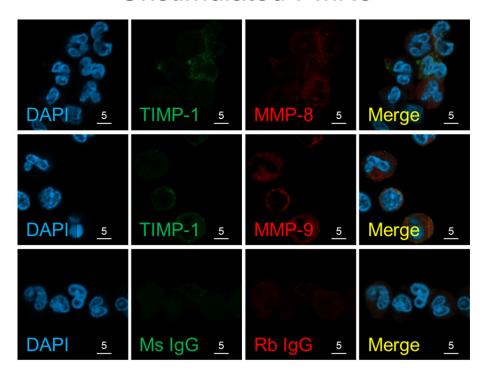


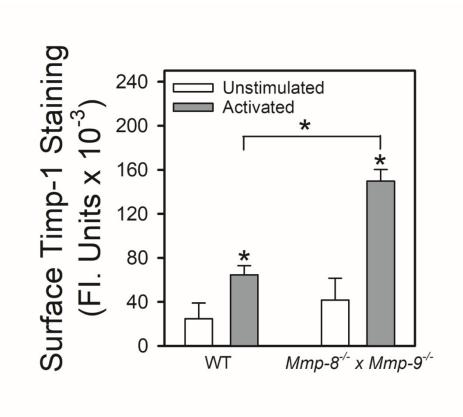
Supplemental Figure 1: Steady state *TIMP-1* mRNA levels in PMNs after stimulated with fMLP, IL-1β, or IL-6. PMNs isolated from healthy human donors (n = 3) were incubated for 30 min at 37°C with or without 10^{-7} M fMLP, 10^{-7} M IL-1β or 10^{-7} M IL-6. Steady state *TIMP-1* mRNA levels were measured using qRT-RT-PCR using *PPI*A as the housekeeping control gene, and the $\Delta\Delta C_T$ method. None of the agonists studied increased *TIMP-1* mRNA in PMNs. Data are mean ± SEM; n = 3.

Supplemental Figure 2

Unstimulated PMNs

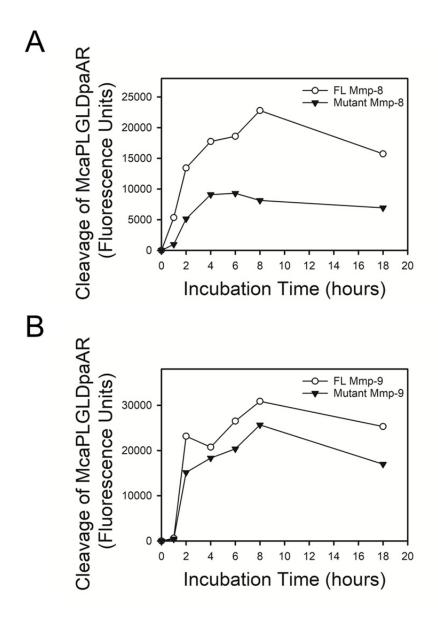


Supplemental Figure 2: Minimal staining was detected on the surface of unstimulated PMNs. PMNs were isolated from peripheral blood samples from healthy male and female human donors as described in Methods. Cells were double immunostained with Alexa 488 and murine anti-TIMP-1 IgG or non-immune murine (Ms) IgG (second panel) and with Alexa 546 and rabbit anti-MMP-8 IgG, or rabbit anti-MMP-9 IgG or rabbit (Rb) IgG (third panel). Nuclei in the cells were counterstained blue using 4',6-diamidino-2-phenylindole (DAPI, first panel). Co-localization of TIMP-1 and MMPs on the surface of the PMNs was assessed by confocal microscopy (see merged images in the fourth panels). The white bars are 5 microns in length. The results shown are representative of PMN preparations from 3 different donors.



Supplemental Figure 3: Timp-1 level is expressed on the surface of $Mmp-8^{-/2}$ x $Mmp-9^{-/2}$ PMNs. PMNs isolated from WT and $Mmp-8^{-/2}$ x $Mmp-9^{-/2}$ mice were incubated at 37°C for 15 min with or without 10^{-6} M PAF and then for 30 min with or without 10^{-6} M fMLP. PMNs were then immunostained with Alexa 488 for surface Timp-1. Data are mean \pm SEM; n = 150-200 cells/group. Asterisk indicates P < 0.001 compared with unstimulated control belonging to the same genotype or the group indicted. The data shown are representative of 3 independent experiments.

Supplemental Figure 4



Supplemental Figure 4: Recombinant full length proMmp-8 and proMmp-9 and mutant proMmp-8 and proMmp-9 lacking the COOH terminal hemopexin domain retain the capacity to be activated and degrade a susceptible substrate: Equimolar amounts (50 nM) of full length (FL) proMmp-8, FL pro-Mmp-9 and mutant forms of these proMmps lacking the COOH-terminal hemopexin domain (Mutant Mmp-8 and Mutant Mmp-9) or assay buffer alone (as a control) were incubated in duplicate with a quenched fluorogenic substrate that is susceptible to cleavage by these Mmps (Mca-Pro-Leu-Gly-Leu-Dpa-Ala-Arg). Cleavage of the substrate was measured using fluorimetry.