Supplemental Data

Nano-zymography using laser-scanning confocal microscopy unmasks proteolytic activity of cell-derived microparticles.

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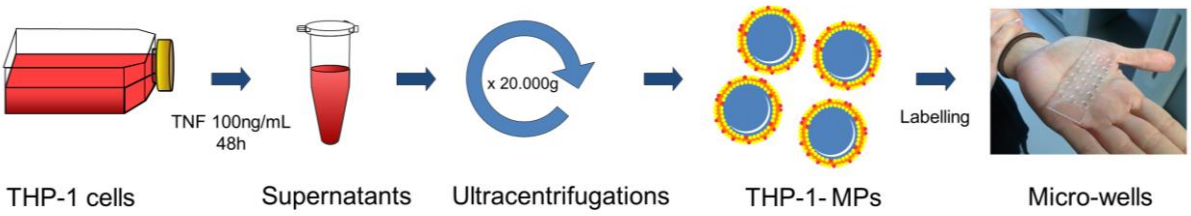

Figure S1. Schematic representation of the method that allows MPs production from THP-1 cells.

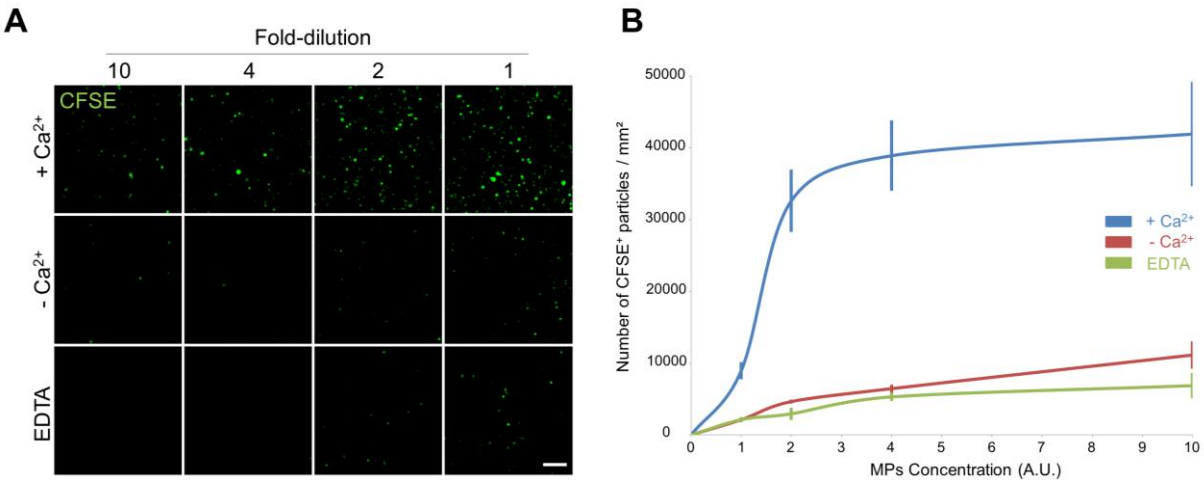

Figure S2. Calcium chelation using EDTA does not further reduce binding of MPs in the absence of added calcium. (A) Representative imaging of CFSE+ MPs in the presence (black) or absence (grey) of calcium and in the presence of EDTA, a calcium chelator. (B) Corresponding quantification revealing that EDTA treatment of the sample does not further reduce MPs binding in the absence of calcium. (n=3 per group) This suggests that the residual binding is not due to trace amount of calcium. (Scale Bar= 10 µm)

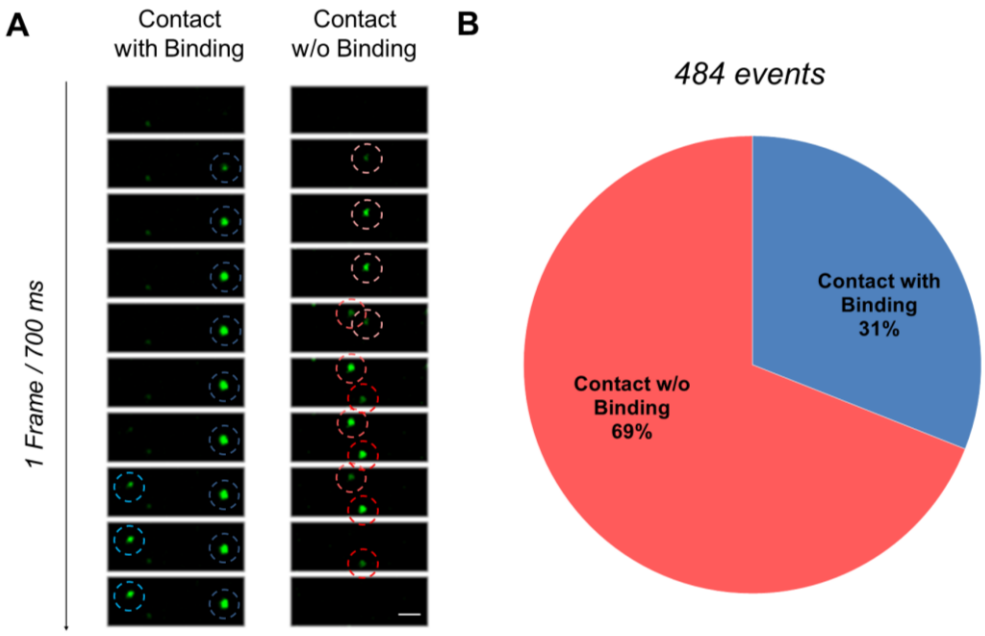

Figure S3. Quantification of the number of annexin-V binding MPs in a sample from mouse plasma. (A) Representative images from a time-lapse at high temporal and spatial resolutions allowing to detect contact of the MPs to the coated micro-wells. MPs were labelled with CFSE and the binding was performed in the presence of calcium. Events were considered as "contact with binding" if the MP remains bound to the micro-wells after the contact. Imaging was performed using LSCM in the first 5 minutes after incubation of the MP sample in the micro-wells. (B) Corresponding quantification showing that 31% of the total number of contacts led to MP binding (n=3 per group). (Scale Bar= 2 µm)

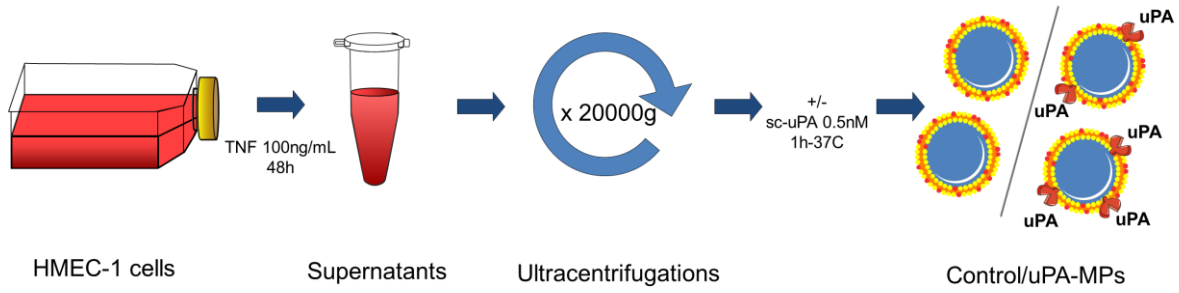

Figure S4. Schematic representation of the method that allows uPA-MPs production from HMEC-1 cells.

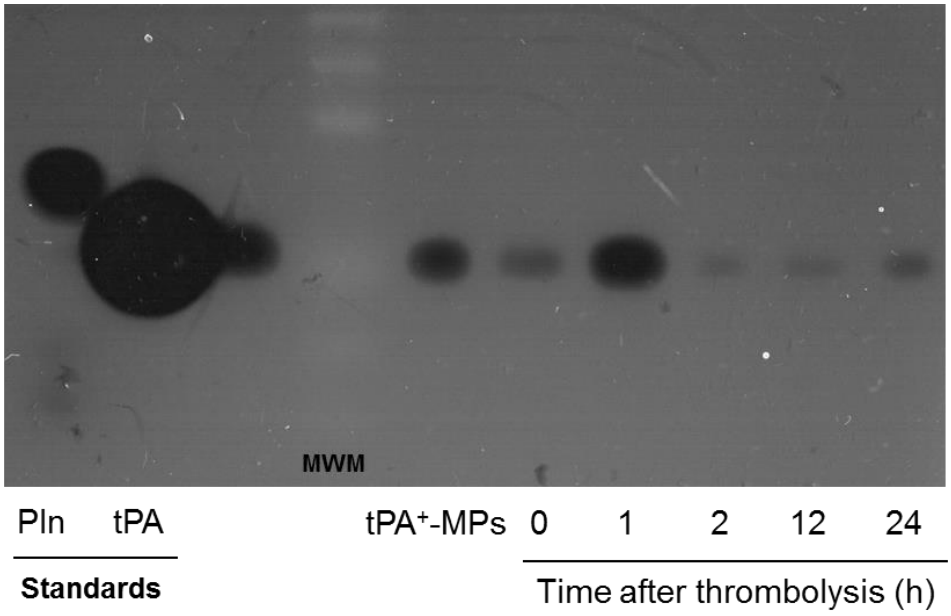

Figure S5. MPs from stroke patients present fibrinolytic activity before and after thrombolysis. Representative fibrin-agar zymography showing that even before thrombolysis (0 time point) and at all other time-points (including the delayed time-point of 24 hours), MPs from this stroke patient present a detectable amount of active tPA. MPs were purified from plasma by sequential centrifugations, lysed using a detergent and their contents were run on a SDS-page gel. Subsequent fibrin-agar zymography was performed as described in the material and methods section. Positive controls (tPA+-MPs) were purified from tPA-transfected HEK293 cell lines.