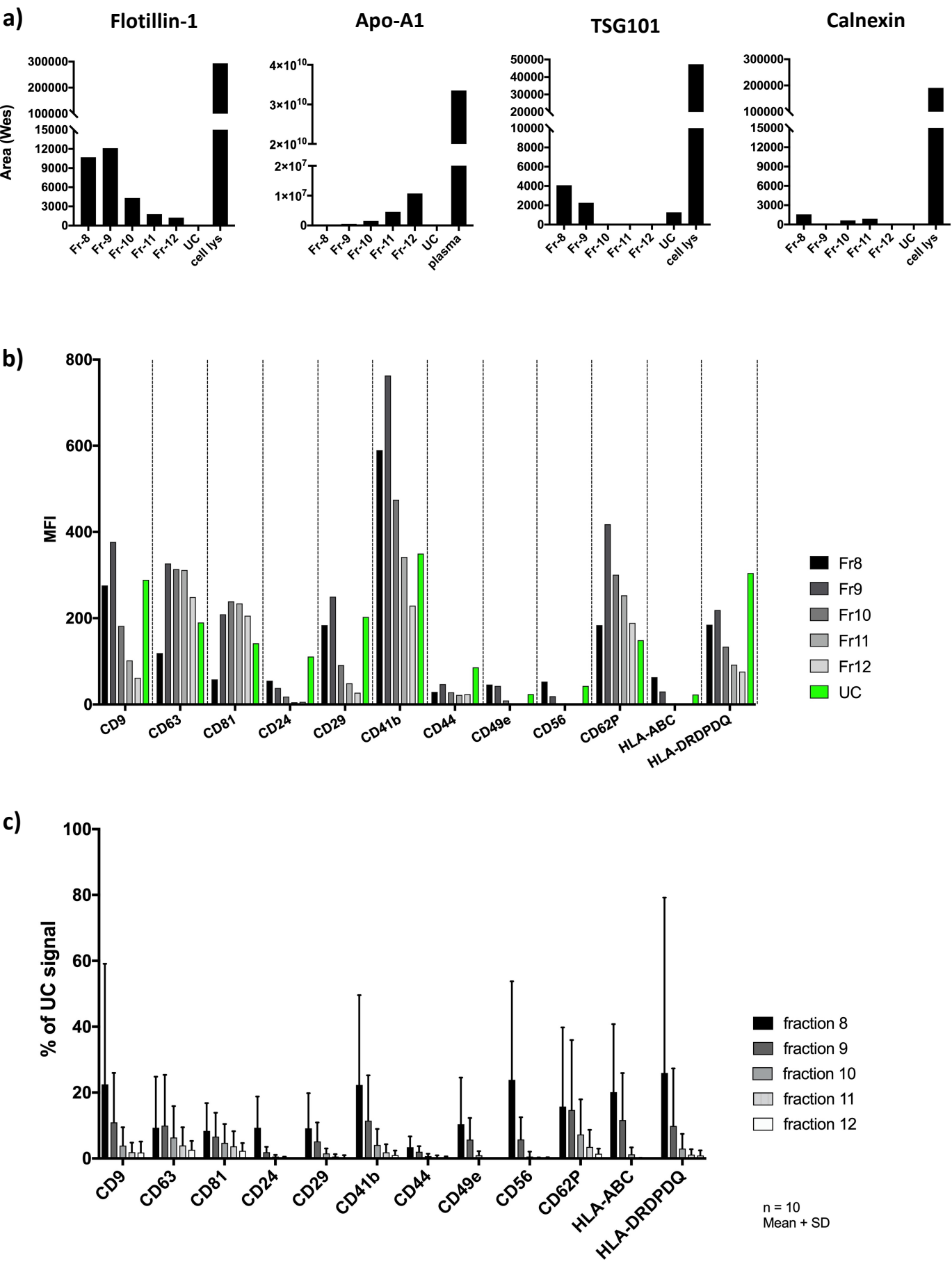


Figure S1



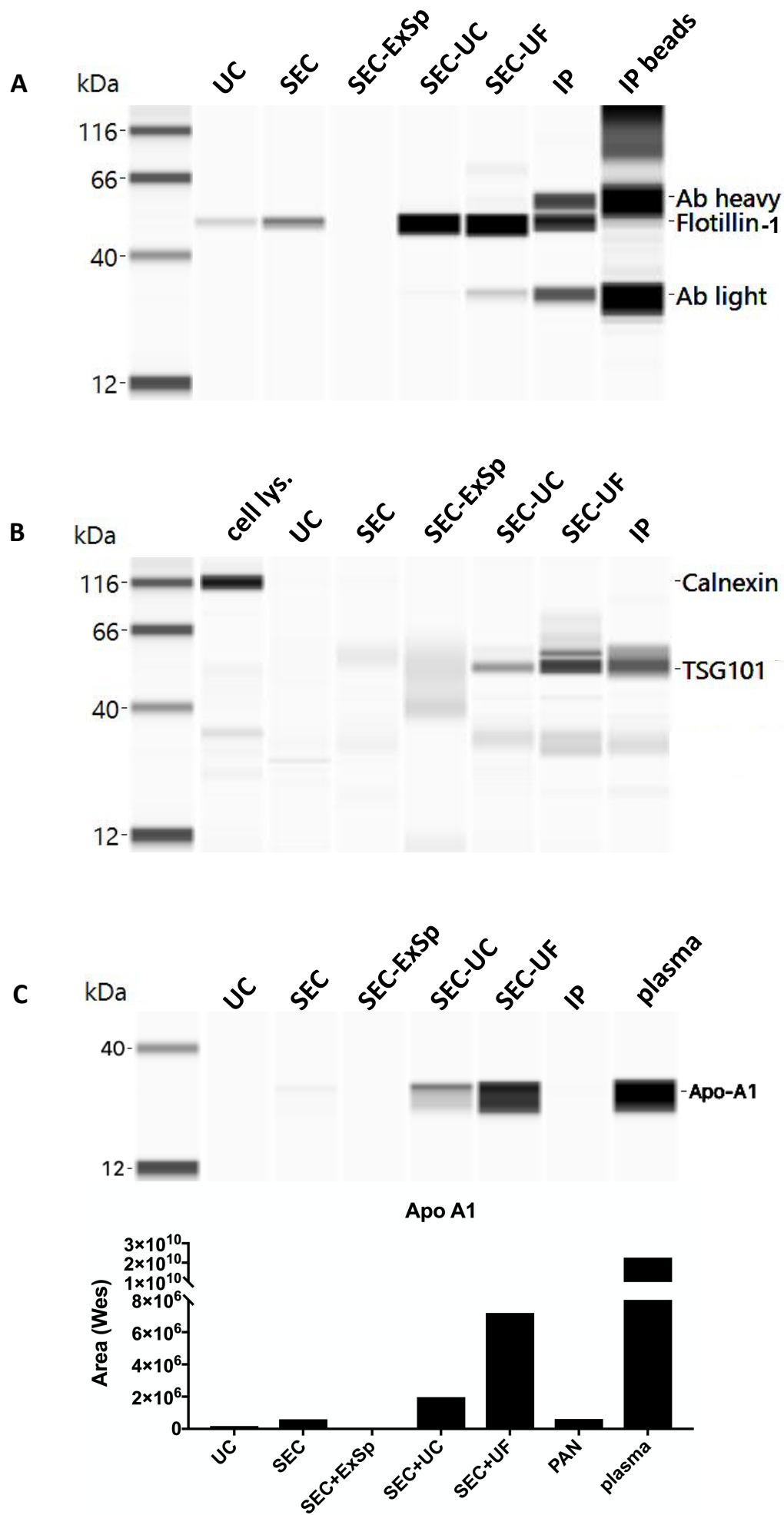
## **Figure S1**

### **SEC- and UC-EV: Quantified immunodetection and MFI values from MACSPlex assay**

(a) Immunodetection of flotillin-1, Apo-A1, TSG101, and calnexin illustrated as calculated area (analyzed with Compass software, ProteinSimple), reflecting the signal intensity. (b) Surface protein analysis with the MACSPlex assay. Depicted is the mean fluorescence intensity (MFI) of one representative experiment out of 10. (c) Surface protein analysis with the MACSPlex assay. Depicted is the mean of 10 experiments as a percentage of the normalized signal from the UC-EVs.

Fr-8 to 12: collected fraction from SEC column, SEC: size exclusion chromatography, UC: ultracentrifugation

Figure S2

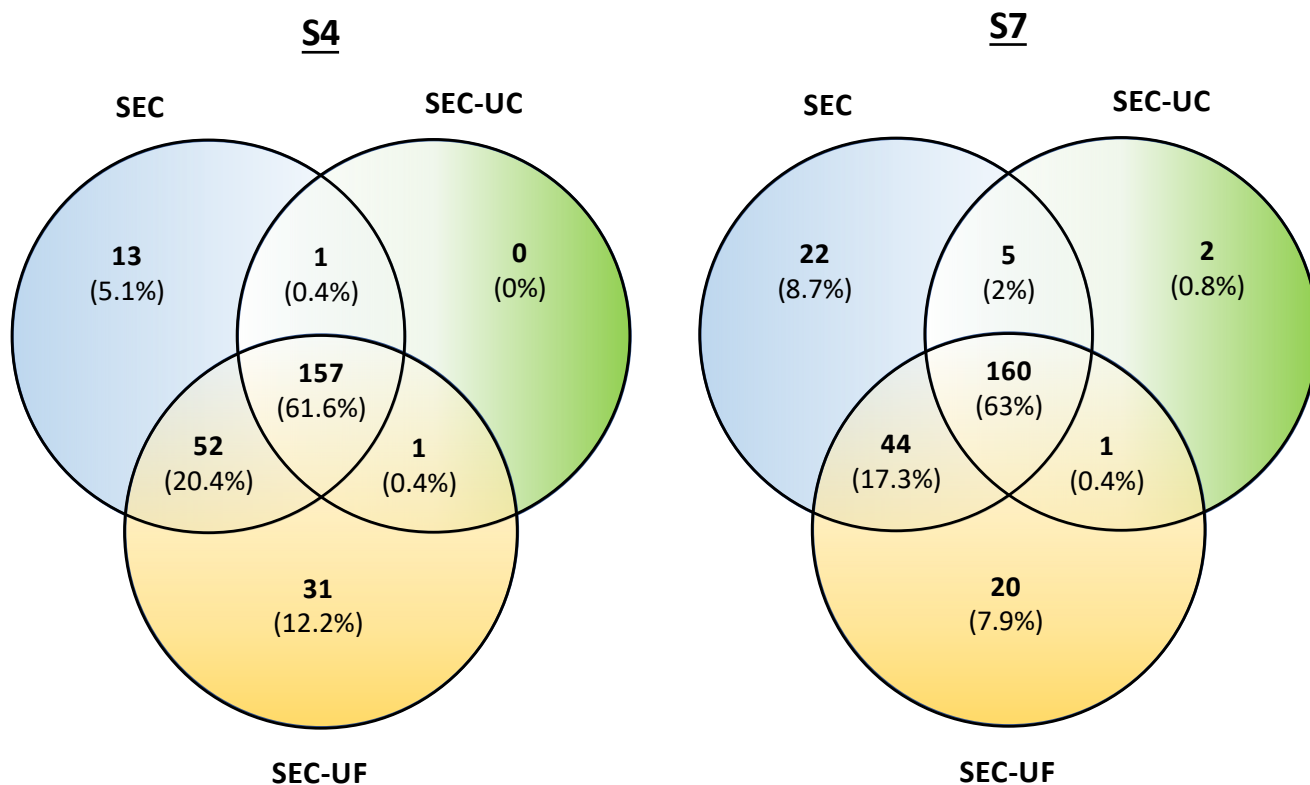


**Figure S2 - EV markers can be detected in all samples but SEC-ExSp-EV**

Immunodetection of the EV marker (A) flotillin-1 and (B) TSG101 and the ER marker calnexin, and (C) apolipoprotein A1 (Apo-A1) shown by Simple Western. The graph illustrates the calculated area (analyzed with Compass for SW software, ProteinSimple), reflecting the signal intensity.

ExSp: precipitation using Exo-spin™ exosome purification kit, IP: immunoaffinity precipitation, SEC: size exclusion chromatography, UC: ultracentrifugation, UF: ultrafiltration

**Figure S3**



**Figure S3**

Venn diagram of proteins detected in EV derived from serum samples 4 and 7, which were isolated with SEC alone or followed by concentration with UC and UF. A high overlap of identified proteins (62.3%) was observed in samples irrespective of the isolation method. Concentration of EVs after SEC isolation results in the identification of up to 12% additional proteins.

**Table S1 - Details on parameters of proteomic analysis**

<b>a)</b>	<b>Reversed phase liquid chromatography (RPLC)</b>	
	<i>instrument</i>	Ultimate 3000 RSLC (Thermo Fisher Scientific)
	<i>trap column</i>	75 µm inner diameter, packed with 3 µm C18 particles (Acclaim PepMap100, Thermo Fisher Scientific)
	<i>analytical column</i>	Accucore 150-C18, (Thermo Fisher Scientific) 25 cm x 75 µm, 2,6 µm C18 particles, 150 Å pore size
	<i>buffer system</i>	binary buffer system consisting of 0.1% acetic acid, (buffer A) and 100% ACN in 0.1% acetic acid (buffer B)
	<i>flow rate</i>	300 nl/min
	<i>gradient</i>	linear gradient of buffer B from 2% up to 25%
	<i>gradient duration</i>	120 min
	<i>column oven temperature</i>	40°C
<b>b)</b>	<b>Mass spectrometry (MS/MS)</b>	
	<i>instrument</i>	Q Exactive plus mass spectrometer (Thermo Fisher Scientific)
	<i>electrospray</i>	Nanospray Flex Ion Source
	<i>operation mode</i>	data-independent
	<b>Full MS</b>	
	<i>MS scan resolution</i>	70,000
	<i>AGC target</i>	5e6
	<i>maximum ion injection time for the MS scan</i>	120 ms
	<i>Scan range</i>	300 to 1650 m/z
	<i>Spectra data type</i>	profile
	<b>dd-MS2</b>	
	<i>Resolution</i>	35,000
	<i>MS/MS AGC target</i>	3e6
	<i>maximum ion injection time for the MS/MS scans</i>	auto
	<i>Spectra data type</i>	profile
	<i>selection for MS/MS</i>	1
	<i>isolation window</i>	see below
	<i>Fixed first mass</i>	-
	<i>dissociation mode</i>	higher energy collisional dissociation (HCD)
	<i>normalized collision energy</i>	27.5

**Table S1**

c)

window	m/z	window size [m/z]
1	400-430	30
2	428-459	31
3	457-483	26
4	481-506	25
5	504-531	27
6	529-554	25
7	552-576	24
8	574-600	26
9	598-624	26
10	622-650	28
11	648-676	28
12	674-704	30
13	702-735	33
14	733-771	38
15	769-810	41
16	808-856	48
17	854-914	60
18	912-1000	88
19	998-1220	222

**Table S1**

**Details on parameters of proteomic analysis** for a) reversed phase liquid chromatography, b) mass spectrometry and c) data independent acquisition (DIA) with 19 different windows.

**Table S2**

miR	Assay ID	Mature miRNA sequence
hsa-miR-103a-3p	478253_mir	5'-AGCAGCAUUGUACAGGGCUAUGA-3'
hsa-miR-484	478308_mir	5'-UCAGGCUCAGUCCCCUCCCGAU-3'
hsa-miR-128-3p	477892_mir	5'-UCACAGUGAACCGGUCUCUUU-3'
hsa-miR-23a-3p	478532_mir	5'-AUCACAUUGCCAGGGAUUUCC-3'
hsa-miR-92b-3p	477823_mir	5'-UAUUGCACUCGUCCCGGCCUCC-3'
hsa-miR-106a-5p	478225_mir	5'-AAAAGUGCUUACAGUGCAGGUAG-3'
hsa-miR-199a-3p	477961_mir	5'-ACAGUAGUCUGCACAUUGGUUA-3'
hsa-let-7a-5p	478575_mir	5'-UGAGGUAGUAGGUUGUAUAGUU-3'

**Table S2**  
miRNA assays and sequences used for qRT-PCR (Thermo Fisher Scientific; Waltham, MA, USA).