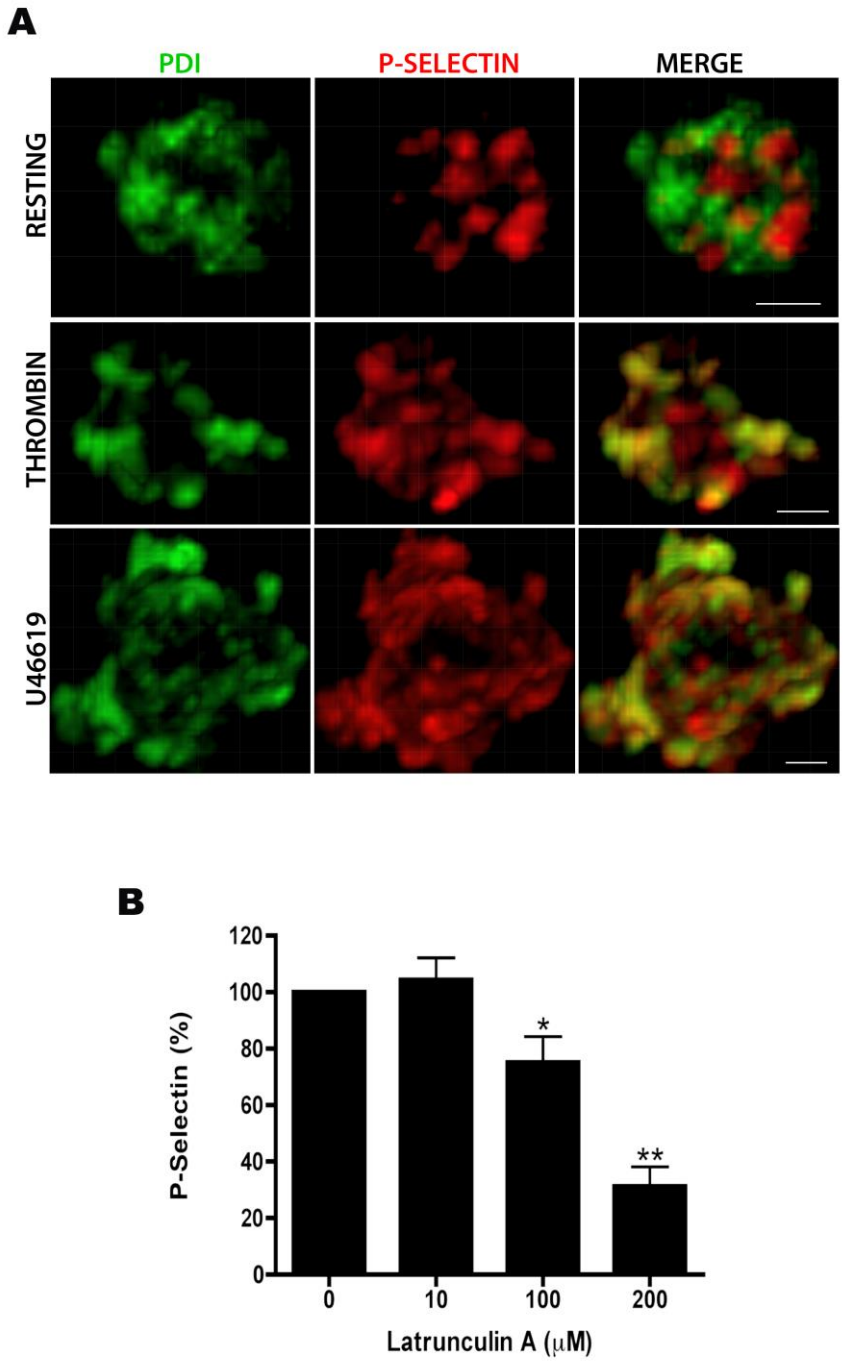

Figure S3 – Thiol isomerase mobilization in activated platelets and concentration-response effect of latrunculin A on P-selectin surface exposure. **A)** IFM imaging of a representative resting platelet and cells activated with thrombin or U46619 immunostained for PDI (green) and P-selectin (red) shows 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.