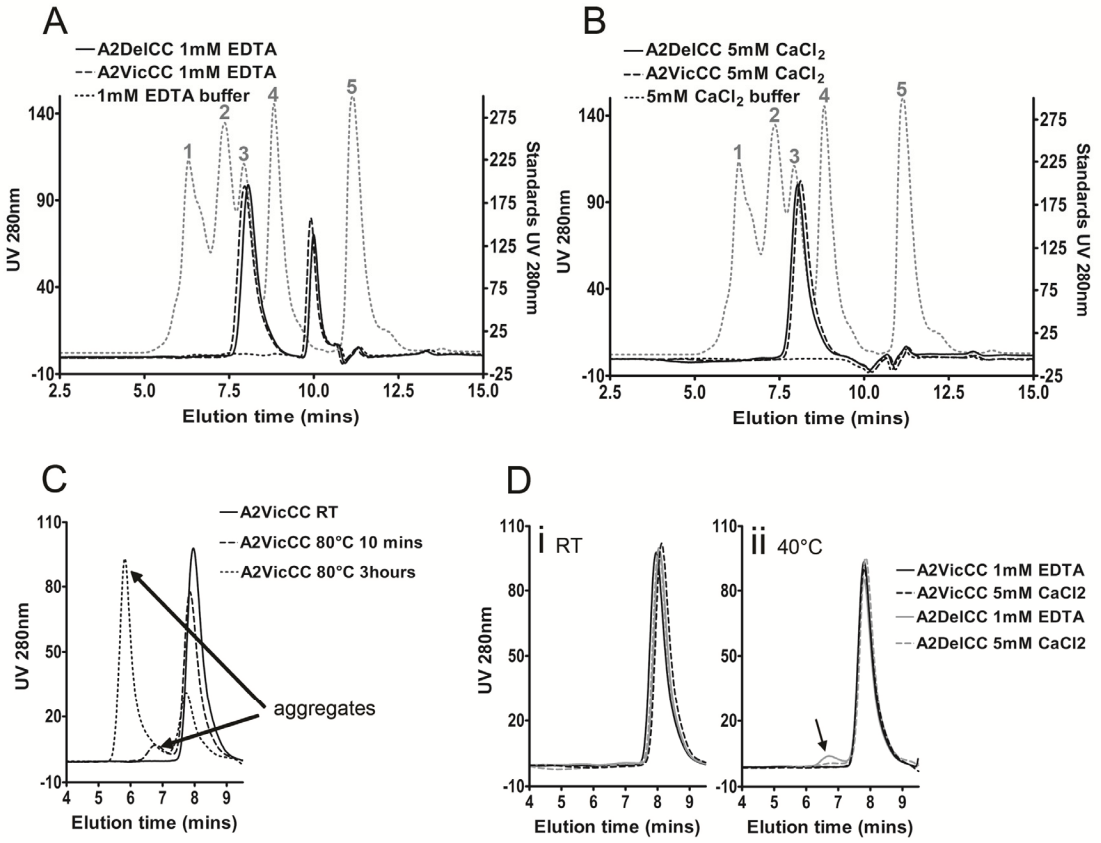
Supplementary Material: Lynch et al "Control of VWF A2 domain stability…."



Legend to Supplementary Figure: SE-HPLC to detect temperature-induced aggregation of purified VWF A2 domain fragments

SE-HPLC was calibrated with a 10x dilution of BioRad Gel Filtration Standards, the labelled peaks (in Panels A and B) represent: 1- Thyroglobulin (MW 660kDa), 2- γ-globulin (MW 158 kDa), 3- Ovalbumin (44 kDa), 4- Myoglobin (MW 17kDa) and 5- Vitamin B_{12} (MW 1.35 kDa). 50µL of 0.5mg/ml purified VWF A2 domains, A2ΔCC and A2VicCC, were loaded in the presence of (A) 1mM EDTA (were a distinct peak is observed at 10mins which is likely to be EDTA in complex with trace cations, as also present when buffer without protein was loaded) and (B) 5mM $CaCl_2$. (C) Induced aggregation of A2VicCC was performed by heating 60µL of 0.5mg/ml of purified VWF A2 at 80°C for 10mins and for 3hours, in a PCR machine just prior to loading onto the SE-HPLC column. (D) 1mM EDTA and 5mM $CaCl_2$ were added to 0.5mg/ml VWF A2ΔCC and A2VicCC and after incubation at room temperature (RT) 50µL was loaded on the column. Monomeric peaks were observed for both protein constructs in both conditions (D-i). After incubation at 40°C for 10mins, a small yet distinct aggregation peak was observed (arrowed) for A2ΔCC in 1mM EDTA (D-ii).