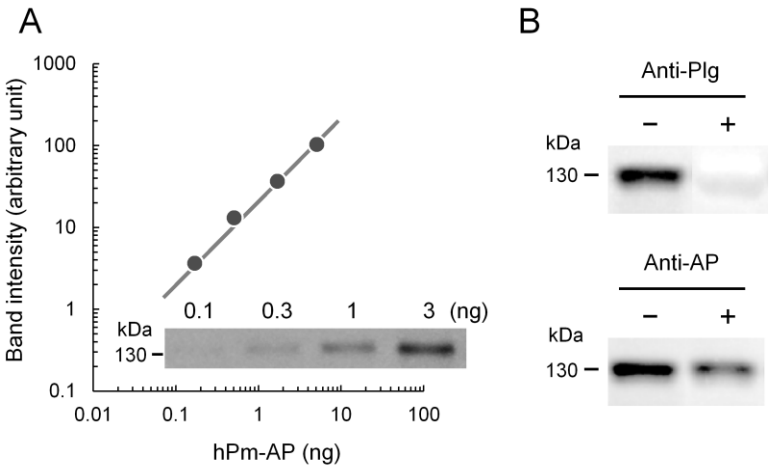
**Additional file 2**




**Supplementary Fig. 1.** The bases of the quantification of Pm-AP and the confirmation of its authenticity.
(A) Human Pm-AP (*hPm-AP*) was resolved on a SDS-polyacrylamide gel containing 2 mg $ml^{-1}$ human fibrinogen and processed for detection of lysis band. The plotted data are the average of duplicate determinations. The intensity of the lysis bands at ~140 kDa was proportional to the amount of hPm-AP at 0.1−3 ng ($r$ = 0.99). (B) Mouse citrated plasma was recalcified (20 mM $CaCl_2$) for 16 h at room temperature to induce Pm-AP formation. After centrifugation, the resulting supernatant (5 µl) was mixed for 30 min with 0.6 mg $ml^{-1}$ of anti-plasminogen IgG (*Anti-Plg*) or anti-$\alpha_2$-antiplasmin IgG (*Anti-AP*). After incubation for 30 min at room temperature, supernatant of the sample (equivalent to 2 µl of the original plasma) was resolved on a SDS-polyacrylamide gel containing 2 mg $ml^{-1}$ casein for zymography. The intensity of the lysis band at ~130 kDa was decreased by the treatments with anti-plasminogen IgG and anti-$\alpha_2$-antiplasmin IgG, demonstrating the authenticity of the ~130 kDa lysis band as Pm-AP. Similar results were obtained when samples were processed for zymography after removal of the immune complexes with protein G-Sepharose. (It is likely that the immune complexes precipitate following the reaction or that the IgG binding prevents the regeneration of plasmin activity during the zymography process.)