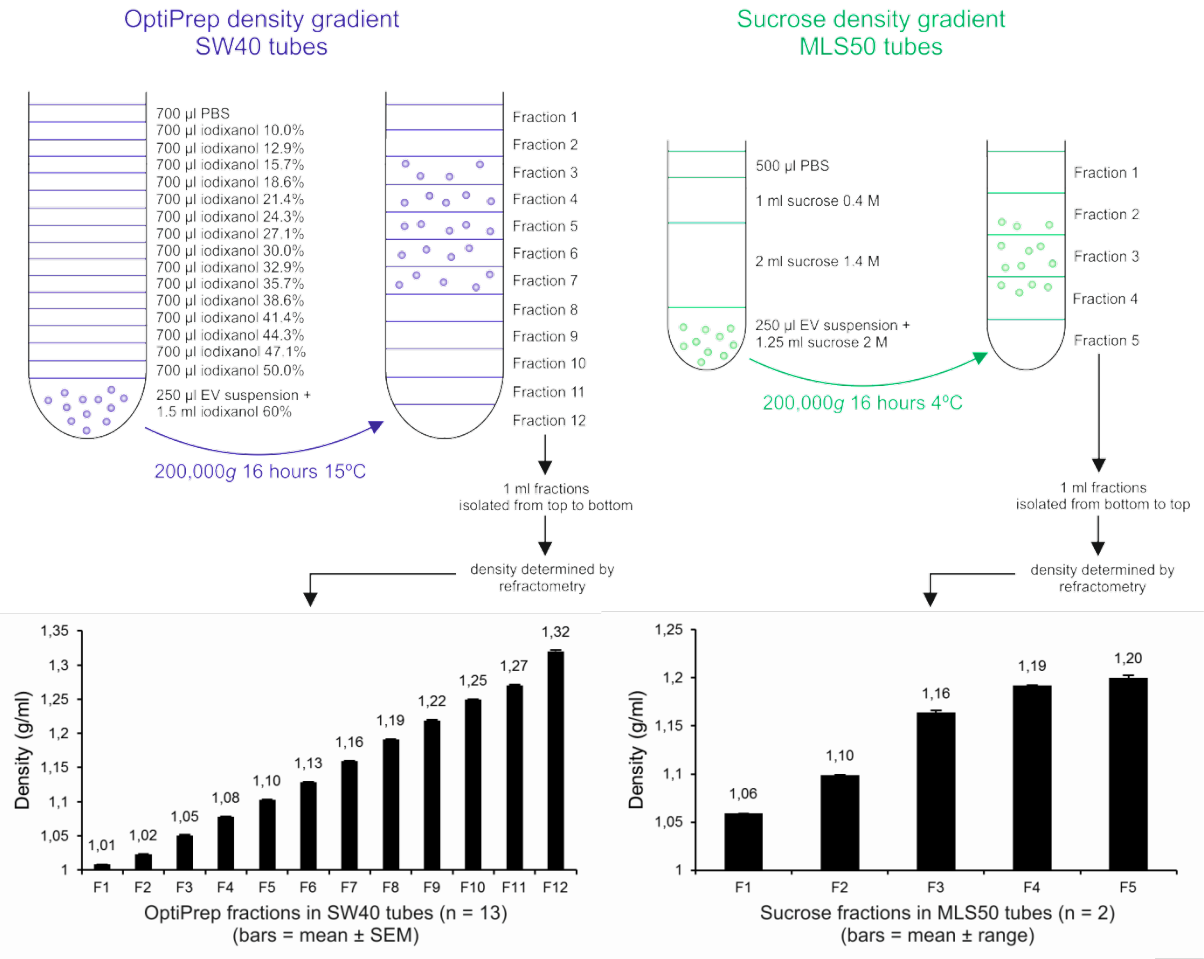
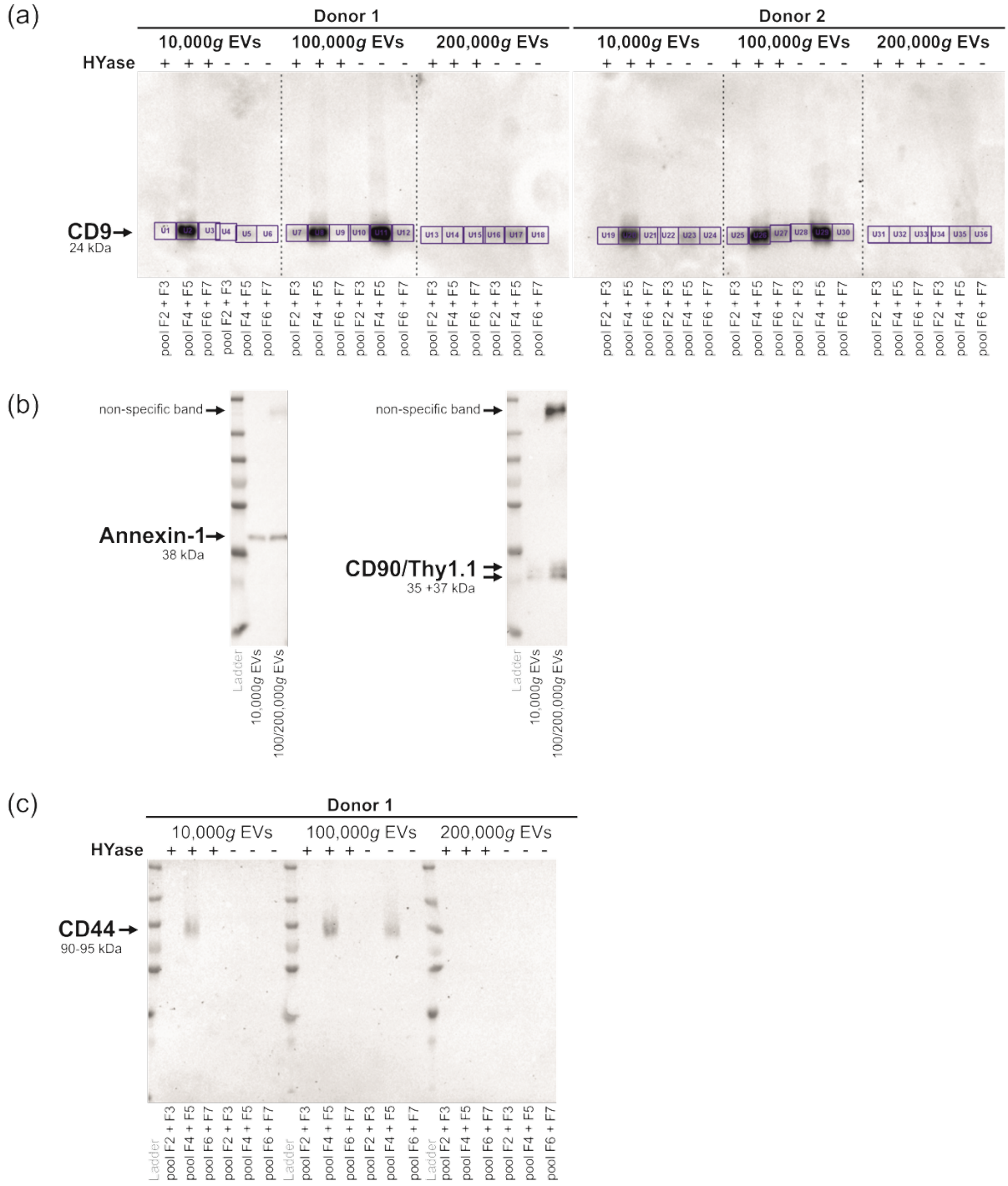
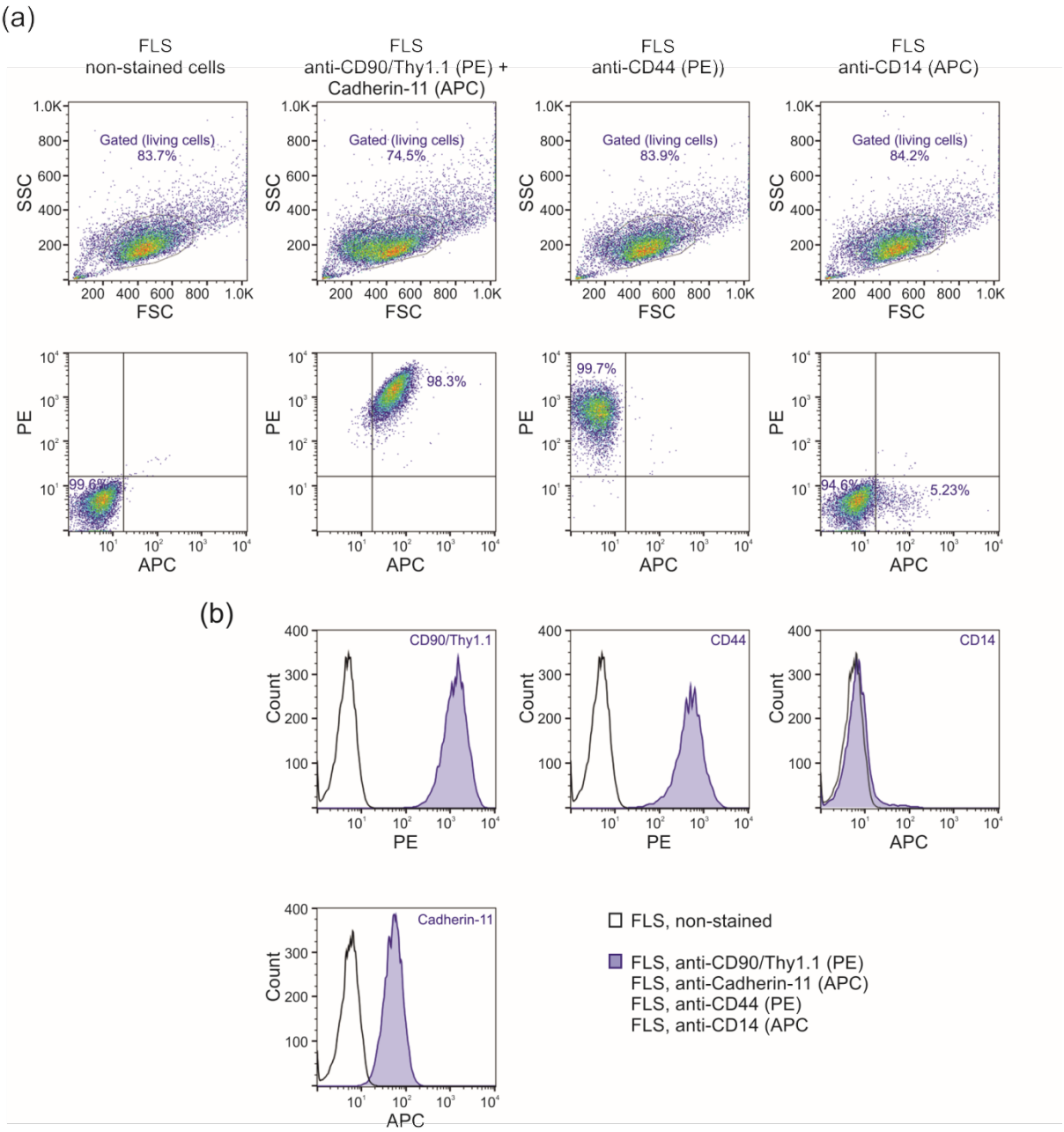


Supplementary Fig. 1. Composition of OptiPrep (iodixanol) and sucrose density gradients in, respectively, SW40 tubes (12.95 ml) and MLS50 tubes (5 ml). In OptiPrep, SF-derived EVs float at densities 1.02-1.16 g/ml with highest concentrations at 1.05-1.10 g/ml. In sucrose, similar EVs float at densities 1.10-1.19 g/ml, with highest concentration around 1.16 g/ml. Different floating densities for EVs in iodixanol vs. sucrose gradients can be the result of gradient design, but can also be introduced by density calculation for the two solutions. For sucrose solutions standard refractive indices were used. For iodixanol, density was calculated using the following formula: 3,4394(refractive index)-3,5870 (32).



Supplementary Fig. 2. Western blotting of SF-derived EV proteins. (a) Raw data corresponding to Fig. 3 including boxes set for band quantification. Detection of CD9 (tetraspanin; EV marker) on EVs from healthy SF. (b) Detection of Annexin-1 (phospholipid binding protein; EV marker; anti-inflammatory maker) and CD90/Thy1.1 (fibroblast marker) on EVs from healthy SF. For this particular analysis EVs were isolated from HYase-treated healthy SF by using centrifugation steps of 10,000*g* (= 10,000*g* EVs), immediately followed by 200,000*g* omitting the 100,000*g* step (= 100/200,000*g* EVs, representing a pool of all EVs that are pelleted if using subsequent 100,000*g* and 200,000*g* centrifugation steps). Isolated EVs were floated in sucrose gradients followed by western blotting. (c) Detection of CD44 (HA receptor; fibroblast-like synovioctye marker) on EVs of healthy SF by western blotting. EVs were isolated from HYase-treated or non-treated healthy SF and floated in OptiPrep gradients. Pools of OptiPrep fractions F2-F7 were analyzed using western blotting (F2 = low density; F7 = high density).



Supplementary Fig. 3. Flow cytometry analysis of primary equine fibroblast-like synoviocytes (FLS) stained for CD90/Thy1.1 and Cadherin-11 (both fibroblast markers), for CD44 (HA-receptor) or for CD14 (macrophage marker). (a) Scatter plots for forward scatter (FSC) x side scatter (SSC) and PE- or APC-signal show that >98% of cells are positive for CD90/Thy1.1 (PE conj.), Cadherin-11 (APC conj.) and CD44 (PE conj.). Only 5% of cells are positive for CD14 (APC-conj.), indicating that the contribution of macrophages in this experiment is negligible. (b) Overlay histograms of non-stained cells versus cells stained against CD90/Thy1.1, Cadherin-11, CD44 or CD14. A complete population shift of stained cells can be seen for CD90/Thy1.1, Cadherin-11 and CD44. The population shift is absent for CD14 staining. Together, these data verify the fibroblast origin of the cells in this experiment.

Materials and methods: Synovial membrane was harvested with owner consent from the femoropatellar joints of adult Warmblood horses euthanized at the Utrecht University Equine Hospital (Fac. Veterinary Medicine, Utrecht University, Utrecht, the Netherlands).All horses were euthanized for reasons other than joint disease. Synovial membrane tissue was digested with Type 1 clostridial collagenase (Worthington, Lakewood, NJ, USA) (210 U/ml in RPMI medium) for 3 hours at 37°C. Cells were pelleted and washed during several centrifugation steps and finally filtered through a 70 µm cell strainer, pelleted and suspended in DMEM/F12 culture medium. FLS were cultured for 3-4 days and stored in liquid nitrogen (passage 1). For flow cytometry experiments, stored cells were cultured until passage 3. For single or double antibody staining, $5x10^5$ cells were incubated with 50 µl staining solution containing the following volumes of antibodies: 7 µl anti-

CD90/Thy1.1 (clone MRC-OX-7, PE conj., Abcam, Cambridge, UK) and 7 µl anti-cadherin-11 (clone 667039, APC conj., R&D Systems, Minneapolis, MN, USA) or 0.25 µl anti-CD44 (clone IM7, PE-conj., eBioscience, San Diego, CA, USA) or 3.5 µl anti-CD14 (clone 61D3, APC conj., eBioscience, San Diego, CA, USA). Fluorescent PE- or APC-signal was measured on a FACS Calibur flow cytometer (Becton Dickinson) and data were analyzed with FlowJo v10.07 software.