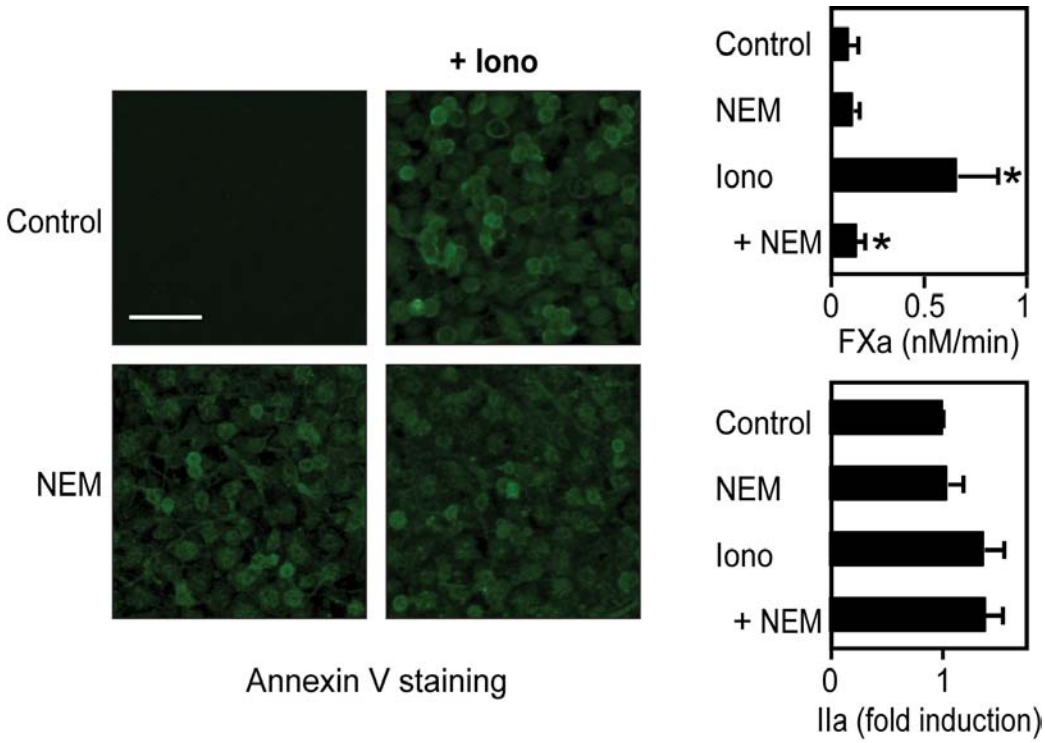
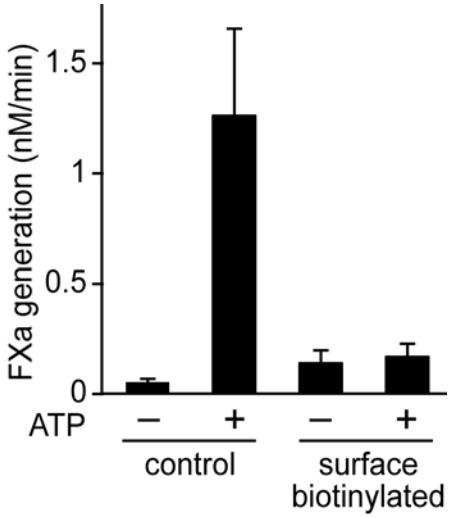
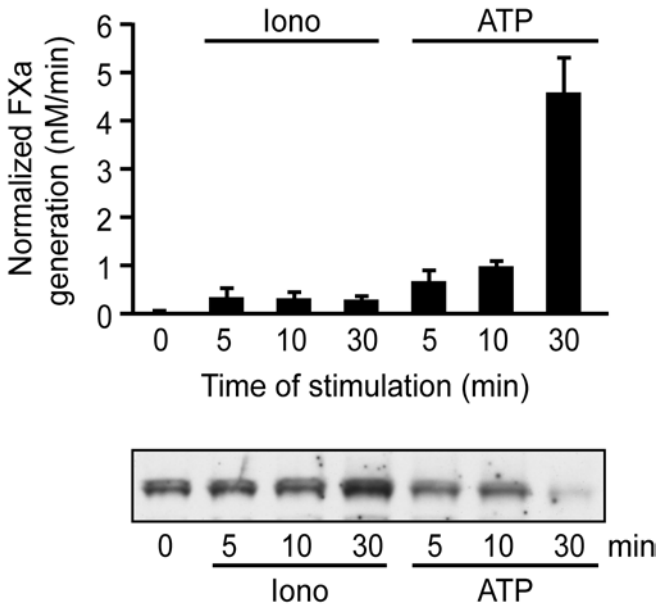
Supplementary Figures



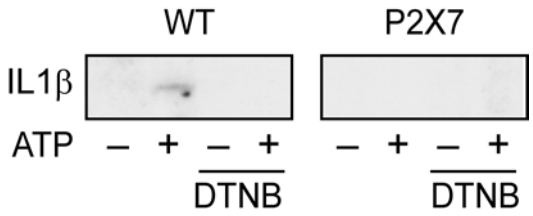
Supplementary Fig. 1: Effect of thiol blockade on TF and prothrombinase activity following ionomycin stimulation of macrophages. We analyzed mechanisms of TF activation in macrophages that express very low TF activity by blocking free thiols with N-ethylmaleimide (NEM) and monitoring phosphatidylserine (PS) exposure by annexin V staining as well as TF and prothrombinase activity. The two bar graphs on the right show TF-dependent generation of FXa activity as compared to prothrombinase activity on the surface of primed macrophages stimulated with the established activator ionomycin (10 μM) for 30 minutes. Where indicated, cells were briefly pretreated with the thiol blocker NEM (1 mM) before ionomycin stimulation. Thiol blockade markedly inhibited TF activation by ionomycin. Control experiments using purified TF reconstituted into PS containing phospholipid vesicles showed that pretreatment with 1 mM NEM did not impair Xa generation (control: 9.4 $\pm$ 2.7 nM Xa/min versus NEM-treated 8.6 $\pm$ 1.2 nM Xa/min), excluding that NEM inactivated interactions of TF with PS to inhibit specifically TF-dependent coagulation. The images on the left show PS detection by annexin V staining after 30 minutes of stimulation.TF activation was not necessarily a consequence of PS exposure, because NEM alone induced surface PS, but not TF activity. In addition, the modestly increased prothrombinase activity following ionomycin stimulation was not inhibited by NEM, indicating that thiol pathways were irrelevant for procoagulant phospholipids exposure but important for regulation of TF activity under these experimental conditions; mean ± SD, $n \geq 4$, *$p < 0.02$ different from control or ionomycin treated cells. Scale bar = 500μm.



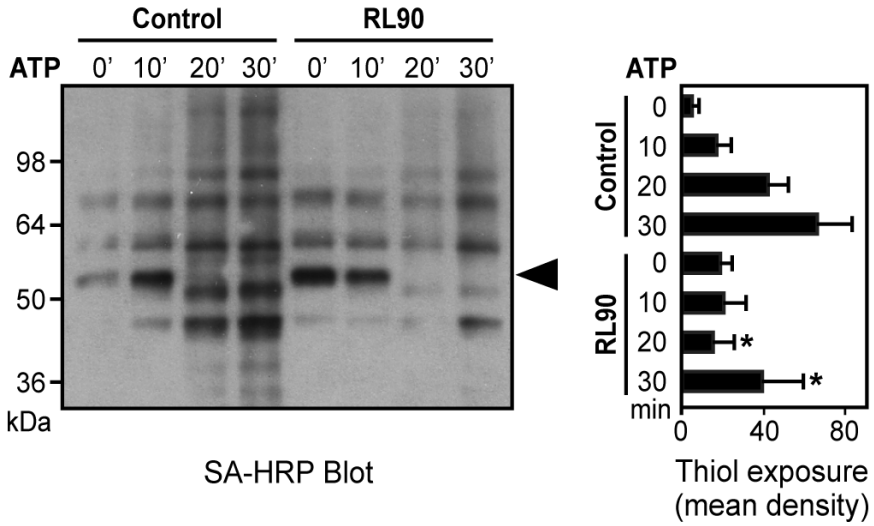
Supplementary Fig. 2: Surface biotinylation abolishes ATP-induced TF activation. In order to address whether TF activation involved translocation of intracellular pools of TF, we covalently modified extracellular amino groups with biotin succinimidyl esters, known to inactive crucial Lys residues required for TF procoagulant activity. Surface biotinylation of unstimulated or ATP-stimulated intact cells followed by cell lysis with octylglucoside revealed the presence of intracellular pools of TF activity that was not changed by ATP stimulation (control: 1.2 $\pm$ 0.2 nM Xa/min versus ATP-stimulated: 1.4 $\pm$ 0.1 nM Xa/min). As shown in the figure, surface biotinylation prior to ATP-stimulation prevented TF activation on intact cells, indicating that ATP-regulated cryptic TF primarily resided in a solvent-accessible surface environment prior to activation.



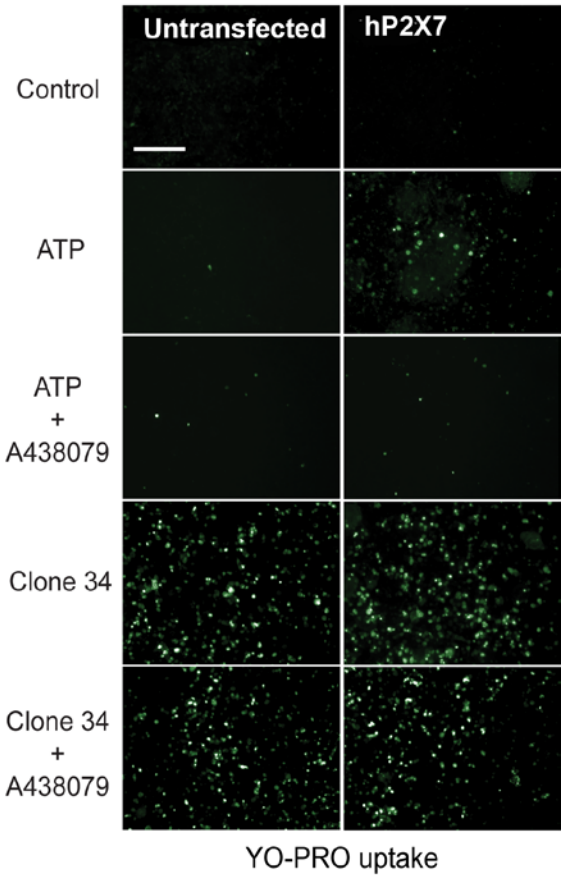
Supplementary Fig. 3: Time course of ATP and ionomycin mediated activation of cell surface TF procoagulant activity of macrophages. The upper panel shows FXa generation normalized for the TF expression levels determined by Western blotting, as shown in the lower panel; n = 3, mean ± SD.

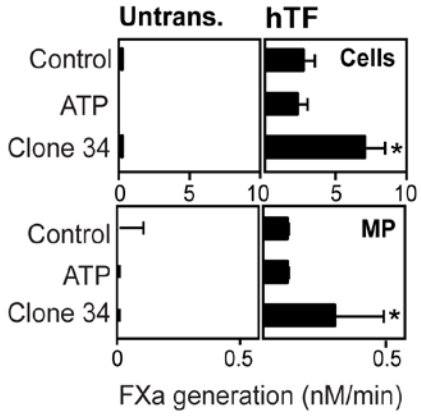


Supplementary Fig. 4: Release of IL-1β from ATP stimulated macrophages. Macrophages were stimulated with 5 mM ATP in the presence or absence of 2 mM DTNB for 30 minutes. Proteins in the MP-depleted supernatants from WT or P2X7$^{-/-}$ macrophages were recovered by TCA precipitation for detection of IL-1β release by Western blotting with 3ZD monocloncal antibody. No IL-1β was detected in the MP fraction. A typical experiment for the release of IL-1β in the supernatant is shown.



Supplementary Fig. 5: Effect of anti-PDI RL90 on the release of MP carrying solvent-accessible free thiols. MPB labeling of MP collected after ATP stimulation for 30 minutes in the presence or absence of RL90 antibody. Blots from 4 independent experiments were quantified for total thiol labeling in the released MP, as shown in the right panel, *$p < 0.003$ different from ATP stimulation at the same time point. RL90 initially increased labeling of a 54 kD band (arrowhead) and significantly delayed the appearance of other thiol labeled bands.





Supplementary Fig. 6: Clone 34 induces YO-PRO uptake, TF cellular activation and TF^{+}MP release independent of P2X7 expression. P2X7-deficient HEK-293 cells were mock-transfected or transfected with human P2X7 (hP2X7) or TF (hTF). P2X7 activation, but not the effect of anti-PDI Clone 34, was blocked with 10 μM of the human P2X7-specific antagonist A438079, demonstrating specificity of the agonists. N = 2-3, mean ± SD, *p<0.001 compared to controls.