Supplemental Figure 1: U33 Fab Sequence.

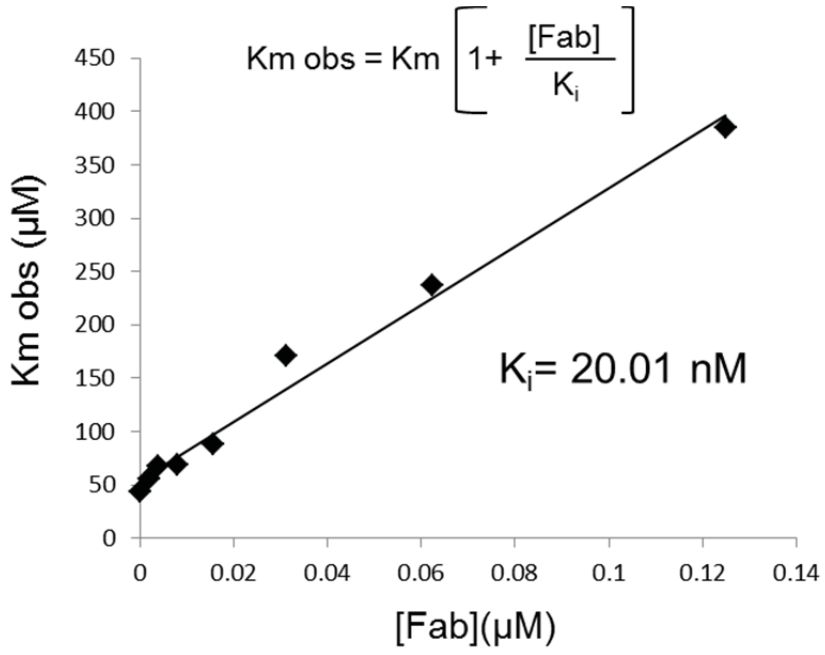
Light chain

EIVLTQSPLSLPVTPGEPASISCRSSQTLMNRNGNNFLDWYLQKPGQSPQLLIYLGSNRAP
GVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCMQRIEFPYTFGQGTKLEIKRTVVAPSVFI
FPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSST
LTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

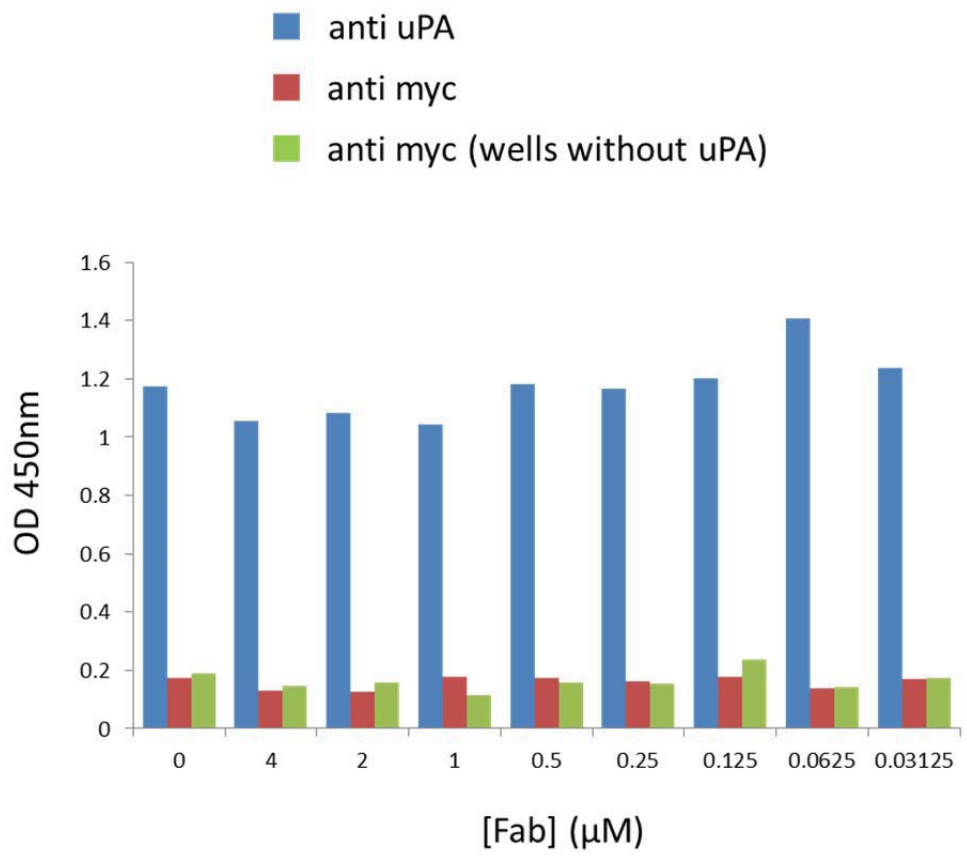
Heavy chain

EVQLVQSGGGLVKPGRSLRLSCTASGFTFGDYAMSWVRQAPGKGLEWVGFIRSKAYGGTTE
YAAVKGRFTISRDDSKSIAYLQMNSLKTEDTAVYYCIRGANWNWGQGTLVTVSSASTKGPS
VFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSV
VTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCAAAHHHHHHGAAEQKLISEEDLNGAA

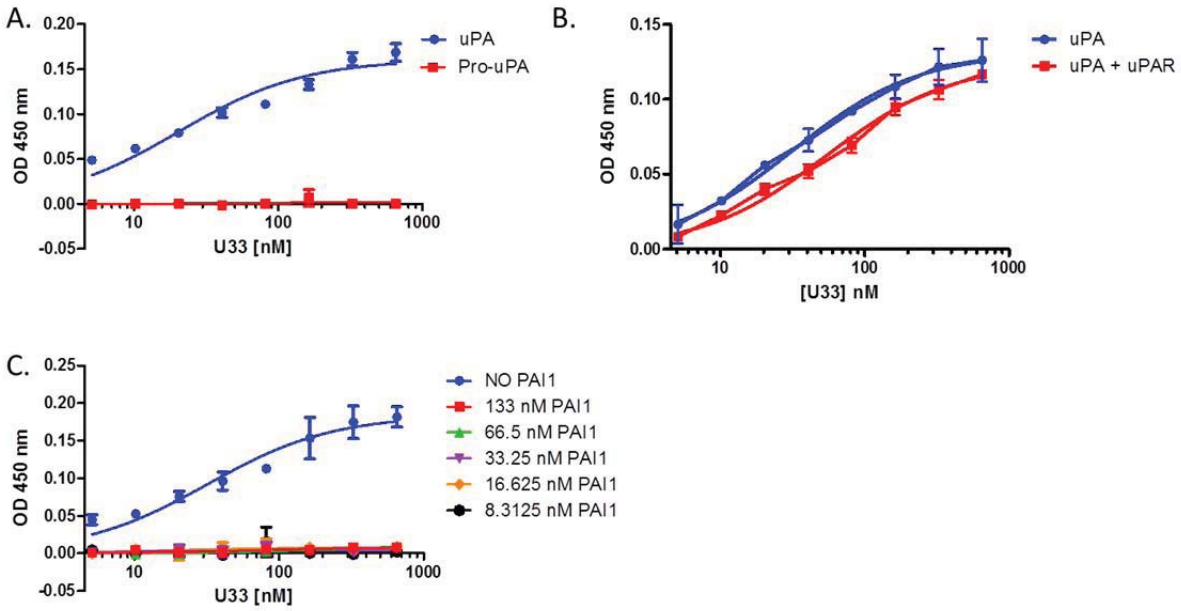
Supplemental Figure 2: Inhibition constant calculation of U33 Fab



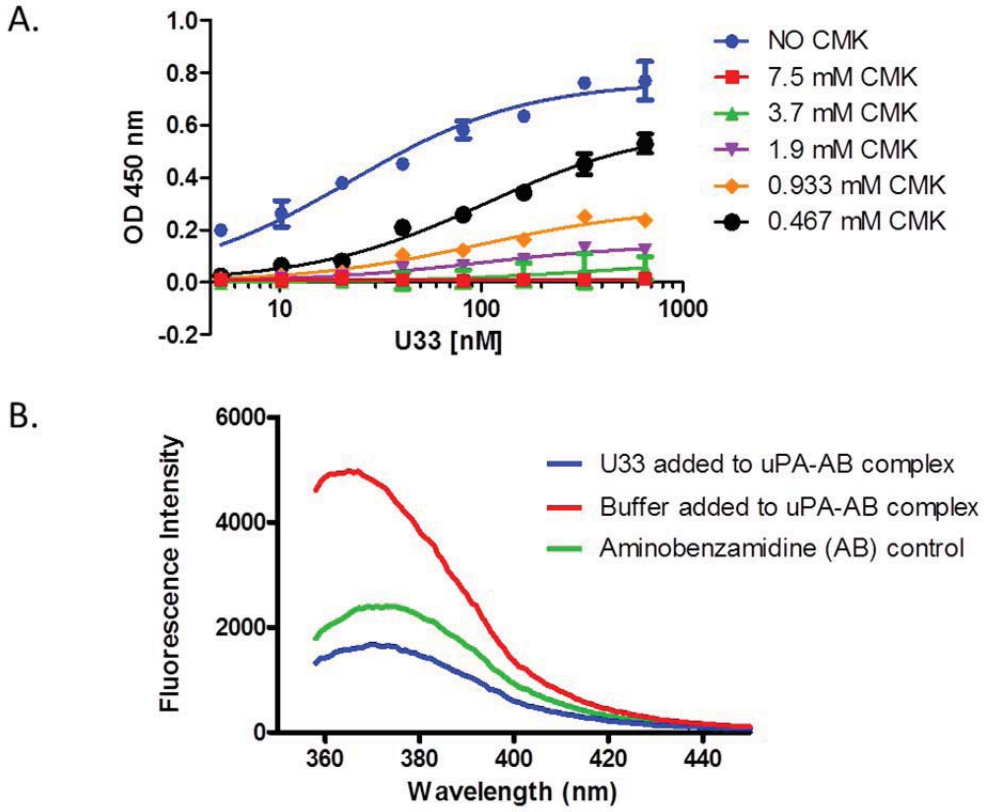
Supplemental Figure 3: U33 does not bind to the uPA-PAI1 complex: Serial dilutions of pure U33 (4 μM to 31.2 nM) were added to the complex uPA-PAI immobilized on plates. The presence of uPA on the plate (blue bars) was detected with an anti-uPA antibody and a secondary antibody conjugated to peroxidase, the absorbance at 450 nm is directly proportional to the concentration of uPA in the well. The U33 Fab has a c-myc tag, the amount of Fab bound to the complex (red bars) was detected using an anti-c-myc antibody, the absorbance at 450 nm is directly proportional to the concentration of uPA in the well. The green bars show the binding of U33 to the plates with PAI1 but without uPA (negative control)



Supplemental Figure 4: Selectivity of U33 IgG for active uPA versus inactive uPA. uPA is found in at least four different forms in vivo. An ELISA assay was used to determine the selectivity of U33 IgG for active soluble uPA (Panels A, B, C), inactive zymogen (Panel A), active uPA bound to uPAR (Panel B), and PAI-1 inhibited uPA (Panel C). The data shows that U33 IgG selectively binds to all active form of uPA but not inactive forms.



Supplemental Figure 5: Dependence of U33 IgG binding on an accessible uPA active site: uPA was pre-treated with active site-directed inhibitors before U33 IgG addition. In Panel A, uPA was pre-treated with increasing concentration of H-Glu-Gly-Arg-CMK, a tripeptide chloromethylketone inhibitor that binds in the S3 to S1 pockets of uPA and forms a covalent bond with catalytic residues. U33 IgG binding was assessed by ELISA. In Panel B, uPA was pre-treated with a saturating amount of aminobenzamidine (AB), a non-covalent inhibitor that binds in the S1 pocket of uPA and emits a fluorescent peak when excited at 325 nm. Addition of U33 to the pre-formed uPA-AB complex decreases fluorescence, suggesting that U33 displaces AB into aqueous solution where it is weakly fluorescent. The AB alone control shows the fluorescence of AB in aqueous buffer. The data from both experiments show that U33 IgG restricts access to the S1 pocket within the uPA active site.



Supplemental Figure 6: Selectivity of U33 IgG for human uPA compared to highly related serine proteases: An ELISA assay was used to determine the selectivity of U33 IgG for human uPA compared to highly related serine proteases. All proteases were active site-titrated to ensure that identical concentrations of active protease were adsorbed to the ELISA plate. At concentrations where U33 IgG saturates human uPA, there is no detectable binding to tPA (Panel A), mouse uPA (Panel B), thrombin, HGFA, hepsin, chymotrypsin, trypsin (Panel C), MT-SP1, and plasmin (Panel D). The data shows that U33 IgG can discriminate between proteases with identical catalytic mechanisms and similar protein folds.