SUPPLEMENTARY MATERIAL

Optimization of human dendritic cell sample preparation for mass spectrometry-based proteomics studies

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Supplementary Figures

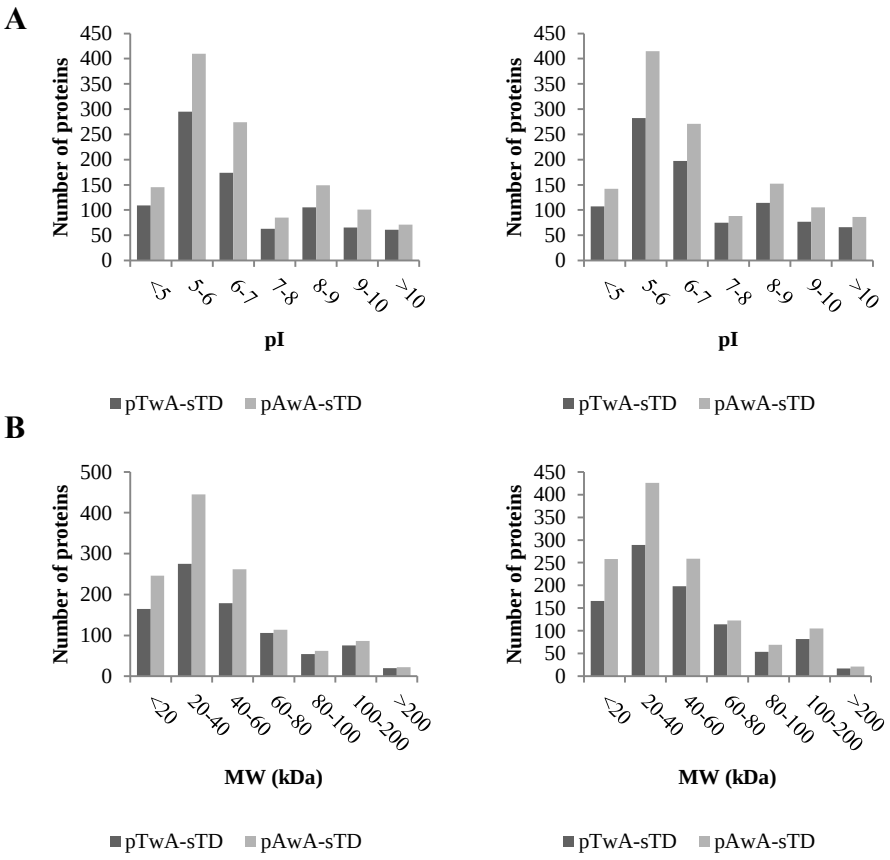

Figure S1. Comparative study of the proteins identified by the workflows using different protein precipitation methods for Triton X-100 cell lysate (Left) and RIPA cell lysate (Right). Data were obtained by merging two experimental replicates for each workflow. pAwA: Acetone precipitation plus acetone-wash step; pTwA: 10% TCA/acetone precipitation method. *The precipitated proteins were then re-solubilised by 1% Na-DOC in 0.5 M TEAB dissolution buffer (sTD)*. (A) Calculated isoelectric point (pI); (B) Average molecular weight (MW).

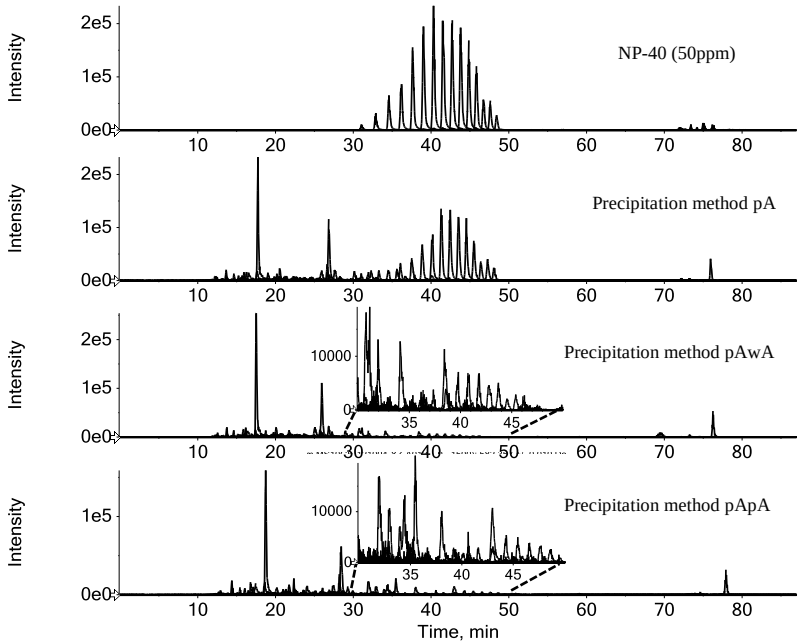

Figure S2. Extracted ion chromatograms (XICs) of the representative ions derived from the detergent residue in the protein extract by RIPA buffer after different acetone precipitation treatments (*the precipitated protein pellets were then re-solubilized by 1% Na-DOC in 0.5M TEAB dissolution buffer*). From top to bottom: NP-40 at 50 ppm; single precipitation by acetone (pA); acetone precipitation plus acetone-wash step (pAwA); double precipitation by acetone (pApA).

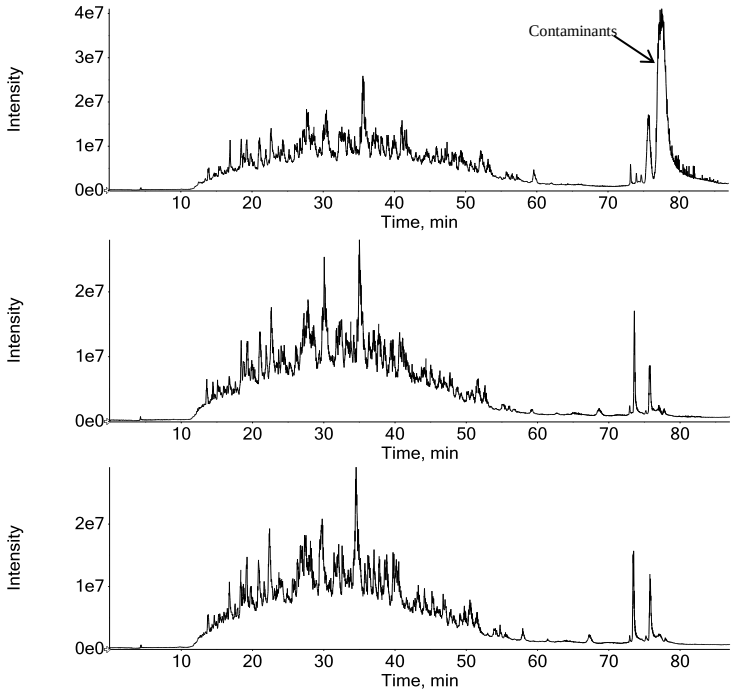

Figure S3. Typical TIC traces of tryptic digest of MDDC protein extract prepared by the workflows with three protein precipitation methods: (top) single precipitation by acetone (pA); (middle) acetone precipitation plus acetone-wash step (pAwA); (bottom) double precipitation by acetone (pApA).

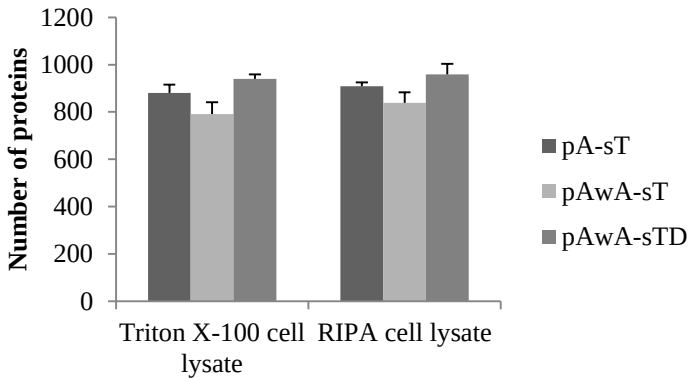

Figure S4. Number of MDDC proteins identified by different sample preparation workflows from Triton X-100 cell lysate and RIPA cell lysate. Data were derived from four experimental replicates and are presented as the mean ± SD. pA: MDDC proteins were precipitated by acetone; pAwA: MDDC proteins were precipitated by acetone, followed by the acetone-wash step; sT: The precipitated proteins were re-solubilised in 0.5 M TEAB; sTD: The precipitated proteins were re-solubilised in 0.5 M TEAB containing 1% Na-DOC.

Supplementary Tables

Table S1. Summary of the LC-MS/MS analysis results of the tryptic digests prepared by different sample preparation workflows for MDDC Triton X-100 cell lysate and RIPA cell lysate. Data reported here are the average results of two experimental replicates for each of the ten different workflows (two technical replicates of each experimental replicate were merged). Identification FDR threshold for MS/MS spectra is 5% (local FDR), for distinct peptides is 5% (local FDR) and for proteins is 1% (global FDR). Only those distinct peptides which are associated with the confidently identified proteins are considered.

Cell lysate	**Sample preparation workflow** (n=2)	**MS/MS spectra**			**Distinct peptides**					**Protein**
		Total	Identified	% of identified	Total	Fully cleaved	% of fully cleaved	Missed cleavage		
								1	≥2	
Triton X-100 (TCL)	pA-sT	54483	26867	49.3	5380	5138	95.5	234	9	856
	pA-sTS	58535	23129	39.5	4302	4142	96.3	155	5	729
	pA-sTD	50172	24553	48.9	5830	5570	95.5	252	8	946
	pAwA-sT	43038	21079	49.0	4995	4886	97.8	107	3	787
	pAwA-sTS	52748	17123	32.5	3583	3494	97.5	88	2	646
	pAwA-sTD	42775	23275	54.4	5706	5479	96.0	222	6	948
	pTwA-sT	38787	15231	39.3	3726	3637	97.6	87	3	597
	pTwA-sTS	56482	16761	29.7	3005	2935	97.7	70	1	506
	pTwA-sTD	37345	17022	45.6	4085	3971	97.2	111	3	666
	pApA-sTD	45694	26053	57.0	5643	5359	95.0	278	6	929
RIPA (RCL)	pA-sT	52845	28093	53.2	5521	5273	95.5	238	11	893
	pA-sTS	55672	24867	44.7	4239	4105	96.8	132	2	759
	pA-sTD	45380	24702	54.4	5721	5479	95.8	233	9	925
	pAwA-sT	42999	22521	52.4	5537	5395	97.4	138	5	865
	pAwA-sTS	46476	19697	42.4	4006	3888	97.1	117	1	757
	pAwA-sTD	43788	25677	58.6	5837	5606	96.0	223	8	1000
	pTwA-sT	39285	16384	41.7	3674	3585	97.6	87	3	618
	pTwA-sTS	54335	17533	32.3	3078	2992	97.2	86	1	542
	pTwA-sTD	36514	17515	48.0	4227	4096	96.9	125	7	711
	pApA-sTD	46244	26521	57.4	5645	5330	94.4	304	11	897

Table S2. Numbers of proteins grouped by different GRAVY index values which were identified by two different protein precipitation methods. Data were obtained by merging two experimental replicates for each workflow. pAwA: acetone precipitation plus the acetone-wash step; pTwA: 10% TCA/acetone precipitation method. *The precipitated proteins were then re-solubilised by 1% Na-DOC in 0.5 M TEAB dissolution buffer (sTD).*

GRAVY index	Triton X-100 cell lysate (TCL)			RIPA cell lysate (RCL)		
	pTwA-sTD	pAwA-sTD	Increase (%)	pTwA-sTD	pAwA-sTD	Increase (%)
-2 - -1.5	9	9	0	11	12	9
-1.5 - -1	37	48	30	47	52	11
-1 - -0.5	215	300	40	235	321	37
-0.5 - 0	446	651	46	458	654	43
0 - 0.5	61	108	77	61	101	66
0.5 - 1	7	13	86	6	10	67
1 - 1.5	0	0	-	0	1	-

Table S3. Peak areas of the XIC traces of the representative ions observed from Triton X-100 buffer, RIPA buffer and their residues remaining in the MDDC protein extract after being treated by three different acetone precipitation methods. Data were obtained by averaging two experimental replicates for each workflow with two technical replicates per experimental replicate. pA: acetone precipitation; pAwA: acetone precipitation plus the acetone-wash step; pApA: double-precipitation by acetone. *The precipitated proteins were then re-solubilised by 1% Na-DOC in 0.5 M TEAB dissolution buffer (sTD).*

Triton X-100					RIPA (NP-40)				
m/z (++)	50ppm	pA-sTD	pAwA-sTD	pApA-sTD	*m/z* (++)	50ppm	pA-sTD	pAwA-sTD	pApA-sTD
296.19	732560	100057	-	-	274.17	165167	-	-	-
318.20	1503534	320703	14689	17554	296.19	555884	46772	-	4854
340.21	2111351	754330	25333	24582	318.20	1152534	161482	9967	14021
362.23	3674198	956854	27210	27775	340.21	1635755	308101	15841	26489
384.24	5045737	1737717	51972	54704	362.23	2739321	554260	24743	32970
406.25	5424502	2384762	64266	70186	384.24	3666326	945256	47953	52585
428.26	5681518	2932911	83963	82910	406.25	4333855	1294866	59851	75319
450.27	5284732	2886622	83167	85789	428.27	3536062	948830	53310	71231
472.29	5162440	2771621	85663	85758	450.28	3947707	1178320	64596	81251
494.30	4725412	2628696	117922	84982	472.29	4032656	1300557	91270	92758
516.31	3533110	2023048	77083	67290	494.31	2414603	916177	66624	54903
538.32	2350792	1376533	62759	45346	516.32	2252307	836132	69585	63326
560.34	1504752	964650	50406	36909	546.85	879493	313827	25534	20645
582.35	856813	608179	42485	21037	560.34	1071945	449136	45437	36975
604.36	418828	348046	22872	12293	582.36	450327	251652	24263	21183
626.37	181229	171773	12176	8353	-	-	-	-	-
Sum	48191508	22966502	821967	725469	**Sum**	32833941	9505365	598972	648512

Table S4. Numbers of hydrophilic proteins (GRAVY negative index) and hydrophobic proteins (GRAVY positive index) detected by the sample preparation workflows using the acetone precipitation method without (pA) or with (pAwA) the acetone-wash step. Data were obtained by averaging four experimental replicates for each workflow. *The precipitated proteins were then re-solubilised by 0.5 M TEAB dissolution buffer (sT).*

Sample	Workflow	GRAVY Negative	Decrease (%)	GRAVY Positive	Decrease (%)
Triton X-100 cell lysate (n=4)	pA-sT	798	9.4	82	16.8
	pAwA-sT	723		68	
RIPA cell lysate (n=4)	pA-sT	823	6.8	85	17.1
	pAwA-sT	767		71	
Average		-	**8**	-	**17**

Table S5. Numbers of hydrophilic proteins (GRAVY negative index) and hydrophobic proteins (GRAVY positive index) detected by the sample preparation workflows using the acetone with wash precipitation (pAwA) method and the precipitated protein pellet was then re-solubilised by the 0.5M TEAB dissolution buffer in the absence (sT) or presence (sTD) of 1% Na-DOC. Data were obtained by averaging four experimental replicates for each workflow.

Sample	Workflow	GRAVY Negative	Increase (%)	GRAVY Positive	Increase (%)
Triton X-100 cell lysate (n=4)	pAwA-sT	723	15.8	68	36.3
	pAwA-sTD	838		93	
RIPA cell lysate (n=4)	pAwA-sT	767	12.9	70.5	31.6
	pAwA-sTD	867		93	
Average		-	**14**	-	**34**