Supplementary Figure Legend

Supplementary Figure 1. Optimisation of detection antibodies for in vitro binding studies of PEGylated A1A2A3-VWF variants.

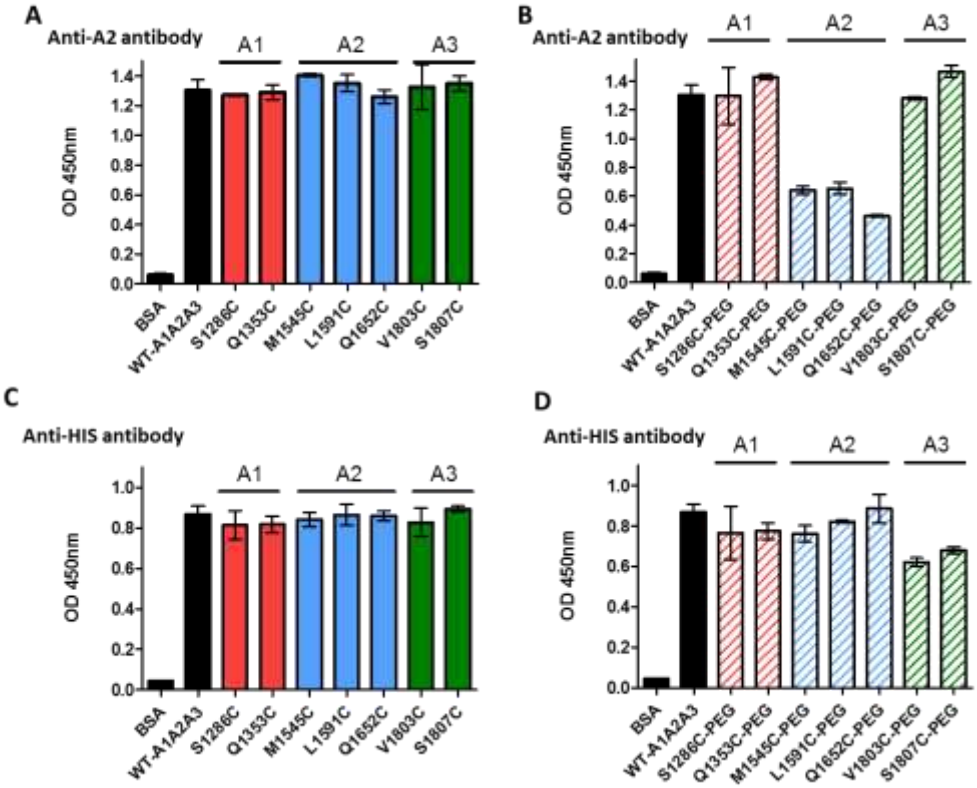
(A) All A1A2A3 cysteine variants were equally detected using polyclonal sheep anti-VWF-A2 and anti-sheep HRP. **(B)** Following PEGylation within the A2-domain, this anti-A2 antibody displayed significantly reduced binding affinity for A2-PEGylated variants. **(C-D)** A polyclonal rabbit biotinylated anti-His tag antibody and strep-HRP were used to ensure equal detection of the A2-PEGylated variants (M1545C-PEG, L1591C-PEG and Q1652C-PEG).

Supplementary Figure 2. PEGylation attenuates A1A2A3-VWF binding to GPIbα in vitro.
Binding of cysteine and PEGylated variants with extended half-lives to GPIbα was assessed using immunosorbant assays in the presence of ristocetin. All data is graphed as mean values ± SEM (* p <0.05, ** p <0.01, *** p <0.0001 respectively).

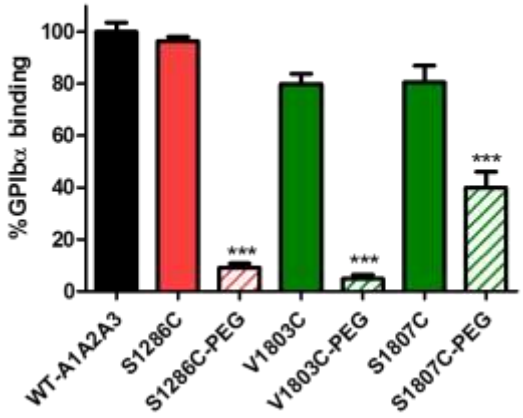
Supplementary Figure 3. Site-specific effects for A1A2A3-VWF PEGylation in modulating binding to collagens III and IV in vitro.
(A-C) Binding of cysteine and PEGylated variants with extended half-lives to collagen type III and collagen type IV **(D-F)** was assessed using immunosorbant assays. All data is graphed as mean values ± SEM (* p <0.05, ** p <0.01, *** p <0.0001 respectively).

Supplementary Figure 4. LRP1 mediates the binding of A1A2A3-VWF to differentiated THP-1 macrophages in vitro. (A) Expression of VWF clearance receptor LRP1 on differentiated THP-1 macrophages was confirmed by flow cytometry. **(B)** Inhibition of LRP1, with an anti-LRP1 antibody, significantly attenuated binding of WT-A1A2A3-VWF suggesting VWF adhesion occurs in LRP1-dependant manner.

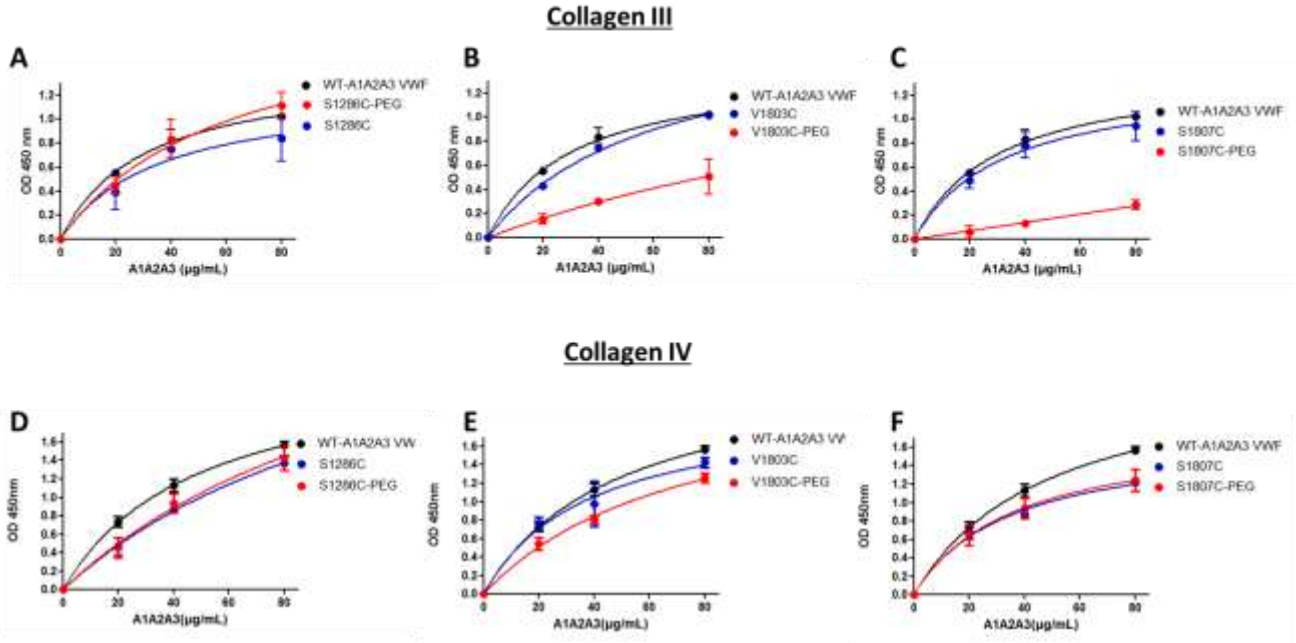
Supplementary Figure 1



Supplementary Figure 2



Supplementary Figure 3



Supplementary Figure 4