SerpinB2 mutants maintain general secondary structure and inhibitory activity

To ensure that any observed changes in LDLR binding were a function of residue replacement and not due to protein misfolding and exposure of alternative receptor binding determinants, SerpinB2 mutants were tested for relative secondary structure using far-UV circular dichroism spectroscopy. All SerpinB2 species were found to have similar ellipticity values to the control SerpinB2 across the full range of wavelengths measured (Figure S1). Additionally, SerpinB2 mutants retained inhibitory activity indistinguishable from control SerpinB2, as assessed by the formation of SDS stable complexes with uPA (data not shown) and solution phase inhibitory assays (Figure S2).

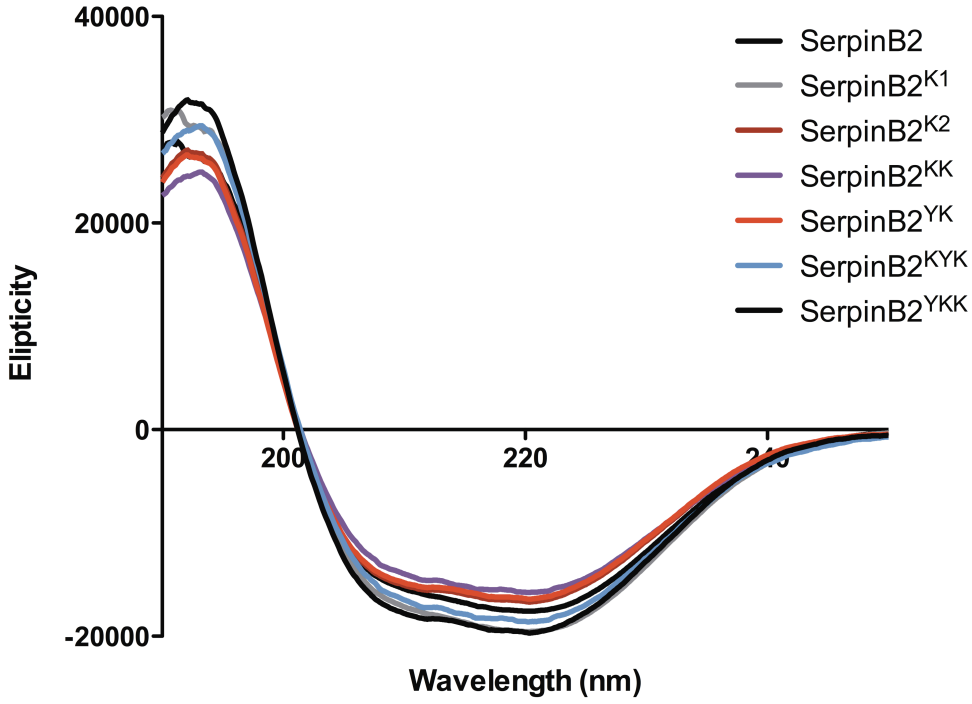

Figure S1: SerpinB2 forms maintained overall secondary structure. Far-UV circular dichroism spectroscopy indicated that the relative secondary structure did not vary between SerpinB2, SerpinB2^{K1}, SerpinB2^{K2}, SerpinB2^{KK}, SerpinB2^{YK}, SerpinB2^{KYK} and SerpinB2^{YKK}. Proteins were analysed using a J-810 spectropolarimeter (Jasco, Easton, MD, USA). Each spectrum is the average of ten scans.

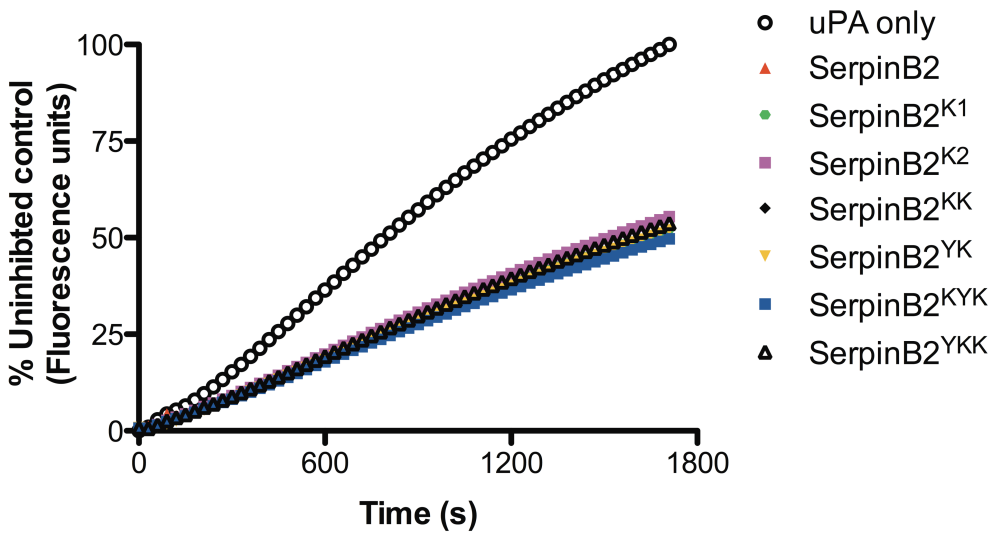

Figure S2: SerpinB2 forms maintained inhibitory activity. SerpinB2 proteins showed similar uPA inhibitory activity as assessed using the fluorogenic uPA substrate Z-Glu-Gly-Arg-AMC. SerpinB2 samples in reaction buffer (20 mM Hepes, pH 7.6, 100 mM NaCl, 0.5 mM EDTA, 0.01% (v/v) Tween 20) were mixed at a 1:1 molar ratio with Z-Gly-Gly-Arg-AMC and pre-incubated at 37°C. HMW-uPA was added at a concentration of 0.675 nM and fluorescence emission measured at 37°C.