

Supplementary Material 3 - Teixeira-Marques *et al.* Improved recovery of urinary small extracellular vesicles by differential ultracentrifugation

Supplementary Table S1 – Calculated optical densitometry values of Western-blot corresponding to UC25min, UC48min and UC60min protocols.

Supplementary Table S2 – Calculated optical densitometry values of Western-blot corresponding to UC48min and UCLEVs protocols.

Supplementary Table S3 – Calculated optical densitometry values of Western-blot corresponding to UC48min and UCwash protocols.

Supplementary Table S4 – Calculated optical densitometry values of Western-blot corresponding to UC48min and dUC and EXO protocols.

Supplementary Table S5 – Calculated optical densitometry values of Western-blot corresponding to UC48min and dUC methods.

Supplementary Table S6 – Calculated optical densitometry values of Western-blot corresponding to UC48min protocol.

Supplementary Table S1. Calculated optical densitometry values of Western-blots correspondent to UC25min, UC48min and UC60min protocols.

Figure 2		CD9					CD81					CD63				
		CD9	inverted	background	inverted	OD	CD81	inverted	background	inverted	OD	CD63	inverted	background	inverted	OD
UC25min	1	119,804	135,20	180,833	74,17	61,03	117,608	137,392	177,216	77,784	59,608	159,973	95,027	179,536	75,464	19,563
	2	129,23	125,77	185,257	69,74	56,03	111,965	143,035	180,068	74,932	68,103	181,112	73,888	181,112	73,888	0
	3	109,412	145,59	185,257	69,74	75,85	121,358	133,642	178,163	76,837	56,805	151,19	103,81	181,112	73,888	29,922
	Mean (n=3)					64,30					61,50533333					16,495
UC48min	1	112,615	142,39	180,833	74,17	68,22	109,668	145,332	177,216	77,784	67,548	89,02	165,98	179,536	75,464	90,516
	2	112,795	142,21	185,257	69,74	72,46	106,252	148,748	180,068	74,932	73,816	181,112	73,888	181,112	73,888	0
	3	106,077	148,92	185,257	69,74	79,18	101,457	153,543	178,163	76,837	76,706	118,611	136,389	181,112	73,888	62,501
	Mean (n=3)					73,29					72,69					51,00566667
UC60min	1	110,431	144,57	180,833	74,17	70,40	97,722	157,278	177,216	77,784	79,494	82,826	172,174	179,536	75,464	96,71
	2	123,09	131,91	185,257	69,74	62,17	115,322	139,678	180,068	74,932	64,746	181,112	73,888	181,112	73,888	0
	3	114,172	140,83	185,257	69,74	71,09	106,23	148,77	178,163	76,837	71,933	129,707	125,293	181,112	73,888	51,405
	Mean (n=3)					67,88					72,05766667					49,37166667

Figure 2		Alix					Flotillin-1					THP				
		Alix	inverted	background	inverted	OD	Flotillin-1	inverted	background	inverted	OD	THP	inverted	background	inverted	OD
UC25min	1	174,536	80,464	174,536	80,464	0	176,574	78,426	178,674	76,326	2,1	99,799	155,201	176,295	78,705	76,496
	2	178,818	76,182	178,818	76,182	0	179,49	75,51	179,49	75,51	0	167,223	87,777	177,814	77,186	10,591
	3	119,419	135,581	178,6	76,4	59,181	159,733	95,267	179,49	75,51	19,757	115,654	139,346	177,814	77,186	62,16
	Mean (n=3)					19,727					7,285666667					49,749
UC48min	1	104,035	150,965	174,536	80,464	70,501	155,498	99,502	178,674	76,326	23,176	90,227	164,773	176,295	78,705	86,068
	2	149,26	105,74	178,818	76,182	29,558	179,49	75,51	179,49	75,51	0	172,038	82,962	177,814	77,186	5,776
	3	102,917	152,083	178,6	76,4	75,683	131,081	123,919	179,49	75,51	48,409	108,565	146,435	177,814	77,186	69,249
	Mean (n=3)					58,58066667					23,86166667					53,69766667
UC60min	1	89,932	165,068	174,536	80,464	84,604	153,593	101,407	178,674	76,326	25,081	87,179	167,821	176,295	78,705	89,116
	2	163,022	91,978	178,818	76,182	15,796	179,49	75,51	179,49	75,51	0	177,814	77,186	177,814	77,186	0
	3	113,602	141,398	178,6	76,4	64,998	138,924	116,076	179,49	75,51	40,566	101,471	153,529	177,814	77,186	76,343
	Mean (n=3)					55,13266667					21,88233333					55,153

Abbreviation: OD – Optical Densitometry value

Supplementary Table S2. Calculated optical densitometry values of Western-blot correspond to UC48min and UCLEVs protocols.

Figure 3; Figure S2 in Supplementary Material 1		CD9					CD81					CD63				
		CD9	inverted	background	inverted	OD	CD81	inverted	background	inverted	OD	CD63	inverted	background	inverted	OD
UC48min	1	113,328	141,67	179,998	75,00	66,67	122,493	132,507	177,459	77,541	54,966	98,054	156,946	176,691	78,309	78,637
	2	83,864	171,14	152,51	102,49	68,65	104,56	150,44	140,728	114,272	36,168	130,786	124,214	149,711	105,289	18,925
	3	129,063	125,94	180,261	74,74	51,20	117,657	137,343	177,205	77,795	59,548	102,83	152,17	175,089	79,911	72,259
	Mean (n=3)					62,17					50,22733333					56,607
UCLEVs (SEVs)	1	116,886	138,11	179,998	75,00	63,11	133,728	121,272	177,459	77,541	43,731	111,487	143,513	176,691	78,309	65,204
	2	152,51	102,49	152,51	102,49	0,00	140,728	114,272	140,728	114,272	0	149,711	105,289	149,711	105,289	0
	7	180,261	74,74	180,261	74,74	0,00	177,205	77,795	177,205	77,795	0	175,089	79,911	175,089	79,911	0
	Mean (n=3)					21,04					14,577					21,73466667
UCLEVs (LEVs)	1	168,647	86,35	179,998	75,00	11,35	177,459	77,541	177,459	77,541	0	155,402	99,598	176,691	78,309	21,289
	2	152,51	102,49	152,51	102,49	0,00	140,728	114,272	140,728	114,272	0	149,711	105,289	149,711	105,289	0
	3	180,261	74,74	180,261	74,74	0,00	177,205	77,795	177,205	77,795	0	175,089	79,911	175,089	79,911	0
	Mean (n=3)					3,78					0					7,096333333

Figure 3; Supplementary Material 1, Figure S2		Alix					Flotillin-1					THP				
		Alix	inverted	background	inverted	OD	Flotillin-1	inverted	background	inverted	OD	THP	inverted	background	inverted	OD
UC48min	1	111,664	143,336	179,998	75,002	218,338	133,636	121,364	178,674	76,326	45,038	145,833	109,167	182,069	72,931	36,236
	2	89,684	165,316	135,417	119,583	284,899	126,715	128,285	161,378	93,622	34,663	90,195	164,805	183,594	71,406	93,399
	3	120,708	134,292	120,708	134,292	268,584	107,303	147,697	159,66	95,34	52,357	92,047	162,953	142,405	112,595	50,358
	Mean (n=3)					257,2736667					44,01933333					59,99766667
UCLEVs (SEVs)	1	117,133	137,867	179,998	75,002	62,865	152,237	102,763	178,674	76,326	26,437	136	119	182,069	72,931	46,069
	2	135,417	119,583	135,417	119,583	0	161,378	93,622	161,378	93,622	0	104,63	150,37	183,594	71,406	78,964
	7	120,708	134,292	120,708	134,292	0	159,66	95,34	159,66	95,34	0	98,268	156,732	142,405	112,595	44,137
	Mean (n=3)					20,955					8,812333333					56,39
UCLEVs (LEVs)	1	134,655	120,345	179,998	75,002	45,343	177,175	77,825	178,674	76,326	1,499	138,994	116,006	182,069	72,931	43,075
	2	135,417	119,583	135,417	119,583	0	161,378	93,622	161,378	93,622	0	117,684	137,316	183,594	71,406	65,91
	3	120,708	134,292	120,708	134,292	0	159,66	95,34	159,66	95,34	0	122,14	132,86	142,405	112,595	20,265
	Mean (n=3)					15,11433333					0,499666667					43,08333333

Abbreviation: OD – Optical Densitometry value

Supplementary Table S3. Calculated optical densitometry values of Western-blot correspondent to UC48min and UCwash protocols.

Figure 4		CD9					CD81					CD63				
		CD9	inverted	background	inverted	OD	CD81	inverted	background	inverted	OD	CD63	inverted	background	inverted	OD
UC48min	1	112,615	142,39	180,833	74,17	68,22	109,668	145,332	177,216	77,784	67,548	89,02	165,98	179,536	75,464	90,516
	2	112,795	142,21	185,257	69,74	72,46	106,252	148,748	180,068	74,932	73,816	181,112	73,888	181,112	73,888	0
	3	106,077	148,92	185,257	69,74	79,18	101,457	153,543	178,163	76,837	76,706	118,611	136,389	181,112	73,888	62,501
	Mean (n=3)					73,29					72,69					51,00567
UCwash	1	153,233	101,767	180,833	74,17	27,60	177,216	77,784	177,216	77,784	0	179,536	75,464	179,536	75,464	0
	2	137,172	117,828	185,257	69,74	48,09	171,155	83,845	181,146	73,854	9,991	181,112	73,888	181,112	73,888	0
	3	155,106	99,894	185,257	69,74	30,15	138,301	116,699	178,163	76,837	39,862	181,112	73,888	181,112	73,888	0
	Mean (n=3)					35,27866667					16,61766667					0

Figure 4		Alix					Flotillin-1					THP				
		Alix	inverted	background	inverted	OD	Flotillin-1	inverted	background	inverted	OD	THP	inverted	background	inverted	OD
UC48min	1	104,035	150,965	174,536	80,464	70,501	155,498	99,502	178,674	76,326	23,176	90,227	164,773	176,295	78,705	86,068
	2	149,26	105,74	178,818	76,182	29,558	179,49	75,51	179,49	75,51	0	172,038	82,962	177,814	77,186	5,776
	3	102,917	152,083	178,818	76,182	75,901	131,081	123,919	179,49	75,51	48,409	108,565	146,435	177,814	77,186	69,249
	Mean (n=3)					58,65333333					23,86166667					53,69767
UCwash	1	174,536	80,464	174,536	80,464	0	178,674	76,326	178,674	76,326	0	164,475	90,525	176,295	78,705	11,82
	2	178,818	76,182	178,818	76,182	0	179,49	75,51	179,49	75,51	0	177,814	77,186	177,814	77,186	0
	3	153,216	101,784	178,818	76,182	25,602	170,845	84,155	179,49	75,51	8,645	130,517	124,483	177,814	77,186	47,297
	Mean (n=3)					8,534					2,881666667					19,70567

Abbreviation: OD – Optical Densitometry value

Supplementary Table S4. Calculated optical densitometry values of Western-blot correspondent to UC48min and dUC and EXO protocols.

Figure 5		CD9					CD81					CD63				
		CD9	inverted	background	inverted	OD	CD81	inverted	background	inverted	OD	CD63	inverted	background	inverted	OD
UC48min	1	132,087	122,91	181,737	73,26	49,65	137,598	117,402	180,845	74,155	43,247	134,058	120,942	177,376	77,624	43,318
	2	153,056	101,94	181,737	73,26	28,68	131,291	123,709	180,845	74,155	49,554	108,933	146,067	177,376	77,624	68,443
	3	144,908	110,09	175,464	79,54	30,56	126,863	128,137	180,845	74,155	53,982	165,027	89,973	177,395	77,605	12,368
	Mean (n=3)					36,30					48,92766667					41,37633333
dUC	1	159,257	95,74	181,737	73,26	22,48	180,845	74,155	180,845	74,155	0	177,376	77,624	177,376	77,624	0
	2	163,48	91,52	181,737	73,26	18,26	178,944	76,056	180,845	74,155	1,901	177,376	77,624	177,376	77,624	0
	3	175,464	79,54	175,464	79,54	0,00	180,845	74,155	180,845	74,155	0	177,395	77,605	177,395	77,605	0
	Mean (n=3)					13,58					0,633666667					0
EXO	1	181,737	73,26	181,737	73,26	0,00	180,845	74,155	180,845	74,155	0	177,376	77,624	177,376	77,624	0
	2	181,737	73,26	181,737	73,26	0,00	180,845	74,155	180,845	74,155	0	177,376	77,624	177,376	77,624	0
	3	175,464	79,54	175,464	79,54	0,00	180,845	74,155	180,845	74,155	0	177,395	77,605	177,395	77,605	0
	Mean (n=3)					0,00					0					0

Figure 5		Alix					Flotillin-1					THP				
		Alix	inverted	background	inverted	OD	Flotillin-1	inverted	background	inverted	Densitometry	THP	inverted	background	inverted	OD
UC48min	1	127,166	127,834	178,504	76,496	204,33	117,66	137,34	175,855	79,145	58,195	114,62	140,38	182,008	72,992	67,388
	2	133,358	121,642	178,504	76,496	198,138	121,952	133,048	175,855	79,145	53,903	154,653	100,347	182,008	72,992	27,355
	3	157,416	97,584	175,819	79,181	176,765	131,063	123,937	176,817	78,183	45,754	166,349	88,651	182,442	72,558	16,093
	Mean (n=3)					193,0776667					52,61733333					36,94533333
dUC	1	136,433	118,567	178,504	76,496	42,071	139,309	115,691	175,855	79,145	36,546	182,008	72,992	182,008	72,992	0
	2	136,122	118,878	178,504	76,496	42,382	152,235	102,765	175,855	79,145	23,62	182,008	72,992	182,008	72,992	0
	3	175,819	79,181	175,819	79,181	0	176,817	78,183	176,817	78,183	0	176,948	78,052	182,442	72,558	5,494
	Mean (n=3)					28,151					20,05533333					1,831333333
EXO	1	178,504	76,496	178,504	76,496	0	175,855	79,145	175,855	79,145	0	163,155	91,845	182,008	72,992	18,853
	2	142,112	112,888	178,504	76,496	36,392	175,855	79,145	175,855	79,145	0	105,351	149,649	182,008	72,992	76,657
	3	175,819	79,181	175,819	79,181	0	176,817	78,183	176,817	78,183	0	82,346	172,654	182,442	72,558	100,096
	Mean (n=3)					12,13066667					0					65,202

Abbreviation: OD – Optical Densitometry value

Supplementary Table S5. Calculated optical densitometry values of Western-blots correspondent to UC48min and dUC methods.

Figure S3 in		CD9					CD81					CD63					Alix				
Supplementary Material 1		CD9	inverted	background	inverted	OD	CD81	inverted	background	inverted	OD	CD63	inverted	background	inverted	OD	Alix	inverted	background	inverted	Densitometry
UC48min	1	85,675	169,33	236,129	18,87	150,45	80,04	174,957	235,112	19,888	155,069	71,628	183,372	232,27	22,73	160,642	66,966	188,034	236,056	18,944	206,978
	2	56,3	198,70	236,129	18,87	179,83	64,844	190,156	235,112	19,888	170,268	48,952	206,048	232,27	22,73	183,318	61,578	193,422	236,056	18,944	212,366
	3	146,161	108,84	236,129	18,87	89,97	110,069	144,931	235,112	19,888	125,043	67,3	187,7	232,27	22,73	164,97	52,927	202,073	236,056	18,944	221,017
	Mean (n=3)					140,08					150,1266667					169,6433333					213,4536667
dUC	1	231,999	23,00	236,129	18,87	4,13	235,112	19,888	235,112	19,888	0	198,441	56,559	232,27	22,73	33,829	217,094	37,906	236,056	18,944	56,85
	2	92,817	162,18	236,129	18,87	143,31	235,112	19,888	235,112	19,888	0	208,904	46,096	232,27	22,73	23,366	175,178	79,822	236,056	18,944	98,766
	3	236,129	18,87	236,129	18,87	0,00	235,112	19,888	235,112	19,888	0	98,586	156,414	232,27	22,73	133,684	99,395	155,605	236,056	18,944	136,661
	Mean (n=3)					49,15					0					63,62633333					97,42566667

Figure S3 in		Flotillin-1					THP					Cytochrome C				
Supplementary Material 1		Flotillin-1	inverted	background	inverted	OD	THP	inverted	background	inverted	OD	Cytochron	inverted	background	inverted	OD
UC48min	1	232,253	22,747	232,253	22,747	0	74,574	180,426	211,818	43,182	137,244	244,41	10,59	244,41	10,59	0
	2	168,556	86,444	232,253	22,747	63,697	60,033	194,967	211,818	43,182	151,785	244,41	10,59	244,41	10,59	0
	3	202,513	52,487	232,253	22,747	29,74	58,239	196,761	211,818	43,182	153,579	244,41	10,59	244,41	10,59	0
	Mean (n=3)					31,14566667					147,536					0
dUC	1	232,253	22,747	232,253	22,747	0	207,759	47,241	211,818	43,182	4,059	244,41	10,59	244,41	10,59	0
	2	232,253	22,747	232,253	22,747	0	85,221	169,779	211,818	43,182	126,597	244,41	10,59	244,41	10,59	0
	3	232,253	22,747	232,253	22,747	0	150,096	104,904	211,818	43,182	61,722	244,41	10,59	244,41	10,59	0
	Mean (n=3)					0					64,126					0

Abbreviation: OD – Optical Densitometry value

Supplementary Table S6. Calculated optical densitometry values of Western-blots correspondent to UC48min protocol.

Figure S6 in		CD9					CD81					CD63					Alix				
Supplementary Material 1		CD9	inverted	background	inverted	OD	CD81	inverted	background	inverted	OD	CD63	inverted	background	inverted	OD	Alix	inverted	background	inverted	OD
UC48min	1	97,206	157,79	178,981	76,02	81,78	97,64	157,36	156,832	98,168	59,192	86,148	168,852	159,423	95,577	73,275	114,808	140,192	175,821	79,179	219,371
	2	96,212	158,79	178,982	76,02	82,77	117,446	137,554	156,832	98,168	39,386	141,654	113,346	159,423	95,577	17,769	118,221	136,779	175,821	79,179	215,958
	3	107,508	147,49	178,983	76,02	71,48	124,478	130,522	156,832	98,168	32,354	108,416	146,584	159,423	95,577	51,007	139,721	115,279	175,821	79,179	194,458
	4	144,7	110,30	178,984	76,02	34,28	156,832	98,168	156,832	98,168	0	159,423	95,577	159,423	95,577	47,350333	169,922	85,078	175,821	79,179	164,257
	5	112,075	142,93	178,985	76,02	66,91	103,549	151,451	156,832	98,168	53,283	87,593	167,407	159,423	95,577	71,83	111,488	143,512	175,821	79,179	222,691
	6	88,331	166,67	178,986	76,01	90,66	82,484	172,516	156,832	98,168	74,348	72,779	182,221	159,423	95,577	86,644	104,409	150,591	175,821	79,179	71,412
	7	97,846	157,15	178,987	76,01	81,14	87,638	167,362	156,832	98,168	69,194	72,63	182,37	159,423	95,577	86,793	99,15	155,85	175,821	79,179	76,671
	Mean (n=7)						72,72					46,82243					62,095476				

Figure S6 in		Flotillin-1					THP					Lamin A/C					Cytochrome C				
Supplementary Material 1		Flotillin-1	inverted	background	inverted	OD	THP	inverted	background	inverted	OD	Lamin A/C	inverted	background	inverted	OD	Cytochron	inverted	background	inverted	OD
UC48min	1	108,245	146,755	145,006	109,994	36,761	113,755	141,245	168,15	86,85	54,395	161,585	93,415	161,585	93,415	0	169,463	85,537	169,463	85,537	0
	2	145,006	109,994	145,006	109,994	0	150,264	104,736	168,15	86,85	17,886	161,585	93,415	161,585	93,415	0	169,463	85,537	169,463	85,537	0
	3	145,006	109,994	145,006	109,994	0	100,696	154,304	168,15	86,85	67,454	161,585	93,415	161,585	93,415	0	169,463	85,537	169,463	85,537	0
	4	145,006	109,994	145,006	109,994	0	168,15	86,85	168,15	86,85	0	161,585	93,415	161,585	93,415	0	169,463	85,537	169,463	85,537	0
	5	139,993	115,007	145,006	109,994	5,013	127,731	127,269	168,15	86,85	40,419	161,585	93,415	161,585	93,415	0	169,463	85,537	169,463	85,537	0
	6	102,413	152,587	145,006	109,994	42,593	133,712	121,288	168,15	86,85	34,438	161,585	93,415	161,585	93,415	0	169,463	85,537	169,463	85,537	0
	7	122,459	132,541	145,006	109,994	22,547	108,262	146,738	168,15	86,85	59,888	161,585	93,415	161,585	93,415	0	169,463	85,537	169,463	85,537	0
	Mean (n=7)						15,27343					39,21143				0					

Abbreviation: OD – Optical Densitometry value