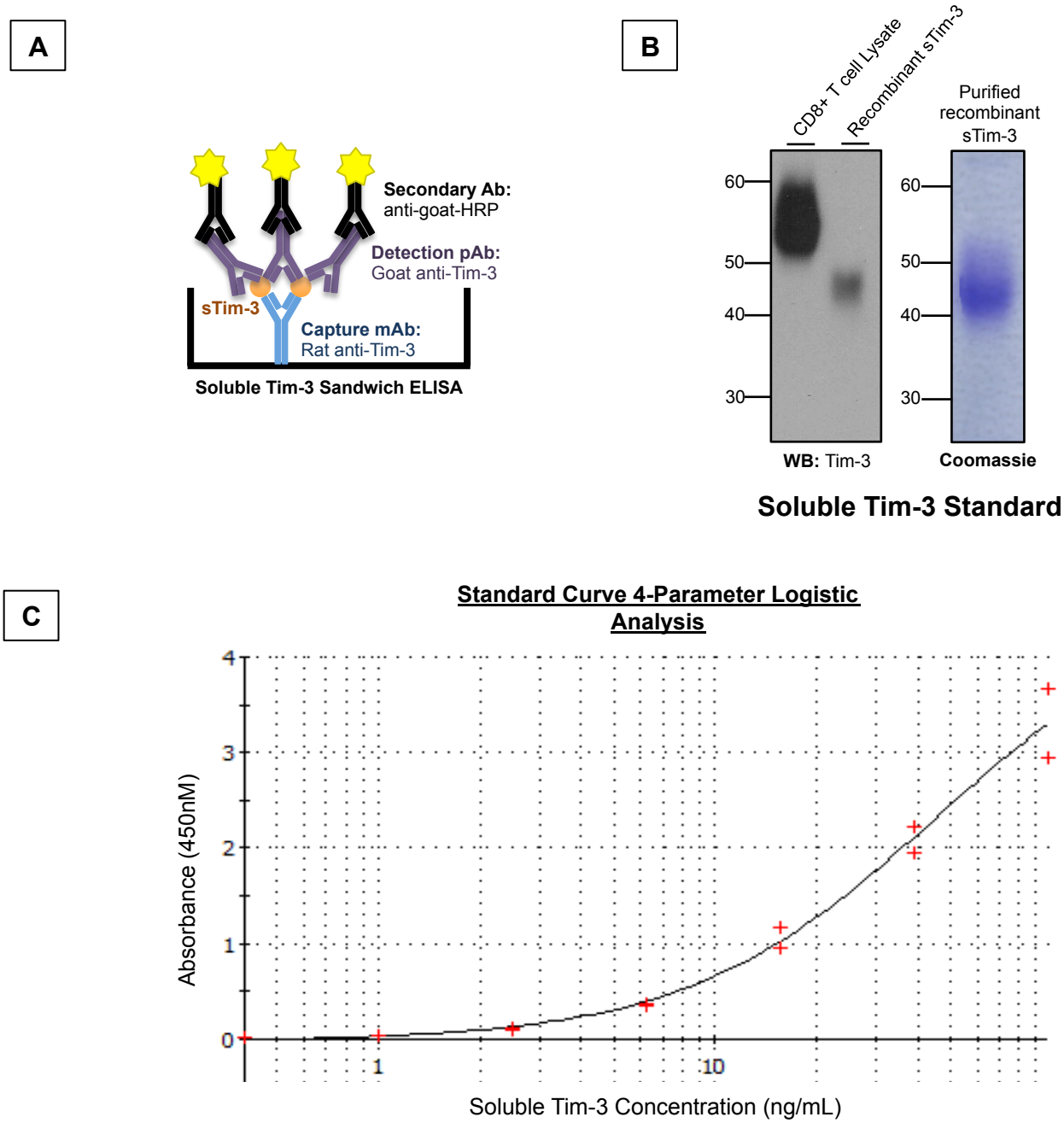
Supplementary Figure 1



Supplementary Figure 1. Soluble Tim-3 ELISA quality control. (A) sTim-3 sandwich ELISA schematic. The purpose of this assay was to develop a system to detect and quantify levels of sTim-3 in human plasma and *in vitro* samples. While a monoclonal Tim-3 antibody was used to capture the antigen, a polyclonal Tim-3 antibody was used to detect the antigen. **(B)** Protein standard quality control. Recombinant sTim-3 consists of the full Tim-3 ectodomain and is fully glycosylated (2 N-linked and 10 O-linked glycans), thus the MW range is from 45-56kDa assuming its glycan contributes 2-3kDa. This protein was produced as described in Jones *et al* (reference). Shown are a western blot confirming the identity of the protein compared to full-length Tim-3 (from CD8+ T-cell lysate) and a reducing denaturing SDS-PAGE of the purified product for purity analysis. **(C)** Standard Curve and 4-Parameter Logistics (4PL) fit. The recombinant sTim-3 is used as the standard for the sTim-3 ELISA. The stock is diluted down to make a 2.5-fold series dilution ranging from 1.0-97ng/mL. A standard curve is run for every plate and 4PL analysis is used to create the standard curve and interpolate sTim-3 concentrations for the plasma samples.