Supplement Material

Tissue factor prothrombotic activity is regulated by integrin-arf6 trafficking

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Supplemental Figures

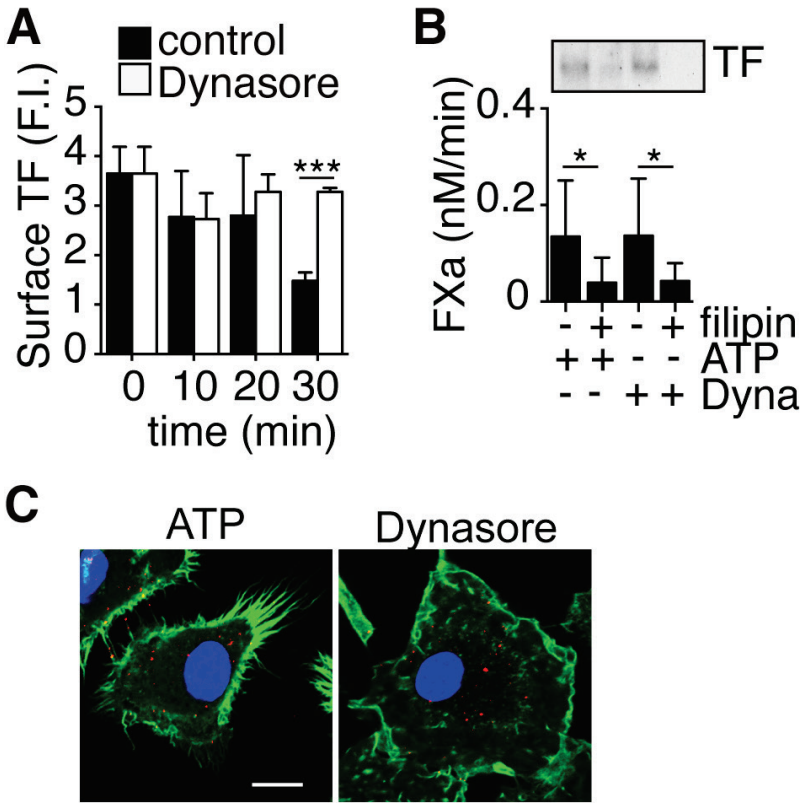

Figure I. Dynasore- and mastoparan- stimulated TF$^+$ MP release. A, FACS detection of cell surface TF on macrophages kept for the indicated times in assay buffer in absence (control, closed bars) or presence (open bars) of Dynasore*; ***p<0.001, t*-test, n=3. **B**, Effect of filipin treatment on the release of procoagulant TF$^+$ MP from ATP- and Dynasore-stimulated cells: Western blot of TF on MP (upper panel) and determination of procoagulant TF MP activity by FXa generation assay (lower panel); **p<0.05* paired *t*-test, n=6. Pairing efficiency test: ATP r=0.7716, *p<0.0361, and Dynasore r=0.922, **p=0.0069. **C**, Macrophages treated for 20 minutes with ATP or Dynasore were stained for F-actin (phalliodin-Alexa488, green), TF (αTF-Alexa647, red) and nuclei (Hoechst, blue). Images were taken on a Zeiss LSM 710 with a 63x Plan-Apochromat NA 1.4 WD 190 mm oil emersion objective and processed using Image Browser Software; scale bar = 10 µm.

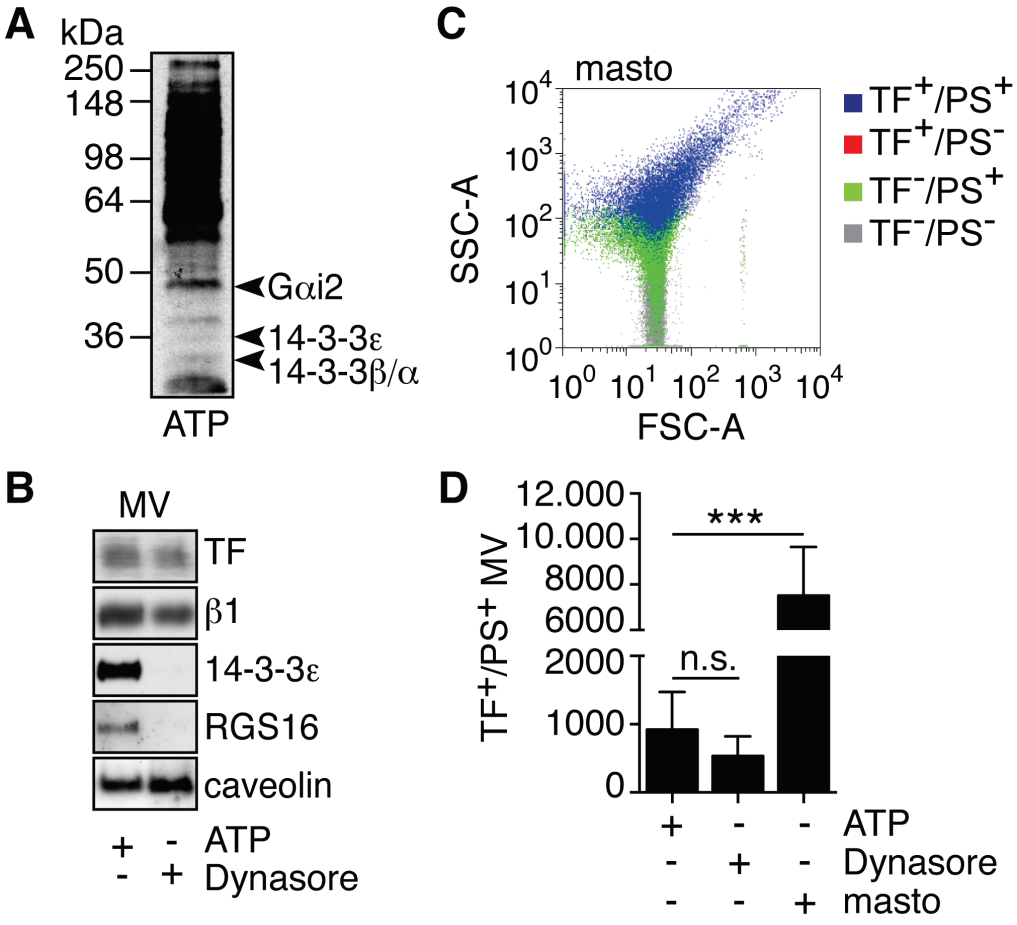

Figure II. ATP and mastoparan stimulate the release of $G\alpha_{i2}$ and TF^+ MP. A, RAW 264.7 macrophage MV thiol-proteome was labeled with MPB and detected by streptavidin blot. **B**, Representative Western blots of TF, integrin β1, 14-3-3ε, RGS16 and caveolin on ATP- or Dynasore-induced MV. **C**, FACS detection of TF- and PS-labeled MV from mastoparan-stimulated cells. **D**, Count of TF^+ PS^+ MP released from macrophages stimulated for 30 minutes with ATP, Dynasore or mastoparan; ****p<0.001* ANOVA (Tukey's), n=6.

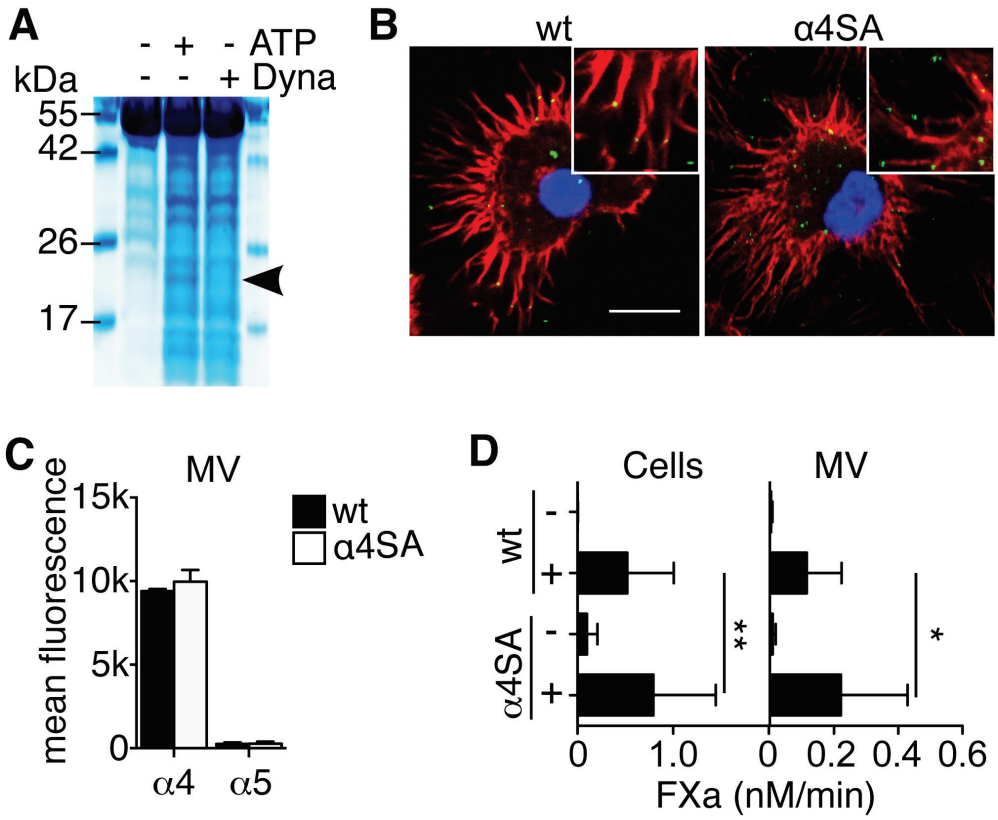

Figure III. Arf6 regulation of TF. A, Coomassie detection of proteins in supernatants from control, ATP and Dynasore-treated cells. The arrowhead indicates the protein band subjected to mass spectrometry. **B**, Filopodia formation and TF localization in control and ATP-stimulated wild-type (wt) and α4SA macrophages. Cell surface TF was labeled with immuno-purified rabbit anti-mouse TF antibody and detected with anti-rabbit-Alexa488 (green). Fixed cells were counterstained for F-actin (phalloidin-Alexa633, red) and nuclei (Hoechst, blue). Inserts show magnification of filopodia. Images were taken on a Zeiss LSM 710 with a 63x Plan-Apochromat NA 1.4 WD 190 mm oil emersion objective and processed using Image Browser Software; scale bar = 10 μm. **C**, Integrin α4 and integrin α5 on ATP-induced MP released from wt (open bars) or α4SA macrophages (closed bars), determined by FACS staining. **D**, FXa generation of cells and MP from ATP-stimulated wild-type or α4SA macrophages; **p<0.05, **p<0.001,* paired *t*-test, n=14. Pairing efficiency test: cells r=0.9251, ***p<0.0001, and MP r=0.7104, **p=0.0022.

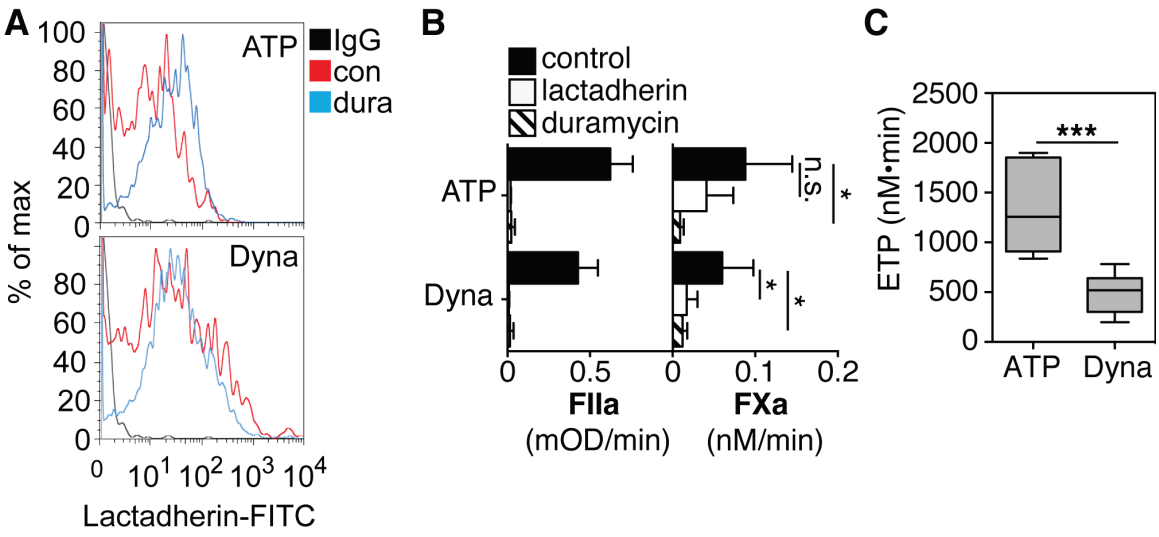

Figure IV: Prothrombotic properties of MP. **A**, FACS analysis of ATP- or Dynasore-induced MP treated with PE-binding duramycin (1 µM) and stained with PS-binding Lactadherin-FITC, or IgG Alexa488. **B**, Prothrombinase activity (left panel) and FXa generation measured with 2 nM FVIIa (right panel) on MP from ATP- or Dynasore-induced MP in the presence of duramycin (1 µM) or lactadherin (50 nM), **p<0.05*, ANOVA (Turkey's), n=3. **C**, Quantification of endogenous thrombin potential (ETP) from curves as shown in Figure 4D, n = 3, ***p< 0.001 t-test.