Supplemental Material

Supplemental Figure 1: Standard curves relating (A) amount of DiO-labeled MP proteins (μg) and fluorescence (R^2 = 0.999) or (B) amount of FITC-labeled fibronectin (Fn-FITC) (μg) and fluorescence (R^2 = 0.999).

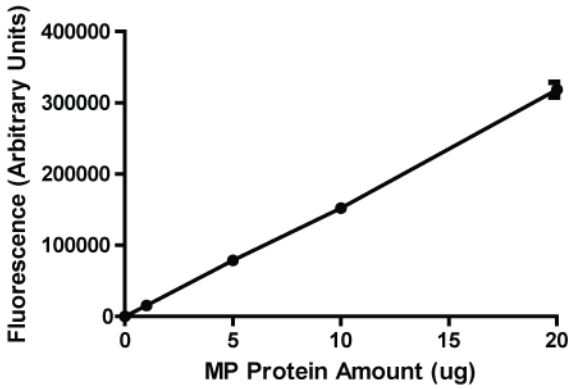
Supplemental Figure 2: Validation of MMP-14, MMP-15, and MMP-16 antibodies for western blot applications. Samples of positive controls (MMP-14-transfected MCF7 cell lysate for MMP-14,HT-1080 lysates for MMP-15 and MMP-16) , microEC total protein (TP), MPs, and Sup (negative control) were analyzed via western blot using MMP-14, MMP-15, or MMP-16 antibody in the presence or absence of their respective neutralizing peptides. Actin and GAPDH were included as loading controls. Bands detected at 66/56 kDa for MMP-14, 72/60 kDa for MMP-15, and 65/63 kDa for MMP-17 were neutralized by blocking peptides, confirming bands' identities as the pro- and active forms of their respective MMPs.

Supplemental Figure 3: Validation of 3 fluorogenic MMP substrates for use in MMP activity assays. MMP activity assays involving 100, 250, or 500 ng/ml MMP-2, MMP-9, MMP-1, MMP-13, or MMP-14 (catalytic domain or full length) (Calbiochem) were analyzed using MMP fluorogenic substrate I, II, or III. Each substrate exhibited different relative sensitivities for the various MMPs tested.

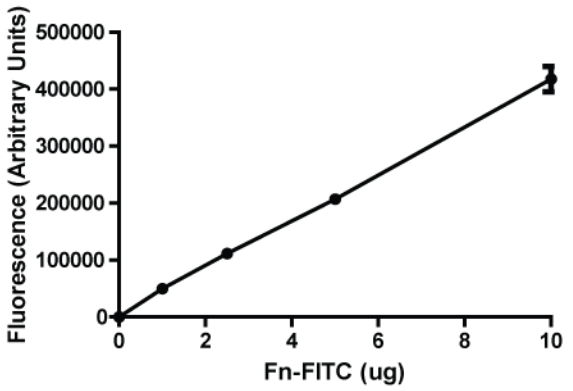
Supplemental Figure 4: Validation of reverse zymography. Samples of TIMP-1, -2, -3, and -4 (20 ng) were analyzed via reverse zymography and TIMP-1, -2, -3, and -4 western blots. TIMP-1 (29 kDa), TIMP-2 (21 kDa), and TIMP-3 (28 kDa) bands were detected in reverse zymograms. TIMP-4 (29 kDa) was not detectable by reverse zymography. Samples of TIMP-1, -2, -3, and -4 were detectable by their respective antibodies with no discernable cross-reactivities, indicating high levels of specificities for the antibodies used.

Supplemental Figure 1

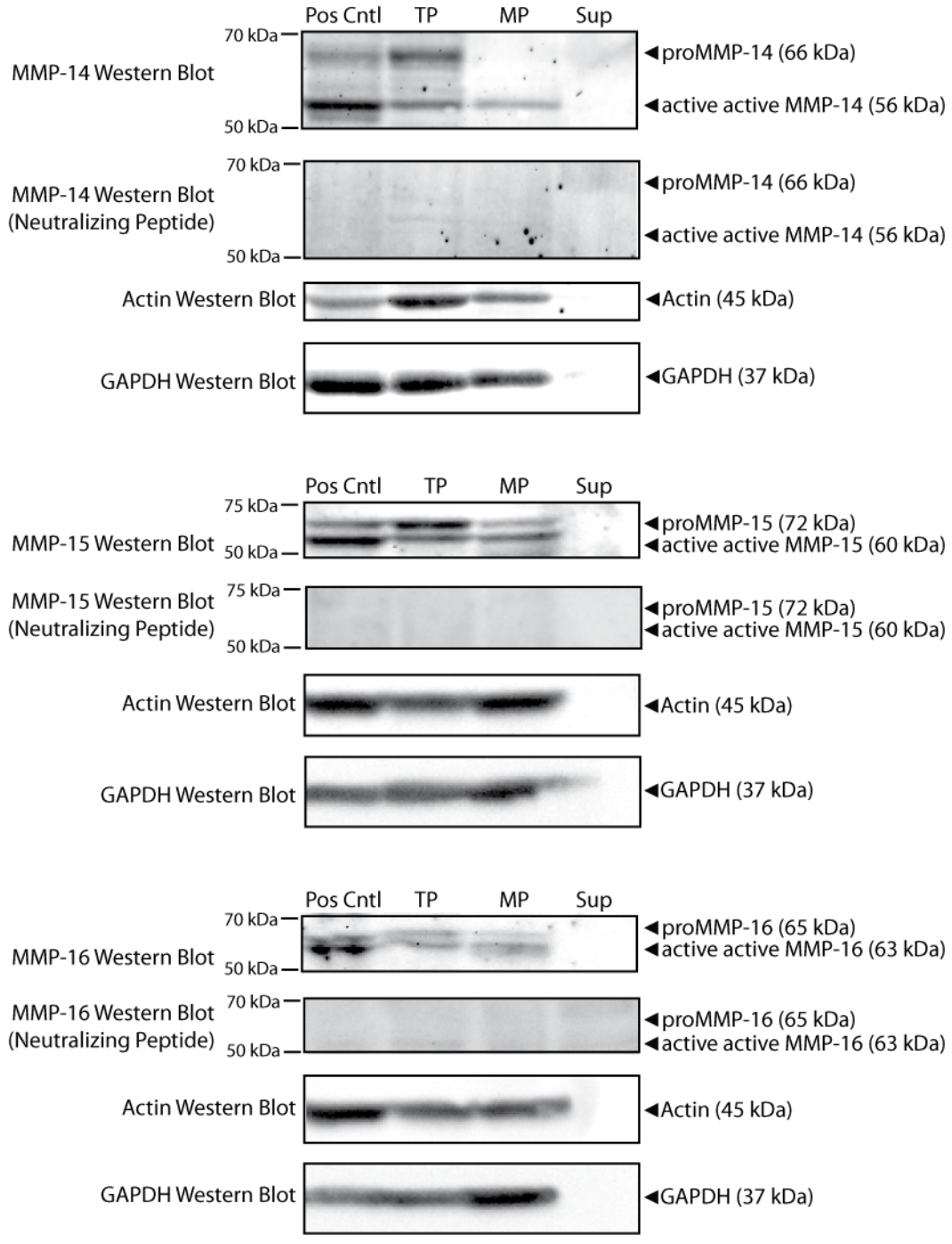
A



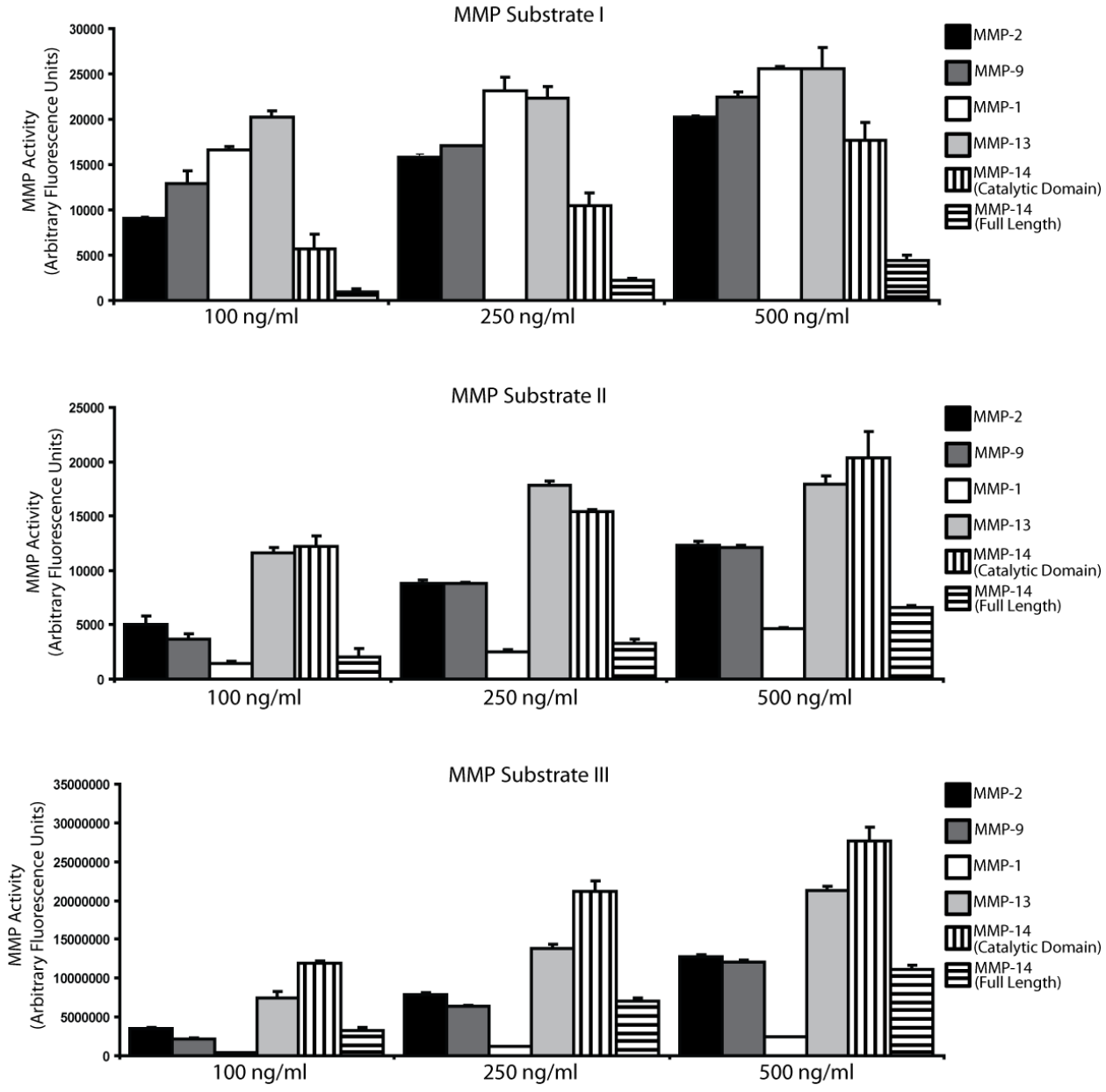
B



Supplemental Figure 2



Supplemental Figure 3



Supplemental Figure 4