Appendix 1

Table S1 shows the characteristics of different mAbs that were used to measure EVs in plasma.

Table S1 Characteristics of different antibodies

Antibody	Clone	Company	Antibody working concentrations (ng/mL)
Anti-CD9 APC	HI9a	Biolegend, San Diego, CA, USA	200
Anti-CD63 APC	H5C6	Biolegend, San Diego, CA, USA	6,666.67
Anti-CD81 APC	1D6	Invitrogen, Waltham, MA, USA	6,666.67
Isotype control IgG1,k/kappa-APC	MOPC-21	Biolegend, San Diego, CA, USA	200/6,666.67
Anti-human TRANCE/RANKL AF488	685857	R&D systems, Minneapolis, MN, USA	4,000
Isotype control IgG2B-AF488	133303	R&D systems, Minneapolis, MN, USA	4,000

Figure S1 shows the tetraspanin identification in PPP and control conditions. In all control samples, no fluorescent events were detected. After detergent treatment (to dissolve EVs) some residual signals were observed. In *Figure S2*, representative samples are shown of a PPP sample and 0.22 µm PBS) before and after detergent treatment. Images of single spots of tetraspanin single-positive samples and tetraspanin and RANKL double-positive samples are shown in *Figure S3*.

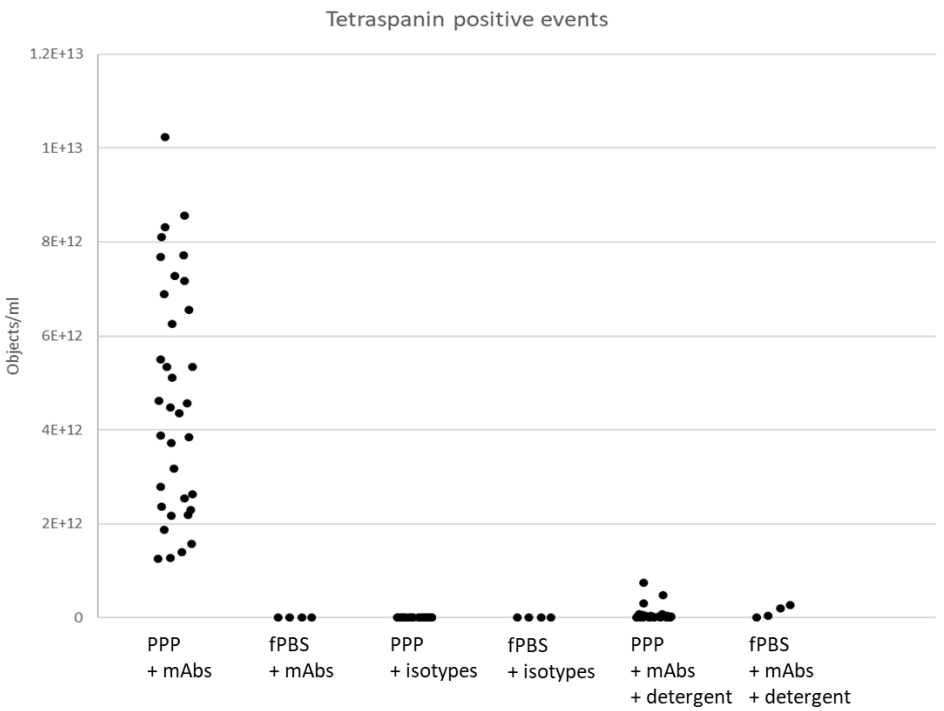

Figure S1 Quantification of tetraspanin (CD9, CD63, CD81) events in PPP samples and fPBS, stained with mAbs or isotypes, before and after detergent treatment. mAbs, monoclonal antibodies.

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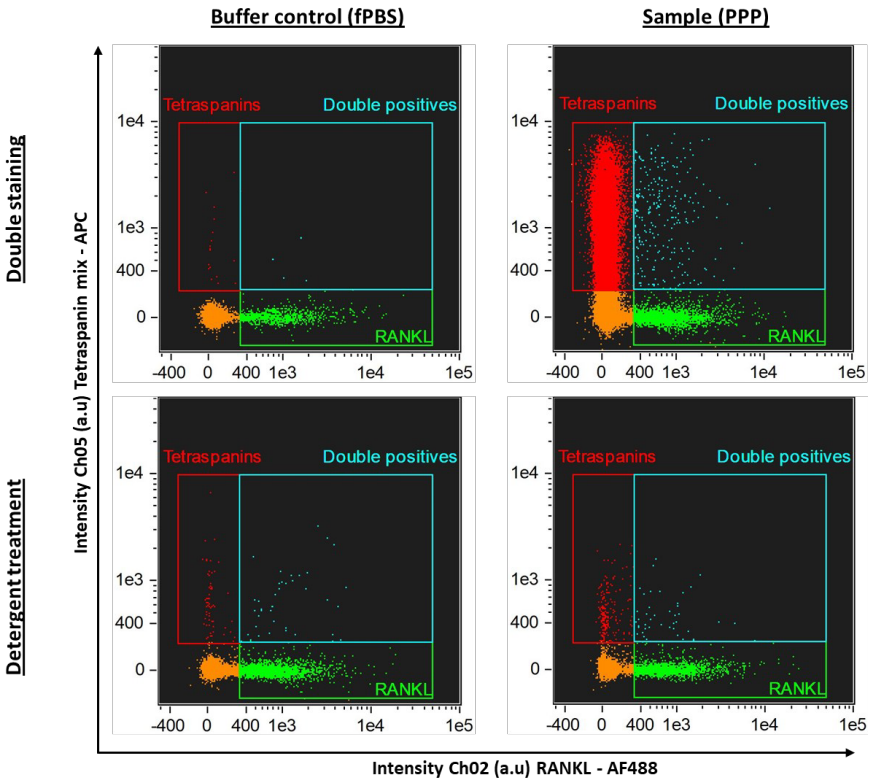

Figure S2 Double-stained (anti-tetraspanin mix containing anti-CD9, anti-CD63, and anti-CD81 conjugated with APC and anti-RANKL conjugated with AF488) fPBS (left panels) or PPP (right panels) before (top panels) and after (bottom panels) detergent treatment. In red and blue all tetraspanin positive events are shown (including RANKL positive events), identifying EVs. Signals in orange and green are tetraspanin negative and not identified as EVs.

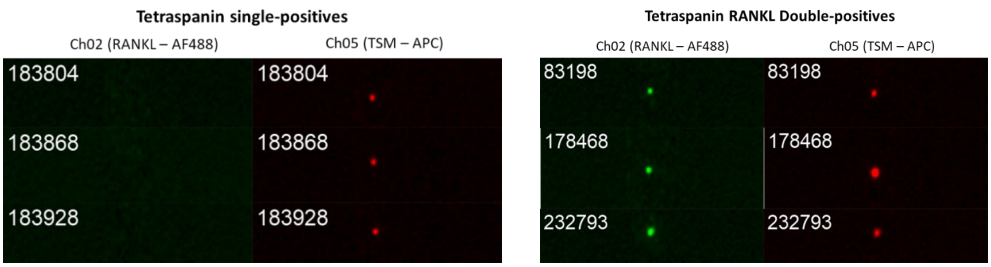

Figure S3 Representative images of tetraspanin single-positive (CD9, CD63, CD81) and double-positive (CD9, CD63, CD81, and RANKL$^+$) objects of a double-stained PPP sample before detergent treatment. The number on the top left of each image denotes the object number assigned during sample acquisition.

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