Magnetic Nanosensor for Detection and Profiling of Erythrocyte-Derived Microvesicles

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Supplementary Figure 1. Membrane filter characterization. The size cutoff of the membrane filter (Vivid Plasma Separation Membrane, Pall Life Sciences) was determined. Samples containing microspheres of different diameters (100 nm to 3 μ m) were prepared. The concentrations of microspheres before and after filtration (C_i and C_f , respectively) were then measured to calculate the penetration (C_f/C_i). A size cutoff of ~1 μ m was established.



Supplementary Figure 2. The microfluidic device for microvesicle (MV) capture and labeling. (A) *MV collection*. pRBC samples are loaded into the inlets (all valves closed). Plasma (containing MVs) is then collected in the reservoir by applying negative pressure to the reservoir (valves #1, 4, 5 open). **(B)** *MV labeling*. The following steps are repeated for 3 cycles. At each cycle, capture microbeads, *trans*-cyclooctene (TCO) monoclonal antibodies (mAb-TCO), and tetrazine-functionalized magnetic nanoparticles (MNP-Tz) are sequentially added to the reservoir. [Step 1] Labeling agents are added into the reservoir containing MVs (all valves closed). [Step 2] The mixture passes through the in-flow chaotic mixer (valves #2, 6 open). [Step 3]. While microbeads are retained on the integrated membrane filter, buffer solution is loaded (into the reservoir) and passed through the channel to wash (valves #2, 6 open). [Step 4] Microbeads are collected in the reservoir by applying reverse flow (valves #3, 6 open).



Supplementary Figure 3. MV detection limit determined by micro-nuclear magnetic resonance (μ NMR). MVs were magnetically labeled by targeting CD235a. From the titration measurements, the detection limit was determined to be ~10⁶ MV/ μ L.



Supplementary Figure 4. Temporal changes in MVs. (A) The MV concentration, as measured by μ NMR, was found to increase with storages time (days). **(B)** The average size of MVs, measured by nanoparticle tracking analysis (NTA), remained unchanged (*P* > 0.95; two-tailed t-test) over time.