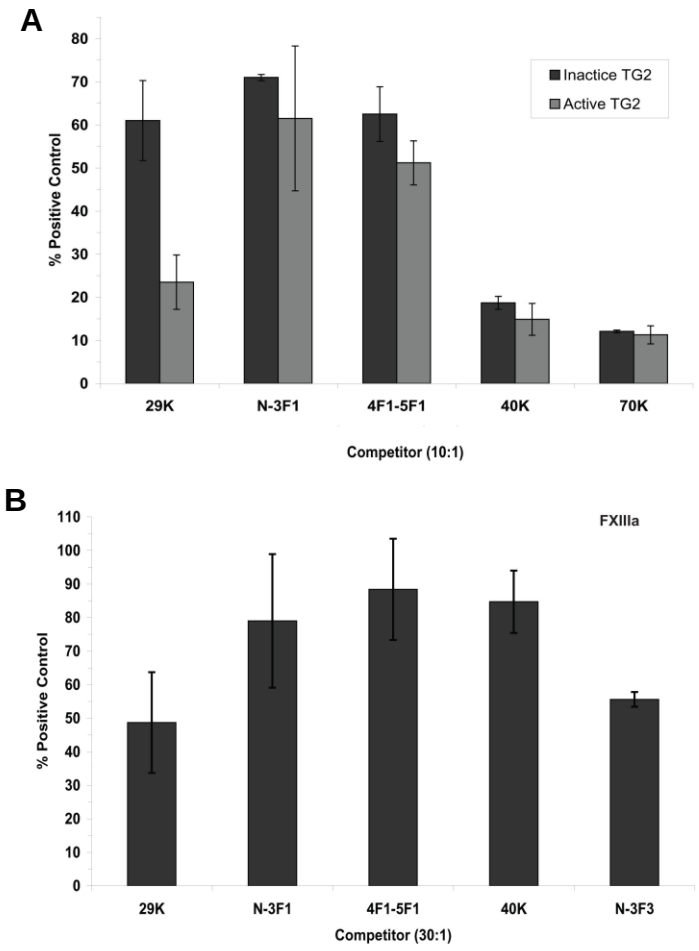
Supplementary Figure 1



Supplemental Figure 1. ELISAs examining abilities of FN fragments or constructs to block binding of TG2 or FXIIIa to substrate-bound FN. ELISAs were based on a published assay that demonstrated competition by soluble 40K for binding of inactive TG2 to substrate-adsorbed FN (26). The bound transglutaminases were detected with monoclonal anti-TG2 (clone 4G3, Santa Cruz Biotechnology) or anti-FXIII (clone AC-1A1. Thermo Fisher Scientific) followed by peroxidase-conjugated goat anti-mouse IgG (H+L) (Jackson ImmunoResearch Laboratories) and then SureBlue TMB peroxidase substrate (Kirkegaard and Perry Laboratories). Results are presented in relation to Ab^{450nm} of control without competitor. (A) TG2, 20 nM in 2 mM EDTA (inactive TG2) or 2 mM calcium chloride (active TG2), was incubated with a 10-fold molar excess of 29K, 40K, 70K, N-3F1, or 4F1-5F1 FN for 1 hr at 22^{o}C before incubation in high-binding microtiter plates previously coated with 20 nM FN. (B) FXIIIa, 50 nM in 2 mM calcium chloride, was incubated with a 30-fold molar excess of the same potential competitors except that N-^{3}F3 was used rather than 70K. Data are mean $\pm$ range or SD of 2 or 3 replicate experiments for each condition.