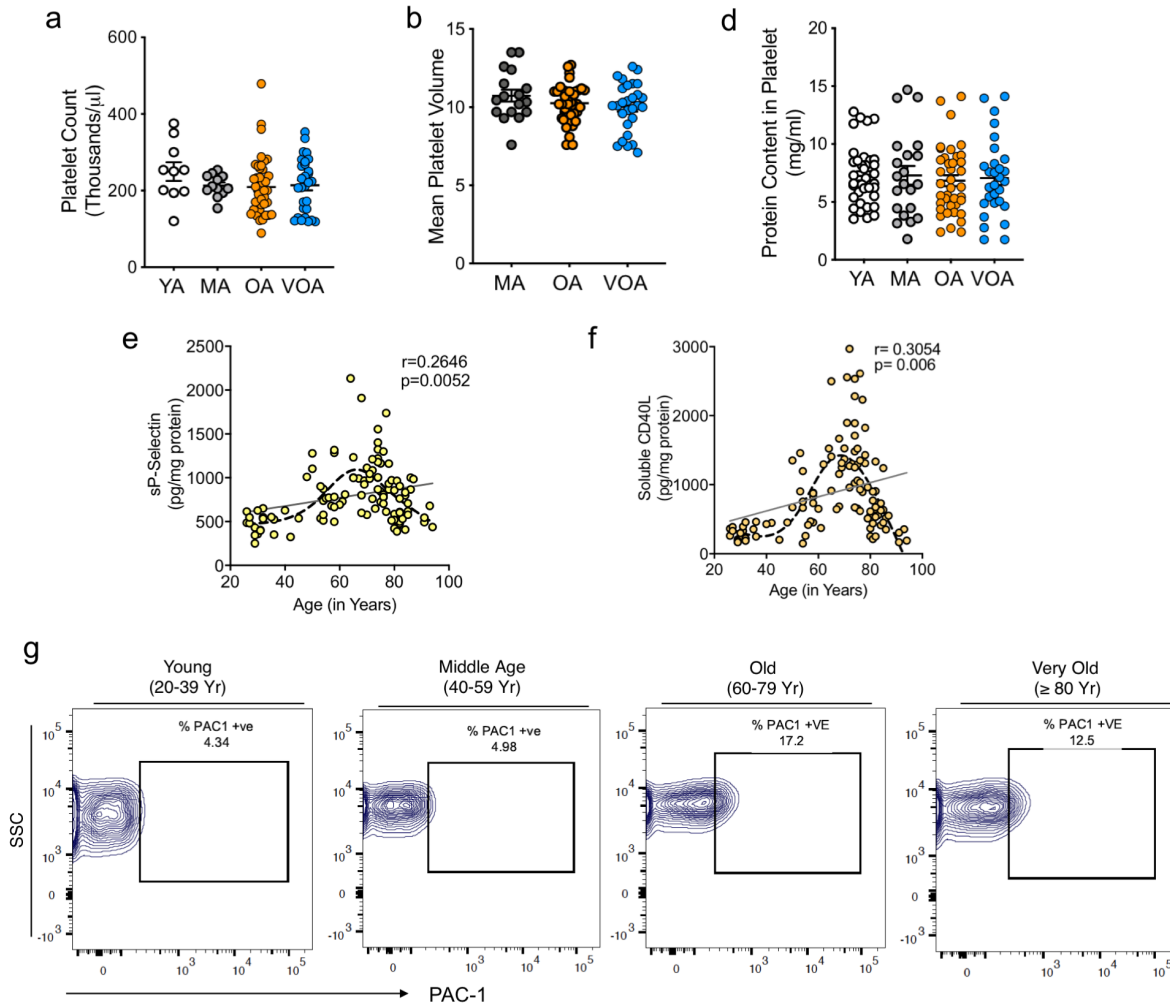


SUPPLEMENTAL FIGURES.

Age associated non-linear regulation of redox homeostasis in the anucleate platelet: Implications for CVD risk patients

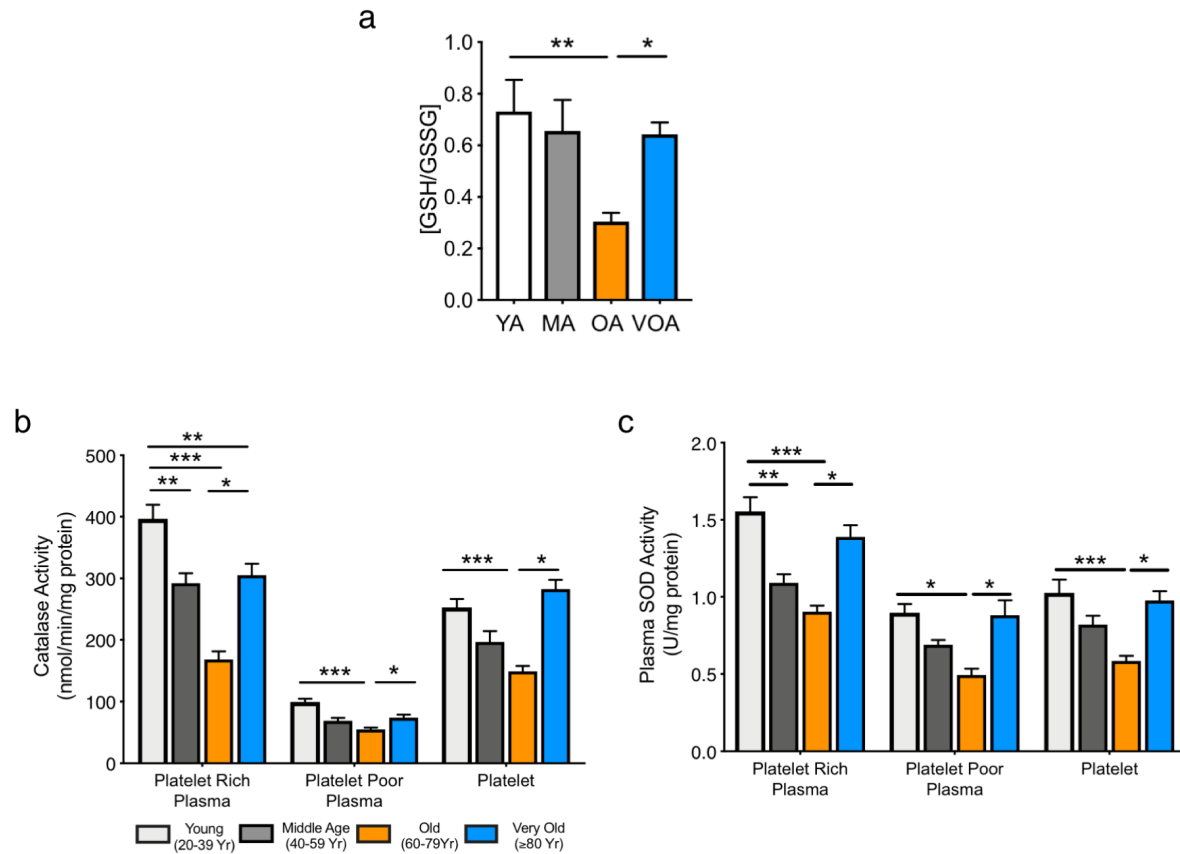
Jain et. al.

Supplementary Figure S1



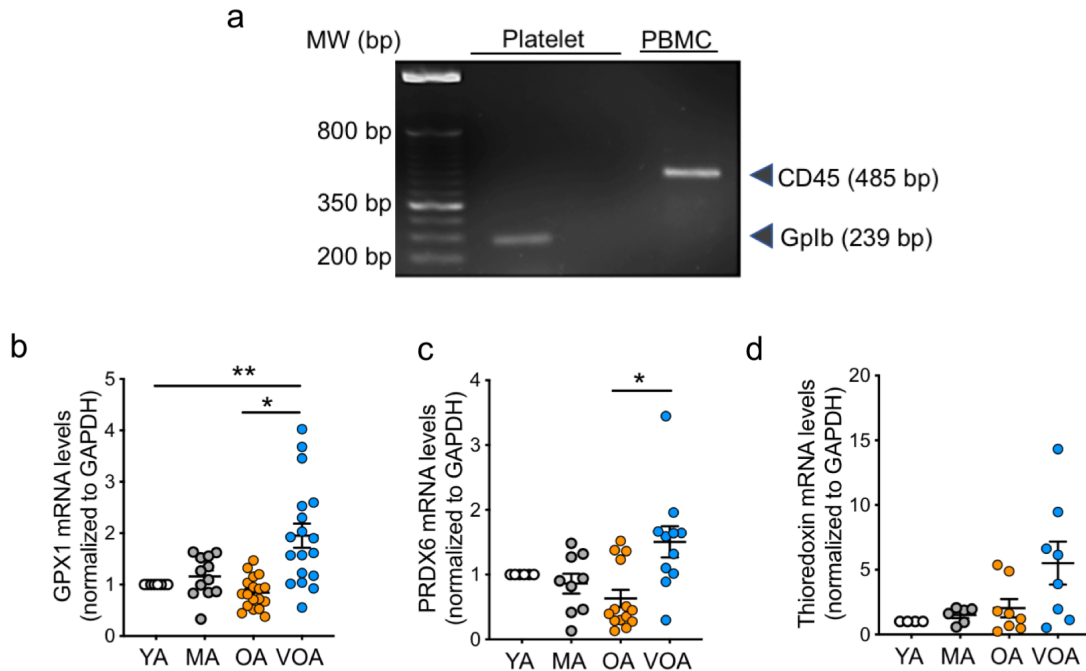
Supplementary Figure 1: Platelet activation is significantly lower in the very old (≥ 80 year) population as compared to the old (60-79 year) population. **a.** Platelet counts across various age subsets. **b.** Mean platelet volume (MPV) across the human cohort. **c.** Platelet protein content in the human cohort. **d-e.** Correlation curve between sP-Selectin and sCD40L, respectively, and patient age. Solid line represents the linear regression curve. Dash line represents the trendline generated using spline curve fitting. **f.** Surface platelet activation (Activated $\alpha_{IIb}\beta_3$) was assessed by the binding of platelets to FITC-conjugated PAC-1 antibody as shown in the representative contour plot. Values expressed as Mean \pm SEM. Experiments performed in a minimum of $n=10$ samples per group.

Supplementary Figure 2



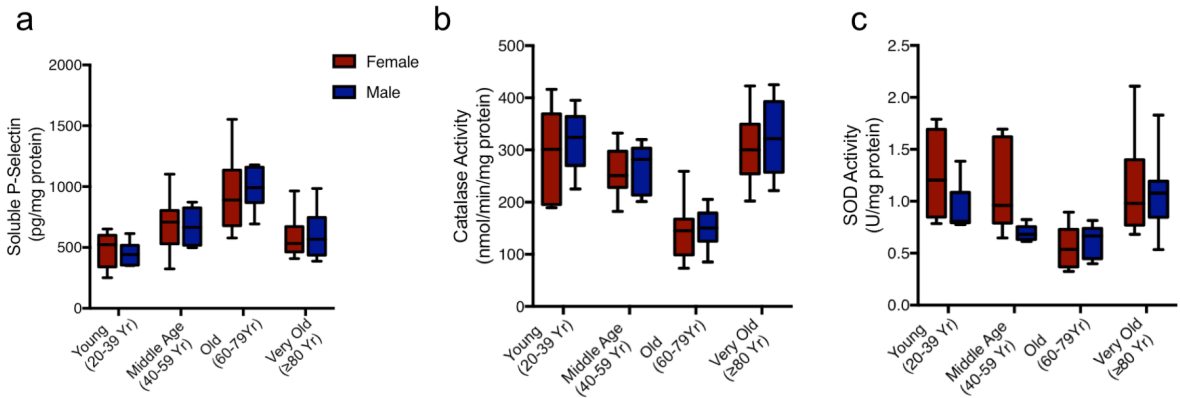
Supplementary Figure 2: Reduced oxidative damage and elevated enzymatic antioxidant levels are in the platelets from the very old (≥80 year) population. **a:** Ratio of reduced to oxidised glutathione, GSH/GSSG is reflective of the redox status of the cell. **b-c.** Catalase and SOD activity measured in the PRP, PPP and platelets in the different age subsets (YA n=12, MA n=12, OA n=20, VOA n=20). Values expressed as Mean ± SEM. * $p < 0.05$; ** $p < 0.005$. Experiments performed in a minimum of n=10 samples per group.

Supplementary Figure 3



Supplementary Figure 3: Increased antioxidant gene expression in the platelets from the very old (≥ 80 year) population. **a.** Agarose gel showing the results of RT-PCR to confirm the absence of leucocyte contamination in the platelet RNA sample. **b-d.** Quantitative Real-time PCR (qRT-PCR) was used to measure mRNA expression levels of *Gpx1*, *Prdx6* and *Thioredoxin* as a validation of the array data. Results are expressed as fold change over Young Healthy controls. Values expressed as Mean \pm SEM. * $p < 0.05$; ** $p < 0.005$. Experiments performed in a minimum of $n=10$ samples per group.

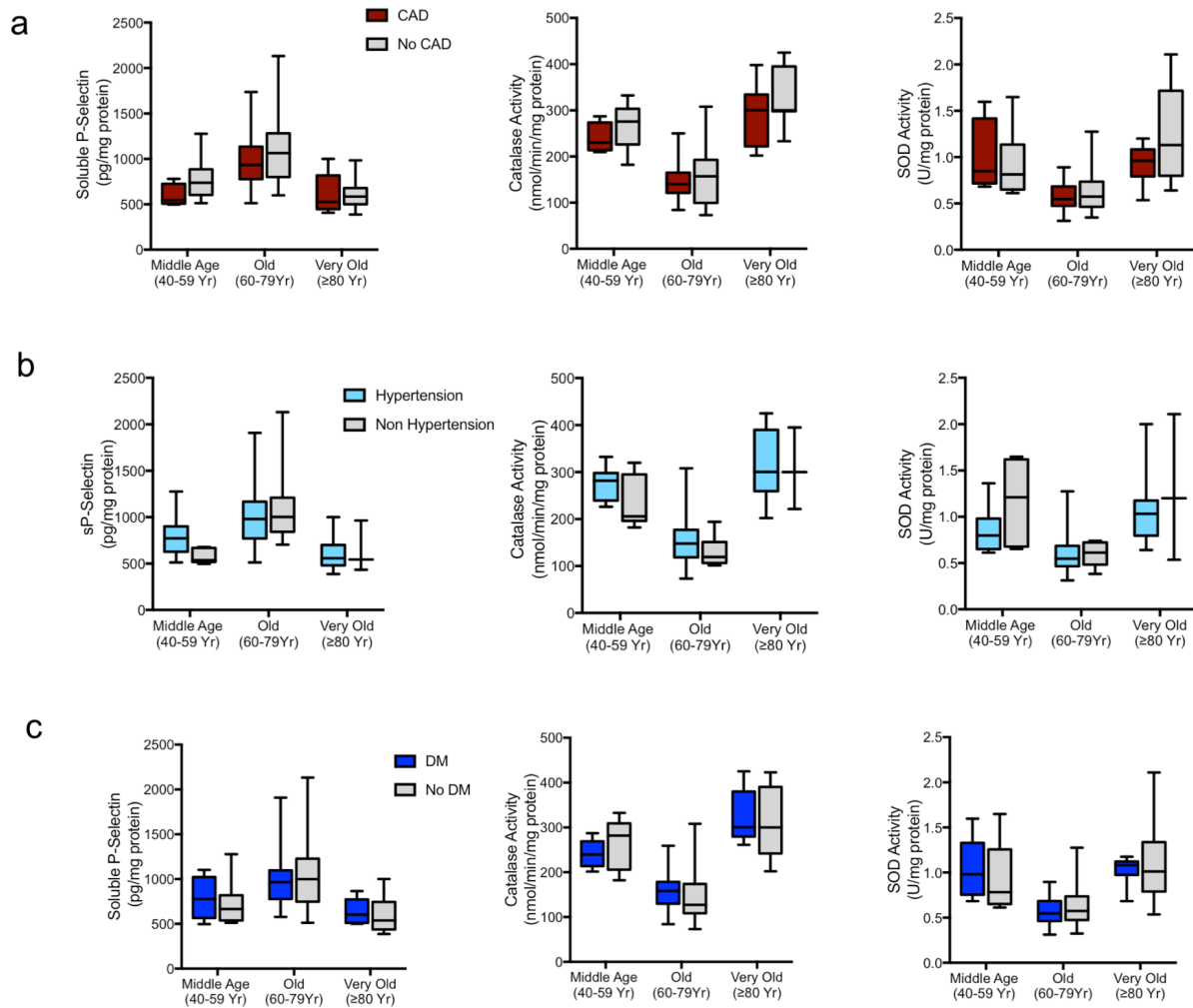
Supplementary Figure 4



Supplementary Figure 4: Levels of sP-selectin and platelet antioxidants are independent of gender.

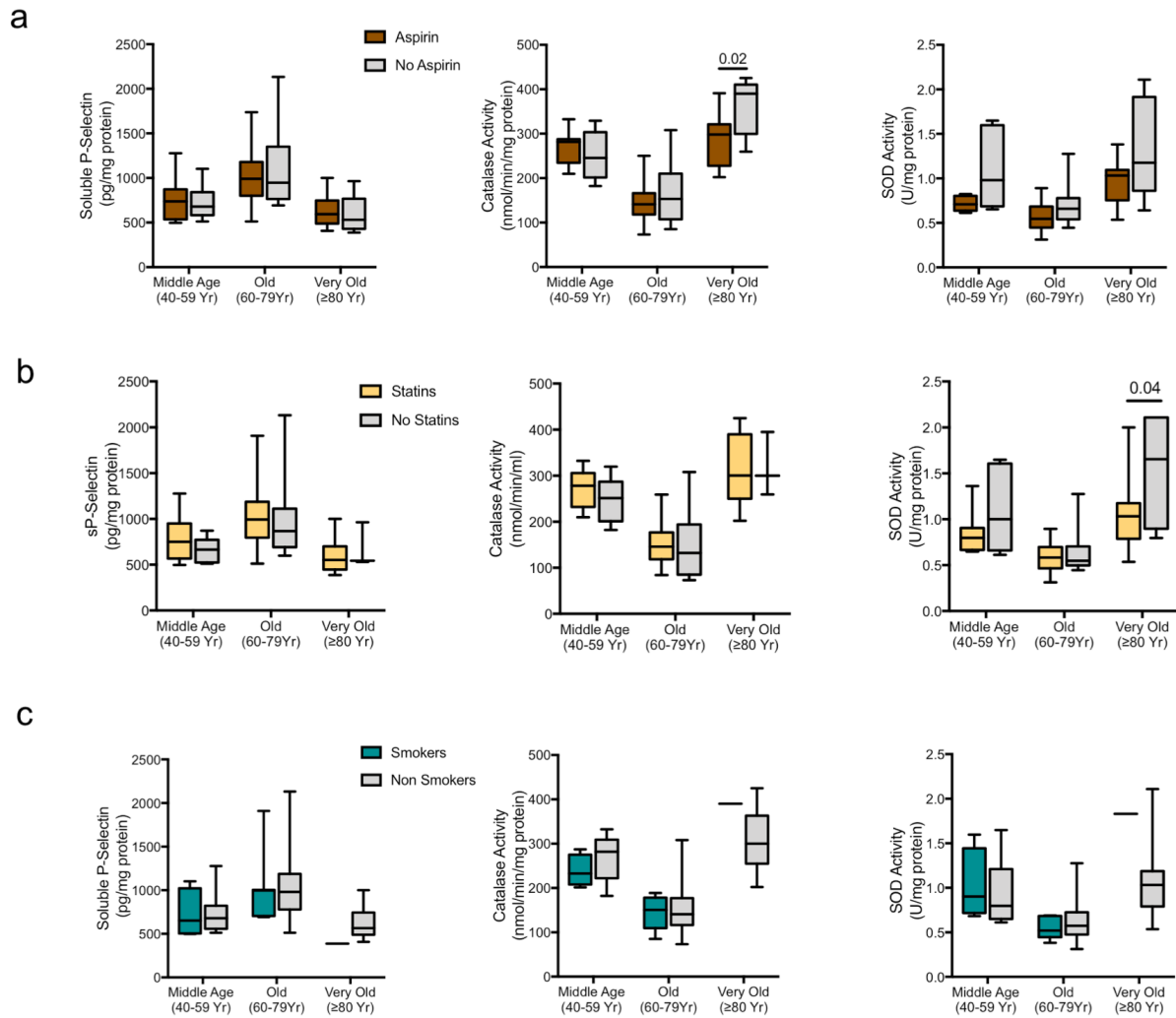
a-c. Graphs depict the differences in the levels of sP-selectin, Catalase activity and SOD activity shown between genders in relation to age. No significant changes seen in the levels in women vs men. Values are expressed as Min to Max ± SEM in the box and whiskers plots

Supplementary Figure 5



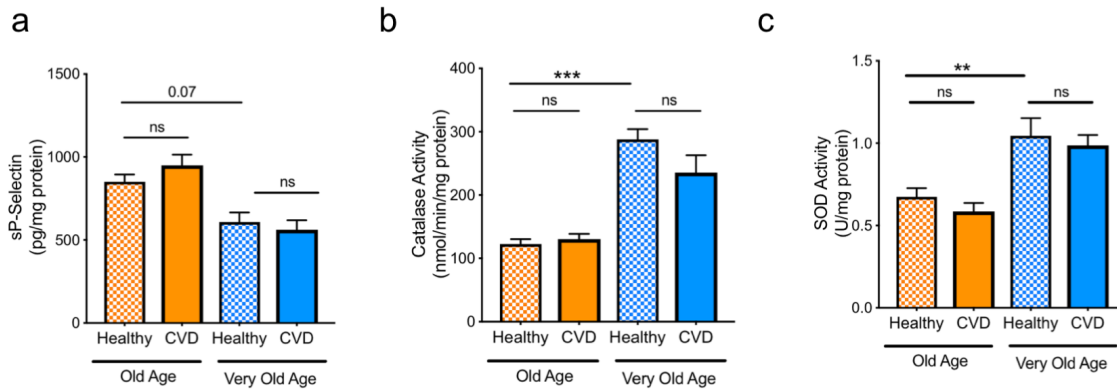
Supplementary Figure 5: Levels of P-selectin and platelet antioxidants are independent of comorbidities, which differ significantly between the cohorts. Each panel represents the levels of sP-selectin, Catalase activity and SOD activity in the cohort of patients recruited from the cardiovascular clinic segregated on the basis of the presence of documented CAD **(a)**, Hypertension **(b)** and Diabetes **(c)**. Values are expressed as Min to Max \pm SEM in the box and whiskers plots.

Supplementary Figure 6



Supplementary Figure 6: Levels of P-selectin and platelet antioxidants are independent of clinical characteristics . Each panel represents the levels of sP-selectin, Catalase activity and SOD activity in the Middle Age, Old Age and Very Old Age groups on the basis of the presence of Aspirin **(a)**, Statins **(b)** and Smoking Status **(c)**. Values are expressed as Min to Max \pm SEM in the box and whiskers plots.

Supplementary Figure 7



Supplementary Figure 7: Levels of sP-selectin and platelet antioxidants are independent of cardiovascular diseases. a-c. Graphs show the levels of sP-selectin, Catalase activity and SOD activity respectively between healthy subjects and CVD patients for the Old Age and very Old Age cohorts. Values are expressed as Mean \pm SEM. ** $p < 0.005$; *** $p < 0.001$. Experiments performed in a minimum of $n=6$ samples per group.