Supplementary Information

A concentration-descending washing strategy with methanol for the enhancement of protein imaging in biological tissues by MALDI-MS

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Contents

Supplementary Information—RESULTS AND DISCUSSION

MeOH-CDWS Washing Strategy Enhances Protein *In situ* Detection on Tissues. To explain why MeOH-CDWS leads to the best *in situ* detection effect (both in terms of protein ion intensity and the number of detectable protein ions), we further compared the *in situ* protein detection from the rat liver tissue sections washed with 70% organic solution (MeOH or iPrOH), 100% organic solution (MeOH or iPrOH), and CDWS (MeOH or iPrOH) (n=3*3). **Fig. S2** and **Table S4** showed that the average ion intensities of detectable proteins through MeOH-CDWS significantly increased by a factor of 4.35, and/or 5.12, respectively, versus 70% MeOH solution and 100% MeOH solution. In addition, the average ion intensities of detectable proteins through iPrOH-CDWS significantly increased by a factor of 2.14, and/or 2.36, respectively, versus 70% iPrOH solution and 100% iPrOH solution. **Fig. S2B** showed that the number of protein ion signals detected by the use of CDWS was significantly more than that detected by 70% organic solution, 100% organic solution (t-tests, $p<0.05$). As shown, a total of 233 ± 3 protein ion signals could be detected from liver tissue sections with MeOH-CDWS versus 91 ± 3 and 73 ± 2 proteins detected by the use of 70% MeOH solution and 100% MeOH solution. Meanwhile, 142 ± 3 detectable protein ion signals were found in liver tissue sections washed by iPrOH-CDWS, and only 86 ± 1 and 69 ± 4 protein ion signals were detected in the washing of 70% iPrOH solution and 100% iPrOH solution. In addition, the signal-to-noise ratio (S/N) of CDWS was generally better than 70% and 100% organic solutions (**Table S4**). Our results showed that MeOH-CDWS significantly enhanced the *in-situ* proteins detection in rat liver tissue sections versus 70% MeOH and 100% MeOH. In addition, the result of iPrOH-CDWS in enhancing protein detection was consistent with the result of MeOH-CDWS. Therefore, we believe that the use of CDWS can improve the performance of MALDI-MSI for *in situ* proteins detection, such as the detectable number and ion intensity of proteins. Furthermore, MeOH-CDWS could be a preferred choice for *in situ* proteins detection than iPrOH-CDWS. We also speculate that MeOH-CDWS leads to the best *in situ* detection effect (both in terms of protein ion intensity and the number of detectable protein ions) for the following reasons: (*i*) 100% MeOH is an effective tissue fixator,[1] by combining with multiple functional groups on the amino acid side chains of the polypeptide chain (*e.g.*, amino, imino, amide, hydroxyl, and sulfhydryl groups) to form complex networks inside and outside protein molecules to play the role of protein fixation, so that proteins no longer migrate and can prevent tissue autolysis and maintain tissue antigenicity.[2] (*ii*) High concentration MeOH solutions (*e.g.*, 100% and 95% MeOH used in this study) are also an effective proton donor solution, which provide a large number of O-H protons to compete with water for protein hydrogen bond formation, further avoiding the possibility of protein delocalization.[3] (*iii*) 70% MeOH solution can not only effectively remove the small molecule compounds on tissues, but also fully expose the proteins on the surface of tissue sections, which is conducive to the subsequent matrix and protein co-crystallization and MS laser desorption ionization.[4]

Optimization of Tissue Washing Strategy for Protein Detection on Tissues. To obtain better profiling and imaging data, serial 12-μm thick homogeneous rat liver parallel tissue sections were selected as the targeted tissue samples for protein detection on tissue, and orthogonal-array testing with three variables, *i.e.*, washing organic solvent selection, washing time, and washing solvent concentration sequence, was performed to optimize the tissue washing strategy. Three washing organic solvents (*i.e.*, MeOH, ACN, and iPrOH); washing time compositions of 0.5, 1.0, and 1.5 min; as well as three washing solvent concentration sequences, *i.e.*, CAWS (70%, 95%, and 100% organic solutions), CDWS (100%, 95%, and 70% organic solutions), and the concentration-descending followed by ascending washing

strategy (CDAWS: 95%, 70%, and 100% organic solutions) were chosen for the tissue washing strategy optimization (**Fig. S5A**). Thus, nine experiments were performed to find the optimal combination of the three variables (**Fig. S5B**). As shown in **Fig. S6 and Fig. S7**, a total of 190 ± 2, 161 ± 2, 91 ± 1, 213 ± 3, 182 ± 2, 124 ± 1, 141 ± 1, 149 ± 2, and 80 ± 1 protein ion signals were successfully detected from each of the nine-rat liver parallel tissue sections washed by the previously mentioned nine types of washing methods, respectively (more details of these detected proteins can be found in **Table S5)**. The largest number of protein ion signals could be detected by using the MeOH-CDWS with 1.0-min washing time. According to the principle of orthogonal-array testing, we could know that the washing organic solvent selection was the dominant factor that influenced the number of detected proteins, while the washing time had the weakest effect in our experiment. Meanwhile, based on the results of orthogonal-array testing, the MeOH-CDWS with 0.5-min washing time was found to be the optimal tissue washing strategy. Meanwhile, we also have performed statistical analysis with the number of proteins detected by the optimal MeOH-CDWS protocol and other combinations obtained from orthogonal experimental design.

We have also performed extra experiments to evaluate the effect of three different solvent temperatures (25°C, -20°C, and -80°C) on the detectable number and ion intensity of proteins in rat liver tissue sections washed by optimized MeOH-CDWS (n=3*3). As shown in **Fig. S8**, the use of 25°C MeOH-CDWS with 0.5-min washing time led to significantly improved protein detection on tissue, with 272 ± 2 protein ion signals being successfully detected from rat liver in a single experiment, compared with 124 ± 2 and 99 ± 2 protein ion signals detected by -20°C MeOH-CDWS and -80°C MeOH with 0.5-min washing time (**Table S6**). In addition, the effect of tissue washing rotation frequency on the detectable proteins was also evaluated. Therefore, we selected different washing rotation frequencies (*i.e.*, 1 rpm, 3 rpm, 5 rpm, 7 rpm, 9 rpm, 11 rpm, and 13 rpm) to explore the effect of rotation frequency on protein *in situ* detection in MALDI-MS as well using 25°C MeOH-CDWS with 0.5-min washing time (n=3*3). As shown in **Fig. S9A**, washing rotation frequency (3 rpm, or 5 rpm), especially 5 rpm significantly enhanced the *in-situ* detection of protein from the rat liver tissue sections. As shown in **Fig. S9B**, 120 ± 2, 270 ± 3, 261 ± 5, 230 ± 4, 210 ± 2, and 182 ± 2 molecular signals were detected with 1 rpm, 3 rpm, 7 rpm, 9 rpm, 11 rpm, and 13 rpm respectively, all of which are lower than the 272 ± 3 molecular signals that were detected with 5 rpm (**Table S5**). As shown in **Fig. S10**, a total of 190 ± 2, 161 ± 2, 91 ± 1, 213 ± 3, 182 ± 2, 124 ± 1, 141 ± 1, 149 ± 2, and 80 ± 1 protein ion signals were successfully detected from nine rat liver parallel tissue sections washed by nine different tissue washing methods including $a_1b_1c_1$, $a_1b_2c_2$, $a_1b_3c_3$, $a_2b_1c_2$, $a_2b_2c_3$, $a_2b_3c_1$, $a_3b_1c_3$, $a_3b_2c_1$, and $a_3b_3c_2$, respectively. Obviously, a total of 272 ± 2 protein ion signals could be successfully detected by using optimized MeOH-CDWS, and the number of detectable protein ion signal is significantly more than previously mentioned nine combinations. Therefore, the optimal tissue washing strategy we obtained was 25°C MeOH-CDWS with 0.5-min washing time and 5 rpm washing rotation frequency, and this optimal tissue washing strategy was used for our subsequent experiments.

Supplementary Information--FIGURES

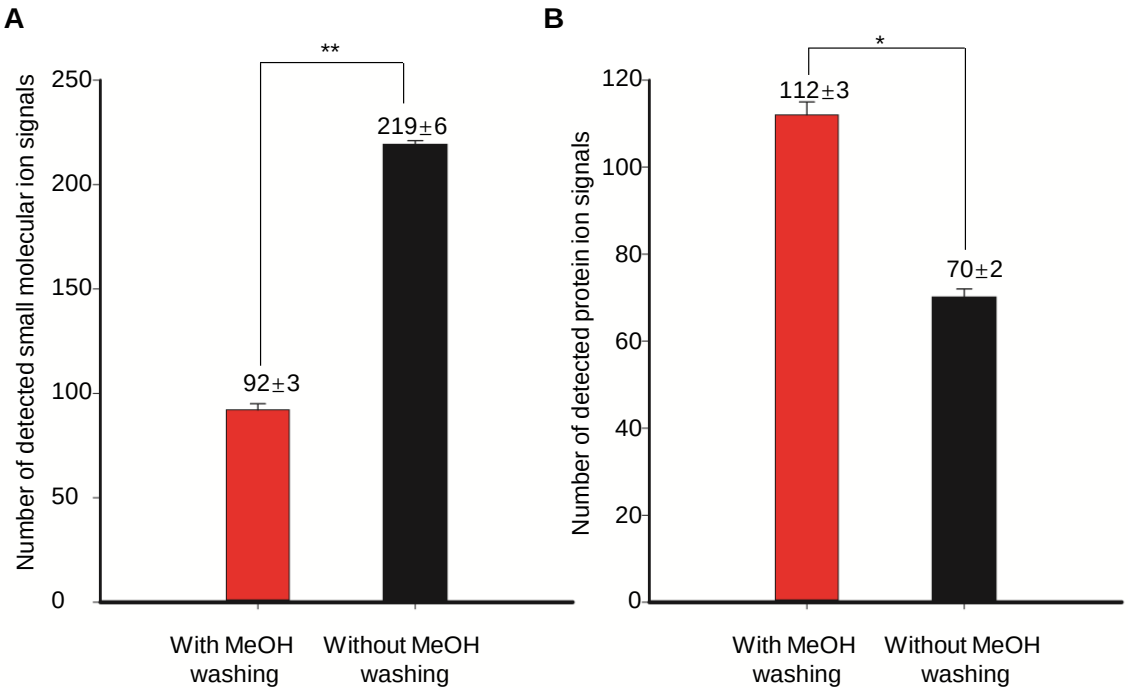

Fig. S1. Evaluation of the influence of MeOH solvent washing on the *in situ* detection of small molecule and protein ion signals from rat liver tissue sections (n=3*3). **(A)** The number of small molecule ion signals (MW, typically below 2,000 Da) detected from the rat liver tissue sections by MALDI-TOF MS using DHB as the matrix, with and without MeOH washing. **(B)** The number of protein ion signals (MW, typically above 2,000 Da) detected from the rat liver tissue sections by MALDI-TOF MS using SA as the matrix, with and without MeOH washing. (*, $0.01<p<0.05$, **, $0.001<p<0.01$).

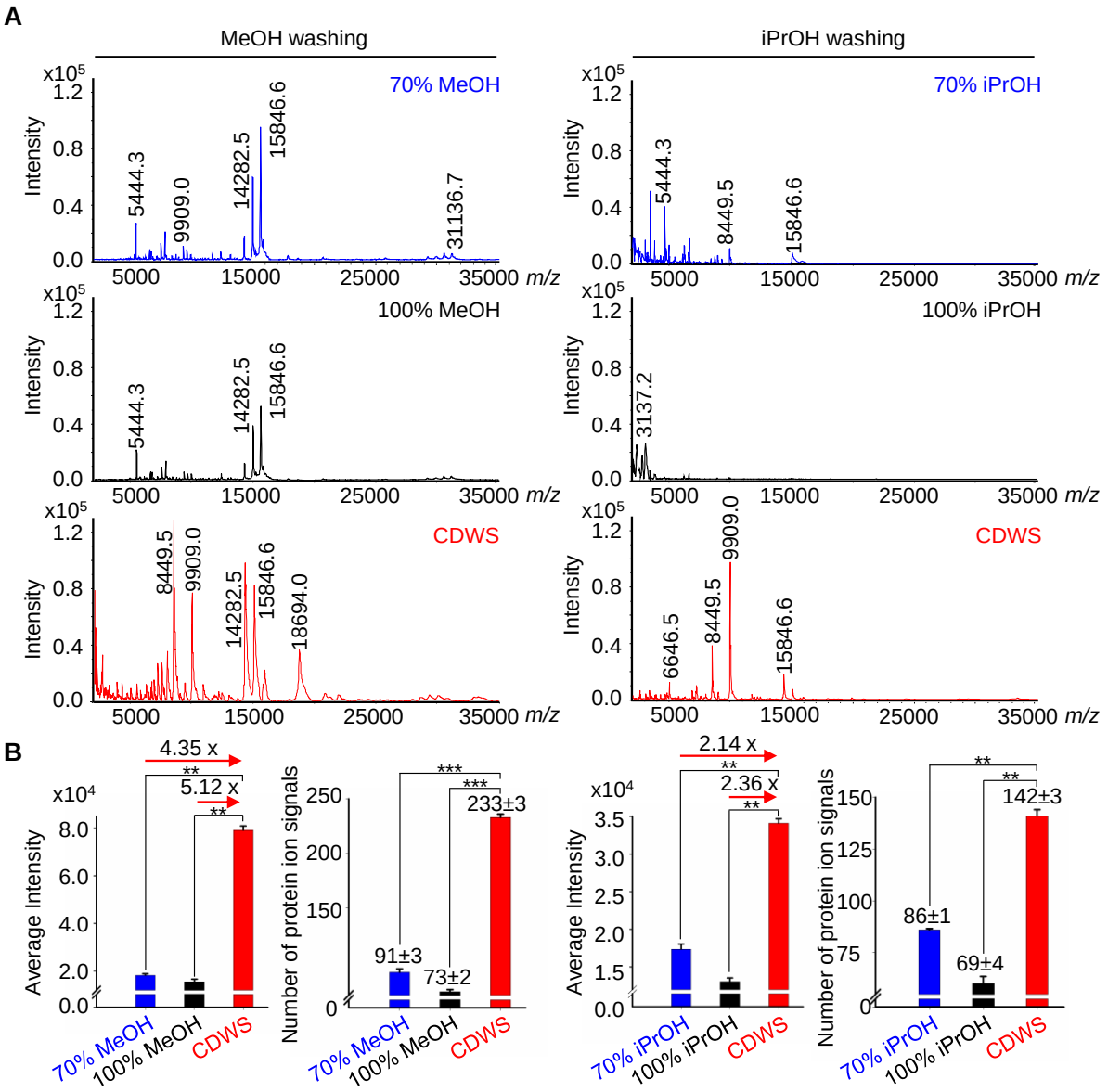

Fig. S2. Evaluation of the *in situ* protein detection from the rat liver tissue sections by MALDI-MS washed with 70% organic solution (MeOH or iPrOH), 100% organic solution (MeOH or iPrOH), and CDWS (MeOH, or iPrOH) (n=3*3). **(A)** MALDI mass spectra acquired from the rat liver tissue sections washed with 70% MeOH or iPrOH (blue), 100% MeOH or iPrOH (black), and MeOH-/iPrOH-CDWS (red), respectively. **(B)** Graphical display of the average intensity and the number of detectable protein ion signals from rat liver tissue sections washed with 70% MeOH or iPrOH (blue), 100% MeOH or iPrOH (black), and MeOH-/iPrOH-CDWS (red), respectively. The used intensities were the average intensities of the protein ion signals detected in three biological replicates. (**, $0.001<p<0.01$, ***, $p<0.001$).

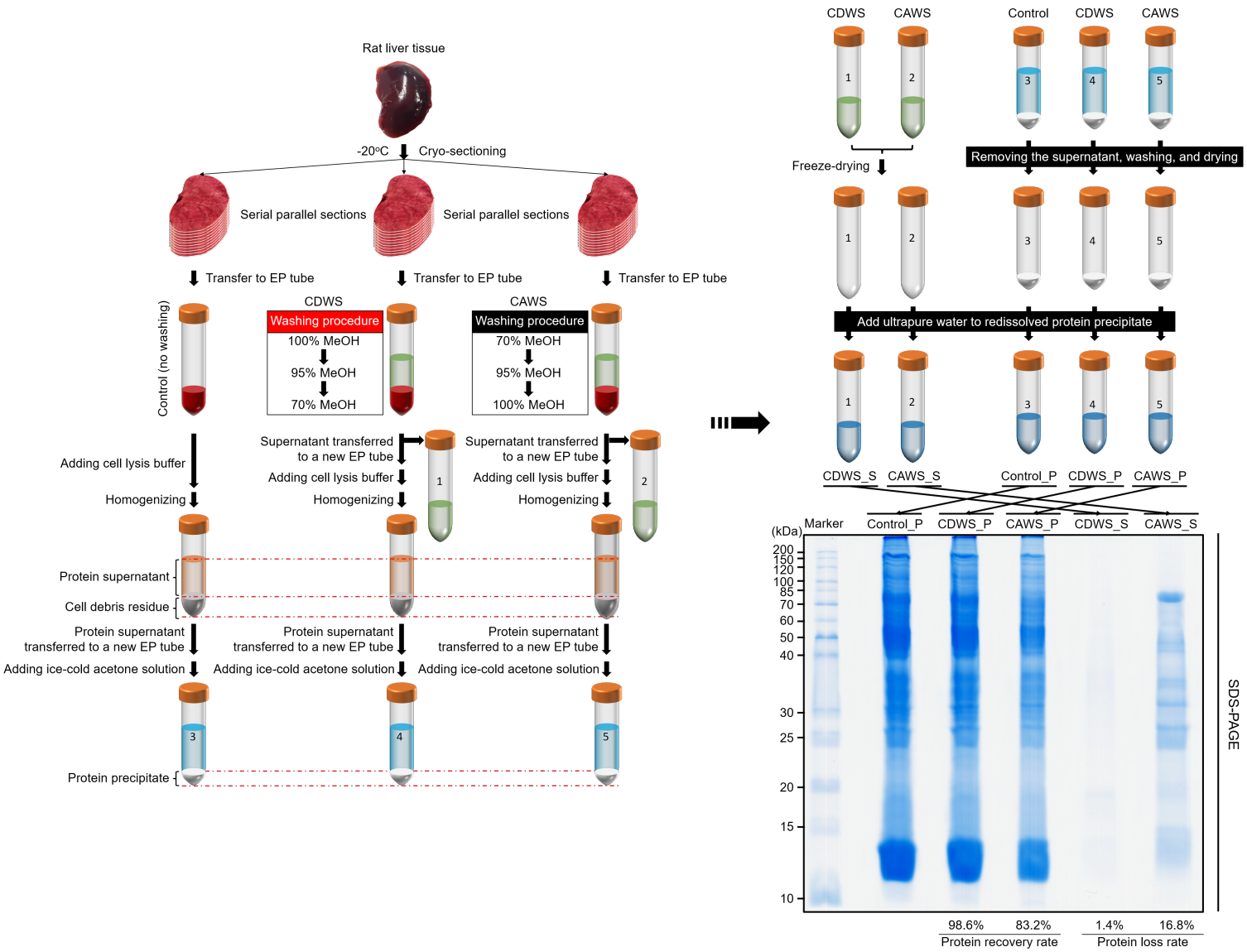

Fig. S3. Evaluation of the protein recovery and loss from rat liver tissue sections washed by MeOH-CDWS and MeOH-CAWS, respectively.

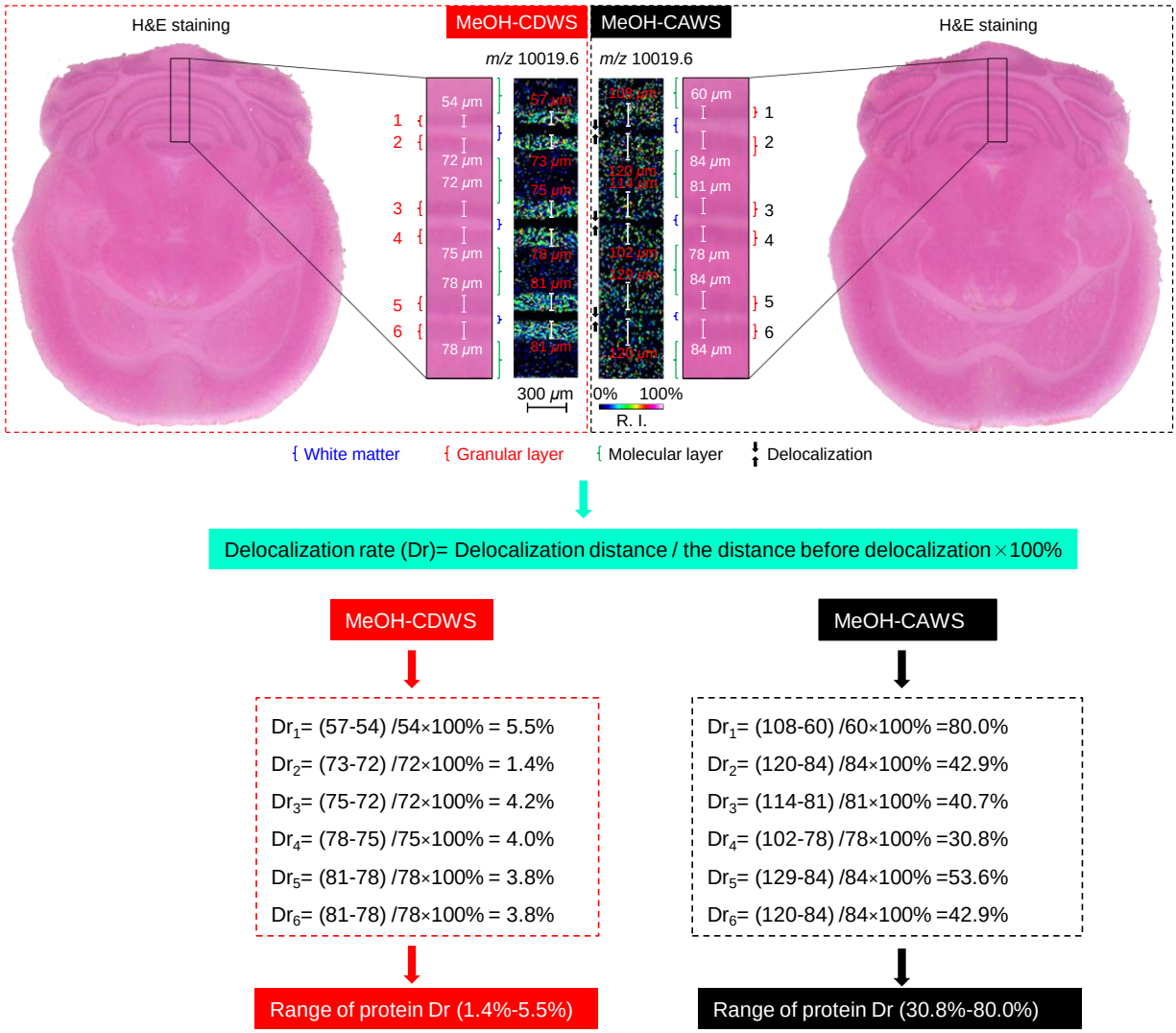

Fig. S4. High-resolution images and pixel broadening evaluation of the protein ion of *m/z* 10,019.6 detected in two parallel rat brain tissue sections by MALDI-MSI with MeOH-CDWS and MeOH-CAWS, respectively. The laser scanning step size is 10 μm.

A

Components	Concentration factors(CFs)		
Washing solvent concentration sequences	a_1	a_2	a_3
Washing organic solvent selection	MeOH (b_1)	ACN (b_2)	iPrOH (b_3)
Washing time	0.5 min (c_1)	1.0 min (c_2)	1.5 min (c_3)

B

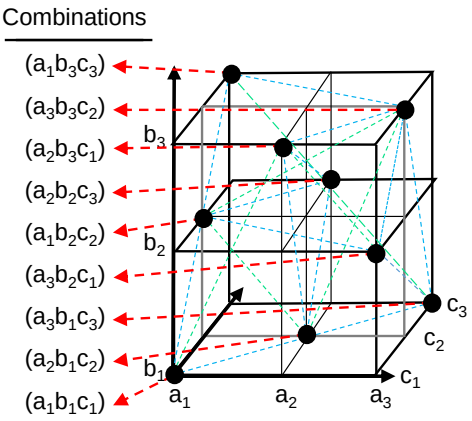

Fig. S5. Design of orthogonal array testing for the optimization of the tissue washing strategy. **(A)** Three variables (*i.e.*, washing organic solvent selection, washing time, and washing solvent concentration sequence) and three levels of each variable were tested. Three washing solvent concentration sequences were composed of a_1, a_2, and a_3. a_1, the concentration ascending washing strategy (CAWS): tissue sections washed by 70%, 95%, and 100% organic solutions; a_2, the concentration descending washing strategy (CDWS): tissue sections washed by 100%, 95%, and 70% organic solutions; a_3, the concentration descending and followed by ascending washing strategy (CDAWS): tissue sections washed by 95%, 70%, and 100% organic solutions. MeOH (b_1), ACN (b_2), and iPrOH (b_3) were selected as the three washing organic solvents. Washing times consist of 0.5 min (c_1), 1.0 min (c_2), and 1.5 min (c_3). **(B)** Nine independent experiment design for orthogonal array testing.

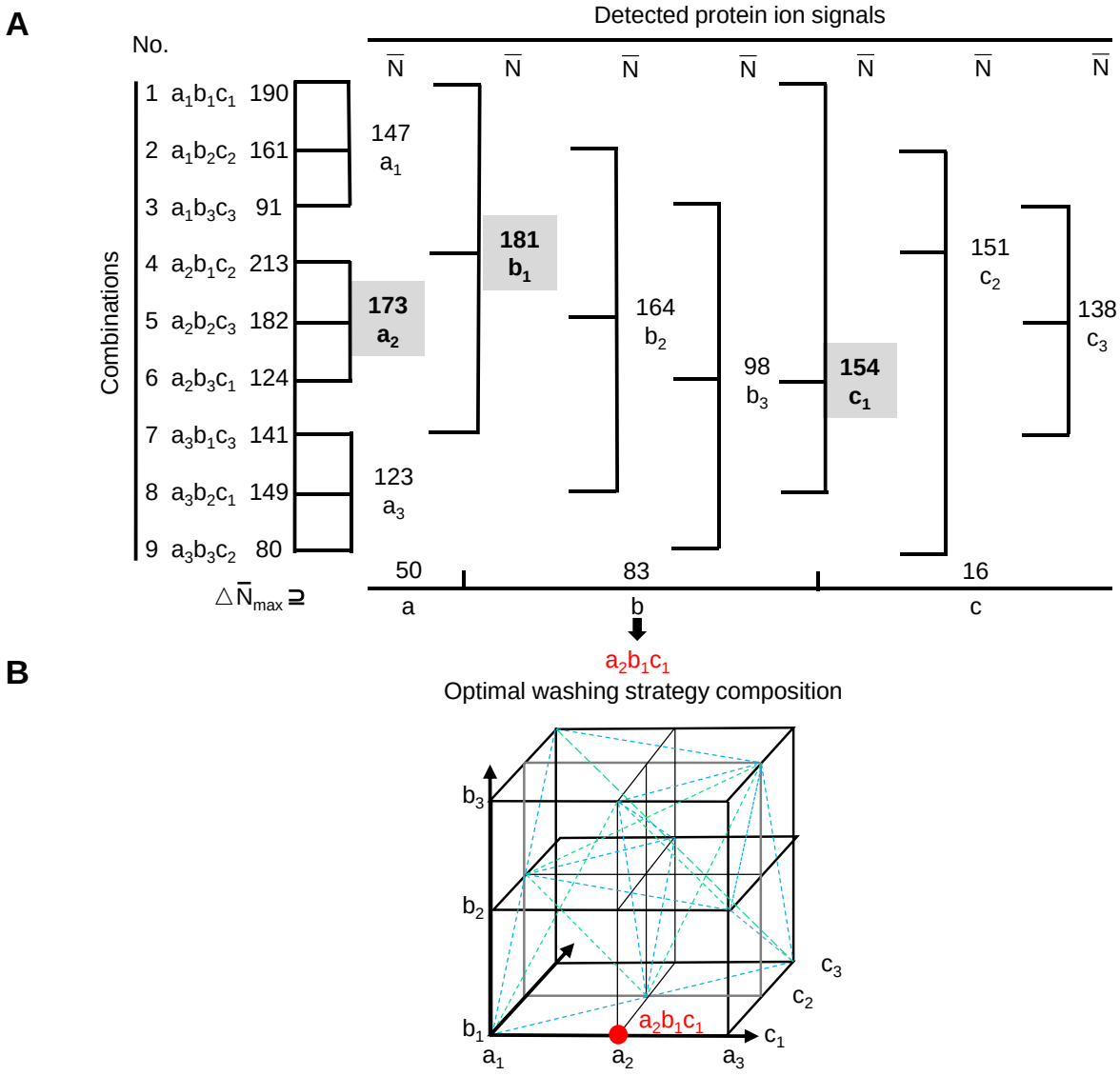

Fig. S6. Establishment of the optimal washing strategy through orthogonal array testing (n=3*3). **(A)** The average number of proteins detected in serial rat liver parallel tissue sections washed by nine different washing methods. **(B)** The red spot shows the composition of optimal washing strategy based on the orthogonal array testing.

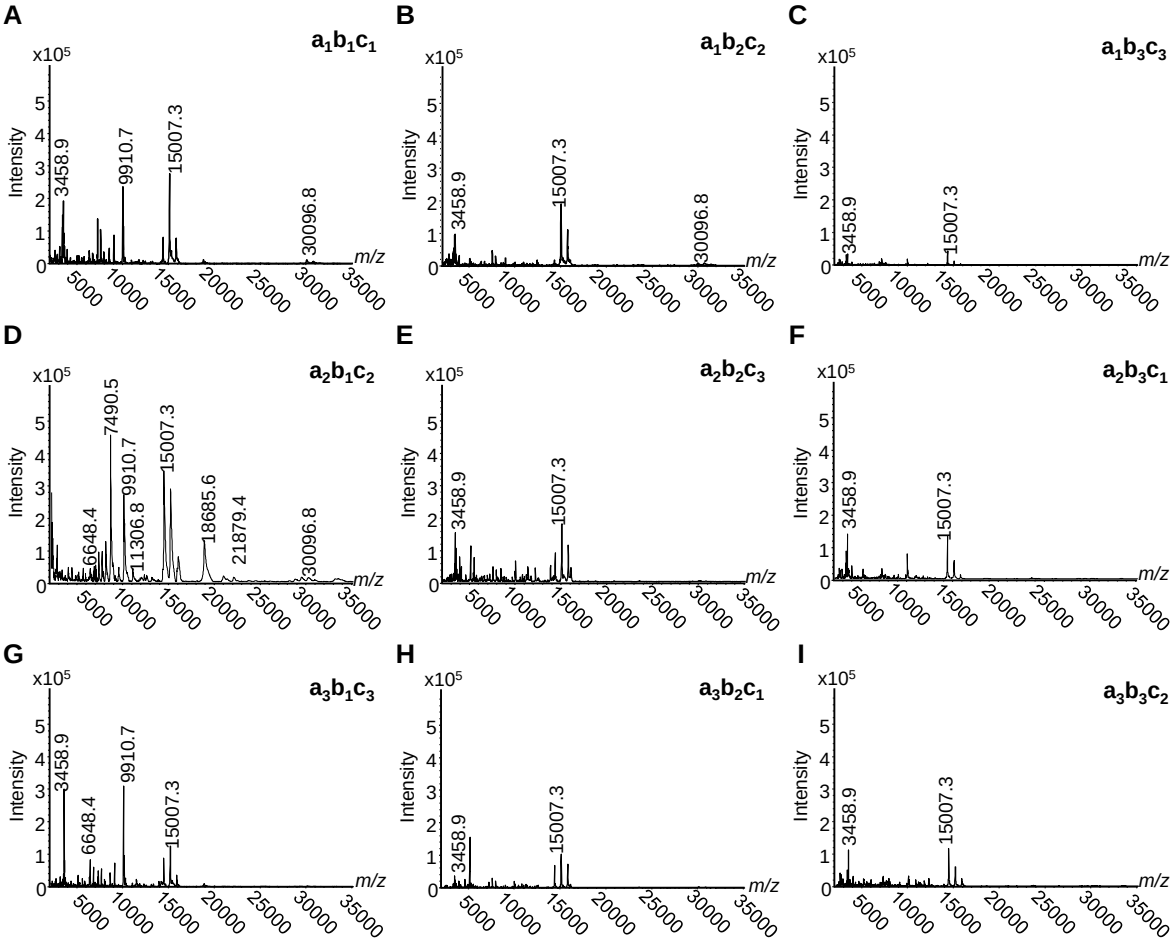

Fig. S7. Mass spectra of proteins detected in serial rat liver parallel tissue sections by positive-ion MALDI-TOF MS, with nine different tissue washing methods including $a_1b_1c_1$, $a_1b_2c_2$, $a_1b_3c_3$, $a_2b_1c_2$, $a_2b_2c_3$, $a_2b_3c_1$, $a_3b_1c_3$, $a_3b_2c_1$, and $a_3b_3c_2$ (n=3*3).

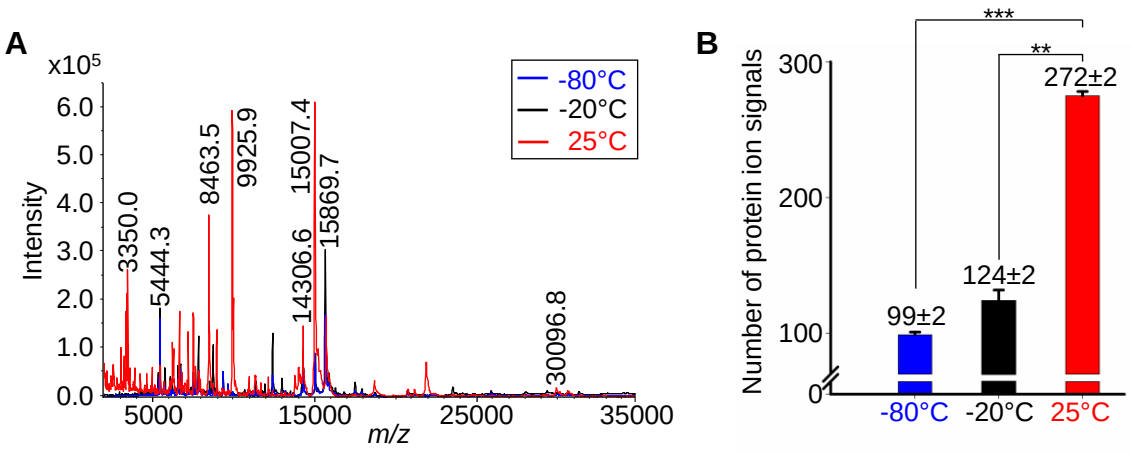

Fig. S8. Comparison of the protein detection from the rat liver tissue sections washed by MeOH-CDWS with three different temperatures (-80°C, -20°C and 25°C) by MALDI-MS in the positive-ion mode using SA as the matrix (n=3*3). **(A)** MALDI mass spectra of proteins acquired from the rat liver tissue sections washed by MeOH-CDWS with -80°C (blue), -20°C (black), and 25°C (red), respectively. **(B)** Histogram showing the number of detectable protein ion signals from the tar liver tissue sections washed by MeOH-CDWS with -80°C (blue), -20°C (black), and 25°C (red), respectively. (**, $0.001<p<0.01$, ***, $p<0.001$).

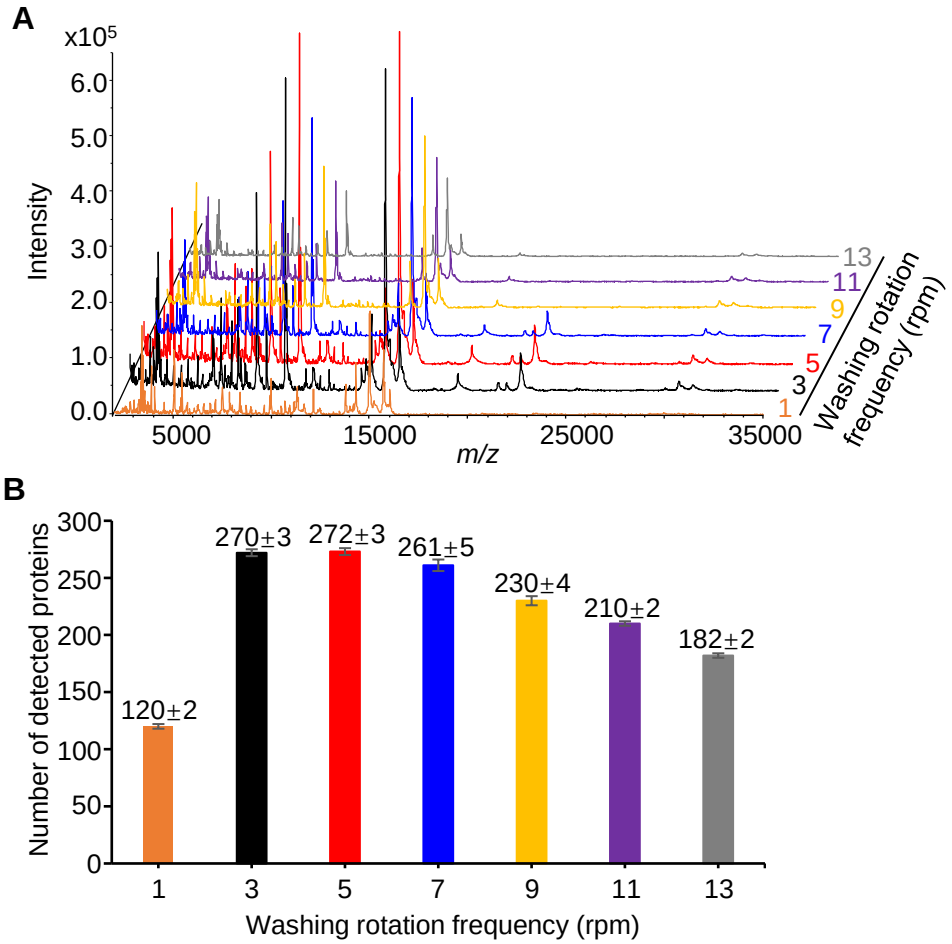

Fig. S9. Mass spectra **(A)** and average number **(B)** of proteins detected in serial parallel-sectioned rat liver tissue sections washed by MeOH-CDWS with different washing rotation frequencies (1 rpm, 3 rpm, 5 rpm, 7 rpm, 9 rpm, 11 rpm, and 13 rpm) at 25°C by (+)MALDI-TOF MS (n=3*3).

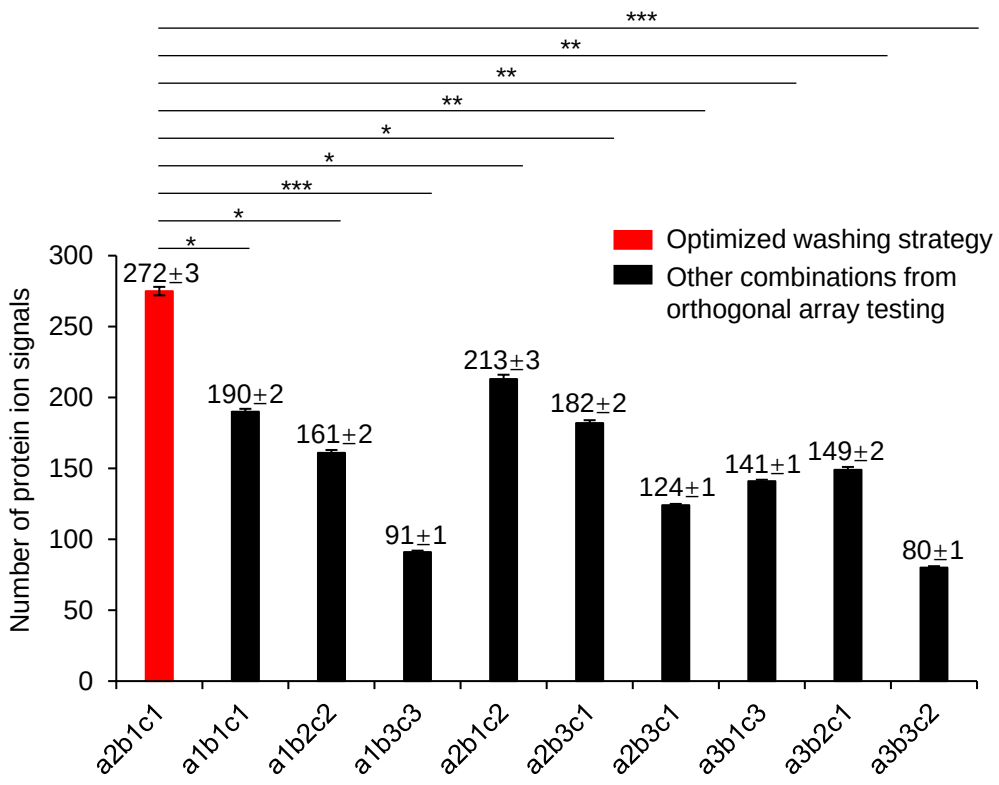

Fig. S10. (+)MALDI-TOF MS *in situ* detection of proteins from serial rat liver parallel tissue sections washed with 10 different tissue washing strategies (n=3*3). The 10 different washing strategies contain the optimized MeOH-CDWS (*i.e.*, $a_2b_1c_1$) and the other nine combinations for orthogonal array testing (*i.e.*, $a_1b_1c_1$, $a_1b_2c_2$, $a_1b_3c_3$, $a_2b_1c_2$, $a_2b_2c_3$, $a_2b_3c_1$, $a_3b_1c_3$, $a_3b_2c_1$, and $a_3b_3c_2$) (*, $0.01<p<0.05$, **, $0.001<p<0.01$, ***, $p<0.001$).

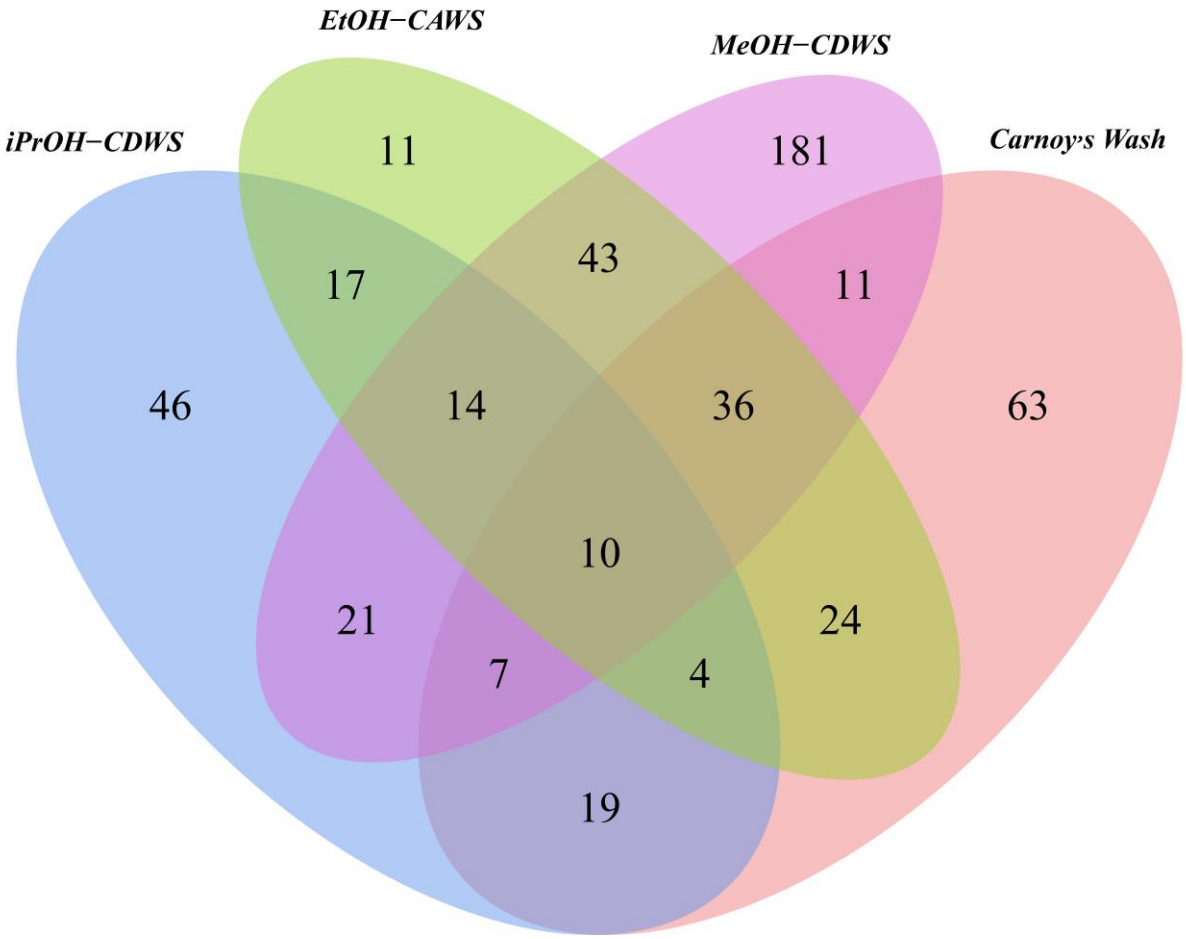

Fig. S11 Venn diagram showing the number of unique proteins detected in rat liver tissue sections washed by MeOH-CDWS, Carnoy's wash, EtOH-CAWS, and iPrOH-CAWS, respectively, by MALDI-MS in the positive-ion mode using SA as the matrix (n=3*3).

Supplementary Information—TABLES

Table S1. The number of small molecule ion signals detected from the rat liver tissue sections by MALDI-TOF MS using DHB as the matrix, with and without MeOH washing (biological replicates, n=3).

Detected protein ion signals (*m/z*)	With MeOH washing			Without MeOH washing		
	1	2	3	1	2	3
104.8				√	√	√
105.2				√	√	√
112.6				√	√	√
113.4				√	√	√
118.1				√	√	√
120.7				√	√	√
125.0				√	√	√
127.1	√	√	√	√	√	√
133.2	√	√	√	√	√	√
137.8	√	√	√	√	√	√
138.3	√	√	√	√	√	√
143.5	√	√	√	√	√	√
146.2	√	√	√	√	√	√
147.0	√	√	√	√	√	
151.0				√	√	√
156.1				√	√	√
159.1				√	√	√
162.1				√	√	√
163.0				√	√	
166.0				√	√	√
167.0	√	√	√	√	√	√
171.0	√	√	√	√	√	
172.1				√	√	
175.3				√	√	√
176.2				√		√
177.3	√	√	√	√	√	
182.0	√	√	√	√	√	√
183.4				√	√	
184.7				√	√	√
185.1				√	√	√
186.6				√	√	√
187.1				√	√	√
188.3				√	√	√
189.1				√	√	√
191.0				√	√	√
194.0				√	√	√
198.1				√	√	√
199.1				√	√	√
201.2	√	√	√	√	√	√
202.0	√	√	√	√	√	√
203.2	√	√	√	√	√	√

Detected protein ion signals (*m/z*)	With MeOH washing			Without MeOH washing		
	1	2	3	1	2	3
204.2		√	√	√	√	√
205.2	√	√	√	√	√	√
206.6	√	√	√	√	√	√
209.3	√	√	√	√	√	√
210.6				√	√	√
215.1				√	√	√
222.1	√	√	√	√	√	√
223.4	√	√	√	√	√	√
224.1	√	√	√	√	√	√
225.3	√	√	√	√	√	√
226.2	√	√	√	√	√	√
227.5	√	√	√	√	√	√
228.0	√	√	√	√	√	
229.9	√	√	√	√	√	√
230.2	√	√	√	√	√	√
231.9	√	√	√	√	√	√
240.1				√	√	√
242.0	√	√	√	√	√	√
243.9	√	√	√	√	√	√
245.9	√	√	√	√	√	√
253.1	√	√	√	√	√	√
258.1	√	√	√	√	√	√
259.1	√	√	√	√	√	√
260.1	√	√	√	√	√	√
270.1				√	√	√
271.0			√	√	√	
272.3	√	√	√	√	√	√
273.9				√	√	√
274.6				√	√	√
275.9	√	√	√	√	√	√
279.1	√	√	√	√	√	√
291.0	√	√	√	√	√	√
302.0	√	√	√	√	√	√
304.6	√	√	√	√	√	√
305.2	√	√		√	√	√
306.1	√	√	√	√	√	√
307.1	√	√	√	√	√	√
312.1	√	√	√	√	√	√
328.0	√	√		√	√	√
334.0	√	√	√	√	√	√
336.0	√	√	√	√	√	√
337.0	√	√	√	√	√	√
344.2				√	√	√
346.1	√	√	√	√	√	√
366.0	√	√	√	√	√	√
367.0	√	√	√	√	√	√
368.0	√	√	√	√	√	√

Detected protein ion signals (*m/z*)	With MeOH washing			Without MeOH washing		
	1	2	3	1	2	3
371.0	√	√	√	√	√	√
373.0	√	√	√	√	√	√
392.1	√	√	√	√	√	√
398.0	√	√	√	√	√	√
400.0	√	√	√	√	√	√
401.9	√	√	√	√	√	√
407.3	√	√	√	√	√	√
430.6	√	√		√	√	√
432.6				√	√	√
433.7				√	√	√
441.9	√	√	√	√	√	√
443.9	√		√	√	√	√
444.9		√	√	√	√	√
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464.9	√	√	√	√	√	√
466.0	√	√	√	√	√	√
468.0	√	√	√	√	√	√
471.2	√	√	√	√	√	√
473.6	√	√	√	√	√	√
476.2	√	√	√	√	√	√
477.5	√	√	√	√	√	√
478.4	√		√	√	√	√
480.9	√	√	√	√	√	√
487.2	√	√	√	√	√	√
495.4	√	√	√	√	√	√
496.2	√	√	√	√	√	√
497.4	√	√	√	√	√	√
498.1	√	√	√	√	√	√
499.2		√	√	√	√	√
510.7	√	√	√	√	√	√
524.4	√	√	√	√	√	√
525.1	√		√	√	√	√
529.9		√	√			
534.3	√	√	√	√	√	√
541.0	√	√	√	√	√	√
544.2				√	√	√
557.2				√	√	√
558.2				√	√	√
559.3				√	√	√
569.6				√	√	√
570.6				√	√	√
578.4	√	√	√	√	√	
613.9	√	√	√	√	√	√
614.2				√	√	√
615.2				√	√	√
616.2	√	√	√	√	√	√

Detected protein ion signals (*m/z*)	With MeOH washing			Without MeOH washing		
	1	2	3	1	2	3
617.2				√	√	√
618.3				√	√	
619.3				√	√	√
620.2				√	√	√
631.2				√	√	√
632.2				√	√	√
648.2				√	√	
710.2				√	√	√
715.8	√	√	√	√	√	√
734.6				√	√	√
749.8				√	√	√
750.8				√	√	√
751.8				√	√	√
753.4				√	√	√
756.2				√	√	√
758.6				√	√	√
759.6				√	√	√
760.7				√	√	√
761.7				√	√	√
772.6				√	√	√
773.8				√	√	√
774.8				√	√	√
775.8				√	√	
776.8				√	√	√
777.9				√	√	√
779.6				√	√	√
780.2	√	√	√	√	√	√
781.6		√	√	√	√	√
782.2				√	√	√
783.7				√	√	√
784.1				√	√	√
785.7				√	√	√
786.7				√	√	√
787.7				√	√	√
788.7				√	√	√
795.6				√	√	√
796.2				√	√	
797.6				√	√	√
798.3				√	√	√
799.6				√	√	√
800.1				√	√	√
801.8				√	√	√
802.9				√	√	√
803.9				√	√	√
804.6				√	√	√
805.7				√	√	√

Detected protein ion signals (*m/z*)	With MeOH washing			Without MeOH washing		
	1	2	3	1	2	3
806.7				√	√	√
807.7				√	√	
808.7				√	√	√
809.7				√	√	√
810.7				√	√	√
811.7				√	√	√
812.7				√	√	√
820.6				√	√	√
821.6				√	√	√
822.7				√	√	√
823.7				√	√	√
824.7				√	√	√
825.7				√	√	√
826.7				√		√
827.7				√	√	√
828.7				√	√	√
829.7				√	√	√
830.6				√	√	√
831.7				√	√	√
832.7				√	√	√
833.7				√	√	√
834.7				√	√	√
835.7				√	√	√
836.7				√		√
837.1				√	√	√
844.6				√	√	√
845.6				√	√	√
846.6				√	√	√
847.7				√	√	√
848.7				√	√	√
849.7					√	√
850.7				√	√	√
851.8				√	√	√
852.7				√	√	√
853.8				√	√	√
872.7				√	√	√
947.2				√	√	√
948.3	√	√	√	√	√	√
949.7		√	√		√	√
950.3	√	√	√	√	√	√
973.7				√	√	√
974.6				√	√	√
1015.6				√	√	√
1069.7				√	√	√
1091.8				√	√	√

Detected protein ion signals (*m/z*)	With MeOH washing			Without MeOH washing		
	1	**2**	**3**	**1**	**2**	**3**
the effective peaks of detected small molecule ion signals	90	93	94	223	222	211
Average of the effective signal peaks of detected small molecule ion signals		92			219	

Note: "√" means the protein ion could be detected in three technical duplicates.

Table S2. The number of protein ion signals detected from the rat liver tissue sections by MALDI-TOF MS using SA as the matrix, with and without MeOH washing (biological replicates, n=3).

Detected protein ion signals (*m/z*)	With MeOH washing			Without MeOH washing		
	1	2	3	1	2	3
2007.6				√	√	√
2034.8	√	√	√	√	√	√
2045.7	√	√	√	√	√	√
2050.1	√	√	√	√	√	√
2069.2	√	√		√	√	√
2083.8	√	√	√	√	√	√
2086.5	√	√	√			
2093.8	√	√	√			
2105.2	√	√	√			
2111.7	√	√	√			
2126.8	√	√	√			
2132.3	√	√		√	√	√
2141.6	√	√	√			
2150.7	√	√	√			
2173.2				√	√	√
2215.1				√	√	√
2279.9	√	√	√			
2284.7	√	√	√			
2303.3	√	√	√			
2328.5	√	√	√			
2332.4	√	√	√			
2345.7	√	√	√			
2349.5	√	√	√			
2365.3	√	√	√			
2376.6	√	√	√			
2385.5	√	√	√			
2392.1	√	√	√			
2397.5	√	√	√			
2401.7	√	√				
2407.4	√	√	√			

Detected protein ion signals (*m/z*)	With MeOH washing			Without MeOH washing		
	1	2	3	1	2	3
2414.7	√	√	√			
2423.9	√	√	√	√	√	√
2430.0	√	√	√			
2439.3	√	√	√			
2448.8	√	√	√	√	√	√
2455.8	√	√	√			
2466.9	√	√	√			
2476.8	√	√	√			
2487.1	√	√	√			
2493.9	√	√	√	√	√	√
2499.0	√		√	√	√	√
2511.8	√	√	√			
2520.8	√	√	√			
2529.8	√		√			
2535.3	√	√	√			
2544.2	√	√	√			
2549.3	√	√	√			
2559.6	√	√	√	√	√	√
2570.4	√	√	√	√	√	√
2594.5	√	√	√	√	√	√
2599.5	√	√	√			
2609.6	√	√	√	√	√	√
2646.3	√	√	√			
2794.8	√	√	√	√	√	√
2800.0	√	√	√			
2818.3	√	√	√			
2833.7	√	√	√			
2846.3	√	√	√			
2860.6	√	√	√			
2869.1	√	√	√			
2880.7	√	√	√			
2886.0	√		√			

Detected protein ion signals (*m/z*)	With MeOH washing			Without MeOH washing		
	1	2	3	1	2	3
2896.5	√	√	√			
2906.2	√	√	√			
2915.3	√	√	√			
2920.1	√	√	√			
2933.5	√	√	√			
2940.7				√	√	√
2963.1				√	√	√
3007.8				√	√	√
3030.0	√	√	√	√	√	√
3039.5	√	√	√	√	√	√
3044.2	√	√	√			
3050.0	√	√	√			
3062.5	√	√	√	√	√	√
3078.6	√	√	√	√	√	√
3082.6	√	√	√			
3088.3	√		√			
3093.0	√	√	√			
3100.5	√	√	√	√	√	√
3118.5	√	√	√	√	√	√
3126.7	√	√	√			
3137.2	√	√	√	√	√	√
3142.9	√	√				
3151.6	√	√	√			
3169.6	√	√	√	√	√	√
3180.1	√	√				
3219.5		√	√	√	√	√
3351.0	√	√	√	√	√	√
3389.8	√	√	√	√	√	√
3456.4	√	√	√	√	√	√
3494.1	√	√	√	√	√	√
3537.1	√	√	√	√	√	√
4663.1	√	√	√	√	√	√

Detected protein ion signals (*m/z*)	With MeOH washing			Without MeOH washing		
	1	2	3	1	2	3
4686.5	√	√	√	√	√	√
4751.6	√	√	√	√	√	√
4792.9	√	√	√	√	√	√
4831.9				√	√	√
4971.1				√	√	√
5009.9				√	√	√
6096.5				√	√	√
6179.6				√	√	√
6202.4				√	√	√
6268.2				√	√	√
6284.6				√	√	√
6324.3				√	√	√
6385.8				√	√	√
6572.2				√	√	√
6658.3	√	√	√	√	√	√
6834.8		√	√	√	√	√
8461.6	√	√	√	√	√	√
8499.9	√	√	√	√	√	√
8518.3	√	√	√	√	√	√
8532.9	√	√	√	√	√	√
8555.6	√	√	√			
8570.2	√	√	√			
8594.3	√	√	√			
9944.8				√	√	√
9987.6				√	√	√
10115.3				√	√	√
10129.7				√	√	√
10169.1				√	√	√
14067.0				√	√	√
14302.3	√	√	√	√	√	√
14324.6	√	√				
14333.3		√	√			

Detected protein ion signals (*m/z*)	With MeOH washing			Without MeOH washing		
	1	2	3	1	2	3
14370.7	√	√	√			
14393.9	√		√			
14403.6				√	√	√
15007.3	√	√	√			
15036.6	√					
15050.8	√		√	√	√	√
15066.7	√	√	√			
15073.9	√					
15084.4	√	√	√			
15096.9	√		√			
15123.8	√	√	√			
15152.1	√	√				
15183.4	√	√	√			
15207.1	√	√	√			
15250.7	√	√	√			
15257.0	√	√	√			
15311.3		√	√			
15869.3				√	√	√
15904.1					√	√
21198.7					√	√
21911.3					√	√
21924.2				√	√	√
22039.9				√	√	√
Number of detected proteins	115	110	110	68	71	71
Average number of detected proteins		112			70	

Note: "√" means the protein ion could be detected in three technical duplicates.

Table S3. The protein ion signals detected by (+)MALDI-TOF MS in serial liver parallel tissue sections washed with MeOH-CAWS, MeOH-CDWS, iPrOH-CAWS, and iPrOH-CDWS for 1.0 min, respectively (biological replicates, n=3).

Detected protein ion signals (*m/z*)	MeOH-CAWS			Average S/N	MeOH-CDWS			Average S/N	iPrOH-CAWS			Average S/N	iPrOH-CDWS			Average S/N
	1	2	3		1	2	3		1	2	3		1	2	3	
2007.6		√	√	19.2					√	√	√	3.7		√	√	8.3
2012.8		√	√	7.2	√	√	√	5.3					√		√	8.5
2024.7									√	√	√	4.9	√	√		9.6
2029.6		√	√	4.5	√			3.5	√	√	√	4.0	√	√	√	8.6
2034.8													√	√		9.2
2045.7		√	√	19.5	√			3.4								
2050.1									√	√	√	6.4		√	√	13.7
2060.7									√		√	3.7	√	√		11.1
2065.4		√	√	8.2					√	√	√	4.6				
2069.2									√	√	√	4.4		√	√	11.3
2073.2									√	√	√	4.4	√		√	11.3
2079.6		√	√	11.8						√	√	4.0				
2083.8		√	√	11.0									√	√		6.9
2090.3									√		√	3.7	√	√		5.8
2099.8		√	√	7.4					√	√	√	4.2		√	√	7.3
2105.2									√	√	√	3.8				
2122.6		√	√	14.2					√	√	√	5.2		√	√	7.1
2126.8		√	√	10.7	√	√	√	3.5								
2141.6		√	√	5.4												
2150.7		√	√	10.5												
2164.1		√	√	8.7												
2173.2		√	√	8.5					√	√	√	13.2		√	√	13.6
2178.1		√	√	7.1	√	√	√	4.1								
2193.9		√	√	17.5						√	√	4.5		√		3.1
2199.6														√	√	4.1
2205.8		√	√	16.1										√	√	4.5
2210.1		√	√	4.5					√	√	√	5.7		√	√	5.3
2215.1		√	√	13.6					√	√		5.6	√		√	5.1

Detected protein ion signals (*m/z*)	MeOH-CAWS			Average S/N	MeOH-CDWS			Average S/N	iPrOH-CAWS			Average S/N	iPrOH-CDWS			Average S/N
	1	2	3		1	2	3		1	2	3		1	2	3	
2221.8									√	√		4.7	√	√	√	5.9
2225.3										√	√	3.8	√		√	6.1
2233.1		√	√	7.4					√	√	√	9.8	√		√	10.2
2247.6		√	√	5.3					√	√	√	7.0	√	√		7.6
2252.5									√	√	√	3.0	√		√	6.8
2256.3										√	√	4.9	√	√		6.8
2260.1									√		√	3.0	√		√	6.2
2264.2									√	√		8.1			√	5.9
2270.1									√	√	√	5.4	√	√		6.5
2274.4									√	√			√		√	6.7
2279.9									√	√	√	4.6	√	√	√	5.6
2284.7		√	√	5.3					√	√	√	5.3	√		√	7.2
2292.4									√	√	√	5.2	√		√	6.7
2297.8									√	√	√	3.4				
2303.3									√	√		4.0	√	√	√	4.2
2308.1		√	√	7.7					√	√	√	3.6	√	√		5.9
2313.6									√	√	√	3.2	√	√		3.7
2320.7									√	√	√	4.9	√	√		3.7
2324.1		√	√	5.6					√	√	√	3.2	√		√	6.1
2332.4									√	√	√	3.3	√	√		5.3
2338.6									√	√		3.2	√	√	√	4.3
2345.7									√	√		3.4	√	√	√	7.0
2349.5		√	√	5.8	√	√		4.1	√	√	√	3.0	√	√	√	6.9
2354.0									√	√	√	3.1	√		√	6.5
2365.3									√	√	√	3.2	√		√	6.8
2370.1									√	√	√	3.2	√		√	6.6
2381.3													√	√	√	6.1
2385.5													√		√	6.3
2397.5									√	√		3.3	√		√	4.1
2401.7									√	√		3.2	√		√	4.8
2407.4									√	√	√	3.2		√	√	5.5

Detected protein ion signals (*m/z*)	MeOH-CAWS			Average S/N	MeOH-CDWS			Average S/N	iPrOH-CAWS			Average S/N	iPrOH-CDWS			Average S/N
	1	2	3		1	2	3		1	2	3		1	2	3	
2423.9		√	√	8.0									√	√	√	6.4
2430.0									√	√	√	3.6	√	√		3.5
2435.7									√	√	√	3.1	√	√		3.6
2448.8									√	√	√	3.4	√		√	3.1
2482.8		√	√	13.5	√	√	√	4.1	√	√	√	3.6	√	√		3.3
2487.1									√	√	√	3.6	√	√		4.0
2493.9		√	√	8.2	√	√	√	3.4	√	√	√	5.6	√		√	8.7
2499.0					√	√		3.2						√	√	4.6
2535.3	√	√	√	6.1	√	√	√	4.8	√	√	√	5.0		√	√	4.8
2555.9		√	√	5.3					√	√	√	5.5		√	√	7.2
2570.4				7.7	√	√	√	3.2						√	√	4.2
2576.1	√		√	3.8						√	√	4.3		√	√	4.2
2585.9					√	√	√	3.2						√	√	4.5
2594.5		√	√	4.9					√	√	√	4.4		√	√	6.3
2599.5															√	5.9
2609.6					√	√	√	3.2						√	√	3.1
2618.7													√	√	√	4.7
2627.0														√	√	3.9
2633.4		√	√	5.3					√	√	√	3.2	√	√	√	4.3
2640.1				4.7						√	√	3.6		√	√	3.4
2684.4		√	√	6.1												
2710.9		√	√	5.6												
2748.2									√	√	√	3.5		√	√	3.7
2754.6		√	√	8.5												
2775.3		√	√	7.8										√	√	5.4
2794.8		√	√	3.5	√	√	√	3.2	√	√	√	3.0	√	√		4.5
2800.0									√	√	√	3.6	√	√		4.7
2804.6														√	√	3.8
2813.7									√	√		3.4		√	√	4.0
2818.3									√		√	3.6	√	√	√	3.9
2838.2									√		√	3.8		√	√	4.2

Detected protein ion signals (*m/z*)	MeOH-CAWS			Average S/N	MeOH-CDWS			Average S/N	iPrOH-CAWS			Average S/N	iPrOH-CDWS			Average S/N
	1	2	3		1	2	3		1	2	3		1	2	3	
2843.3									√	√	√	3.1	√	√	√	3.9
2849.9									√	√	√	3.1	√	√	√	4.8
2863.5													√		√	3.1
2869.1									√	√		3.1	√	√	√	3.2
2896.5		√	√	8.0						√	√	3.4	√	√	√	3.8
2915.3		√	√	10.3												
2933.5									√	√	√	3.3				
2952.9		√	√	6.1												
2956.1		√	√	5.6												
2984.8									√	√	√	3.5				
3003.1									√	√	√	4.8				
3025.5	√	√		5.4												
3034.8	√	√	√	23.9	√	√	√	7.9	√	√	√	4.2	√	√	√	12.4
3058.3		√	√	5.1												
3073.5		√	√	13.4	√	√	√	5.6					√	√	√	6.0
3100.5		√	√	9.9					√	√		3.6	√	√	√	4.4
3108.9		√	√	13.6									√	√	√	4.2
3137.2	√		√	39.4												
3148.8	√	√	√	7.6										√	√	4.1
3161.3													√		√	3.1
3165.8													√	√		3.1
3202.6	√	√	√	6.8												
3214.4	√	√	√	6.0	√	√	√	3.3	√	√	√	3.5	√	√	√	6.1
3256.2					√	√	√	3.3						√	√	4.5
3273.6										√	√	3.0	√	√	√	3.3
3297.1									√	√	√	3.1	√	√	√	3.2
3348.4					√	√	√	13.8					√	√		3.6
3389.8					√	√	√	16.7						√	√	3.3
3409.5					√	√	√	8.7					√	√	√	3.4
3428.1					√	√	√	9.3					√	√	√	4.3
3446.6					√	√	√	9.6	√	√	√	4.9		√	√	4.5

Detected protein ion signals (*m/z*)	MeOH-CAWS			Average S/N	MeOH-CDWS			Average S/N	iPrOH-CAWS			Average S/N	iPrOH-CDWS			Average S/N
	1	2	3		1	2	3		1	2	3		1	2	3	
3456.4	√	√	√	14.4	√	√	√	8.6	√	√	√	24.9	√	√	√	38.8
3475.4	√	√	√	18.7	√	√	√	8.0		√	√	4.3	√	√	√	4.7
3483.8									√	√		3.5	√		√	4.6
3494.1	√	√	√	20.9	√	√	√	5.0	√	√	√	8.3	√	√	√	9.3
3519.8	√	√	√	6.6												
3537.1	√	√	√	6.7	√	√	√	4.5					√	√	√	4.3
3566.7					√	√	√	3.5								
3609.9										√	√	3.6	√	√		3.0
3690.8	√	√	√	6.3												
3789.5	√	√	√	30.9					√	√	√	6.5	√	√	√	12.4
3814.4	√	√	√	5.0						√	√	4.7	√	√	√	5.4
3829.4		√	√	7.3									√	√	√	4.3
3883.6					√	√	√	4.2					√	√	√	3.6
4226.4					√	√	√	3.9					√	√	√	3.5
4241.4		√	√	7.2									√	√	√	3.2
4285.7		√	√	5.4					√	√	√	3.9	√	√	√	4.9
4489.1										√	√	4.0	√	√	√	4.5
4529.2									√	√		4.2	√		√	3.1
4539.5		√	√	8.9												
4618.7		√	√	20.2	√	√	√	25.0	√	√	√	23.2	√	√	√	31.3
4641.4		√	√	31.5					√	√	√	4.8	√	√	√	6.1
4657.0		√	√	22.7					√	√	√	11.9	√	√	√	11.3
4678.7		√	√	19.2					√	√	√	6.5	√	√	√	6.9
4691.5		√	√	11.4					√	√	√	4.0	√	√	√	4.7
4719.6		√	√	7.1												
4738.8		√	√	5.6												
4746.5		√	√	19.1					√	√	√	6.7	√	√	√	8.8
4785.5									√	√	√	3.5	√	√	√	3.3
4796.5		√		3.3					√	√	√	3.7	√	√	√	3.2
4948.0		√	√	7.0												
4956.2					√	√	√	3.1						√	√	3.2

Detected protein ion signals (*m/z*)	MeOH-CAWS			Average S/N	MeOH-CDWS			Average S/N	iPrOH-CAWS			Average S/N	iPrOH-CDWS			Average S/N
	1	2	3		1	2	3		1	2	3		1	2	3	
4961.9	√	√	√	26.9					√	√	√	8.4	√	√	√	10.8
5000.9		√	√	8.0					√	√	√	5.0	√	√	√	4.3
5443.6	√	√	√	7.4	√	√	√	3.4					√	√	√	4.7
6021.3	√	√	√	8.2	√	√	√	3.3					√	√		3.3
6096.5		√	√	13.7					√	√	√	3.3	√	√	√	5.8
6135.5		√	√	6.5												
6169.6		√	√	15.6					√		√	3.6	√	√	√	4.1
6210.5	√	√	√	17.4					√		√	3.7	√	√	√	4.7
6223.4	√	√	√	27.7	√	√	√	9.8	√	√	√	4.7	√	√	√	10.6
6237.9					√	√	√	5.3	√	√	√	4.9		√	√	3.4
6243.6	√	√	√	13.0	√		√	4.1					√	√	√	3.3
6251.5					√	√	√	3.9					√	√		3.6
6259.6					√	√	√	4.0					√	√	√	3.5
6266.1	√	√		10.2	√	√		4.2					√	√		3.1
6274.4	√	√	√	18.3	√	√	√	8.9	√	√	√	5.6	√	√	√	5.9
6282.2	√	√	√	12.8									√	√	√	3.6
6288.1													√	√	√	3.7
6314.9					√	√	√	3.4					√	√		3.8
6561.5													√	√		3.6
6572.2		√	√	6.4	√	√	√	3.3					√	√	√	7.0
6611.0													√		√	3.1
6646.5					√	√	√	16.1	√	√	√	15.1	√	√	√	14.8
6658.3	√	√	√	25.3									√	√		4.0
6683.8									√	√	√	4.7	√	√	√	4.1
6689.6	√	√	√	7.5	√	√		3.0					√	√	√	3.3
6824.9					√	√	√	4.5						√	√	3.4
7003.2													√	√		3.6
7023.1	√	√	√	18.4												
7143.2					√	√	√	3.4						√	√	3.7
7190.2					√	√	√	12.7					√	√		3.2
7490.5					√	√	√	8.5								

Detected protein ion signals (*m/z*)	MeOH-CAWS			Average S/N	MeOH-CDWS			Average S/N	iPrOH-CAWS			Average S/N	iPrOH-CDWS			Average S/N
	1	2	3		1	2	3		1	2	3		1	2	3	
7505.0					√	√	√	16.6								
7520.5					√	√	√	5.0								
7526.4					√	√		5.7								
7535.2					√	√		3.1								
7540.4					√	√	√	3.9								
7544.3	√	√		9.2												
7548.0	√	√	√	7.6												
7594.1					√	√	√	3.2								
7607.0					√	√	√	3.4								
7649.9					√	√	√	6.1								
7854.3					√	√	√	5.2								
7873.2					√	√	√	3.4								
7880.8					√	√	√	3.0								
7933.6					√	√	√	3.6								
7973.1	√	√	√	6.4												
8380.3					√	√	√	3.4								
8389.3													√	√	√	3.4
8408.6					√	√	√	3.3					√		√	3.6
8414.8					√	√	√	3.1								
8449.5	√	√	√	8.5	√	√	√	68.1	√	√	√	5.3				
8488.9					√	√	√	13.0								
8510.6					√	√	√	8.0								
8527.7					√	√	√	6.1								
8532.2						√	√	5.8								
8546.5					√	√	√	4.7								
8550.2						√	√	4.9								
8590.4					√	√		3.3								
8599.3					√	√	√	3.0					√	√		4.6
8706.1													√	√	√	5.1
8720.0													√	√	√	3.1
8741.6													√		√	3.1

Detected protein ion signals (*m/z*)	MeOH-CAWS			Average S/N	MeOH-CDWS			Average S/N	iPrOH-CAWS			Average S/N	iPrOH-CDWS			Average S/N
	1	2	3		1	2	3		1	2	3		1	2	3	
8754.3													√		√	3.1
8844.3	√	√	√	7.7									√	√	√	3.4
8941.5													√	√	√	5.5
8954.2					√	√	√	14.2								
8981.6					√	√	√	3.5								
9287.8	√	√	√	22.7									√	√	√	3.5
9326.8	√	√	√	7.4												
9339.6	√	√	√	5.9												
9880.2					√	√	√	6.0								
9905.6	√	√	√	6.3	√	√	√	10.9	√	√	√	3.8	√	√	√	9.8
9909.0	√	√	√	21.0	√	√	√	12.3					√	√		8.3
9921.8	√	√		5.7												
9931.1	√	√	√	6.2	√	√	√	13.2					√	√		6.8
9945.3	√	√	√	12.9	√	√	√	16.1					√	√	√	5.1
9969.9	√	√	√	10.5									√		√	4.7
9976.7					√	√	√	23.1					√	√	√	4.3
9987.2													√	√	√	3.3
10012.7					√		√	6.4								
10016.1	√	√	√	13.3									√	√	√	3.4
10027.5													√	√	√	3.6
10034.7					√	√	√	4.3								
10057.3	√	√	√	6.6												
10078.1					√			3.3								
10114.9	√	√		5.8												
10145.4					√	√	√	3.9								
10943.9					√	√	√	5.4								
11306.8					√	√	√	6.2								
11318.5					√	√	√	5.3								
11343.9						√	√	5.7								
11359.7	√			4.2	√		√	4.6								
11365.7	√			4.1		√	√	4.6								

Detected protein ion signals (*m/z*)	MeOH-CAWS			Average S/N	MeOH-CDWS			Average S/N	iPrOH-CAWS			Average S/N	iPrOH-CDWS			Average S/N
	1	2	3		1	2	3		1	2	3		1	2	3	
11382.9					√	√	√	3.7								
11396.5	√			4.4		√	√	3.1								
11403.4																
11683.2					√	√	√	4.1								
12131.1					√	√	√	3.4								
12167.9					√	√	√	3.9								
13774.6					√	√	√	7.1								
13810.3	√			4.1		√	√	4.3								
13815.5					√	√	√	4.6								
13820.6	√			4.3												
13830.8	√			4.2												
13835.9					√	√		3.3								
13844.3						√		3.3								
13897.1					√		√	3.1								
13936.1					√	√		3.0								
13951.9	√			3.1		√	√	3.9								
13970.0	√			3.9												
13979.3					√	√	√	4.3								
13997.6	√			4.5												
14006.4					√	√	√	9.8								
14021.5	√			4.3			√	6.7								
14030.1					√	√	√	7.2								
14035.2							√	7.8								
14046.4	√			4.6												
14050.4	√			4.6												
14058.0	√			4.2												
14067.0					√	√	√	6.1								
14081.4	√			4.5												
14086.6	√			4.6	√	√	√	7.5		√	√	3.2				
14106.5					√	√	√	4.7								
14117.3	√			4.6	√	√	√	5.4								

Detected protein ion signals (*m/z*)	MeOH-CAWS			Average S/N	MeOH-CDWS			Average S/N	iPrOH-CAWS			Average S/N	iPrOH-CDWS			Average S/N
	1	2	3		1	2	3		1	2	3		1	2	3	
14140.8					√	√	√	4.5								
14161.0	√			3.9		√	√	6.3								
14164.2					√	√	√	6.6								
14203.3	√			3.6	√	√	√	5.1		√	√	3.6				
14244.4					√	√	√	4.4								
14259.9	√			3.1												
14282.5	√	√	√	7.5	√	√	√	57.6		√	√	3.4				
14302.3					√	√	√	8.7								
14324.6					√	√	√	48.6								
14344.7					√	√	√	38.3		√	√	3.2				
14361.4	√			3.9	√	√	√	5.9								
14370.7	√			3.7		√	√	5.7								
14381.9					√	√	√	28.5								
14389.7	√			3.4		√		5.1								
14403.6					√	√		4.4		√	√	3.1				
14413.0					√	√	√	3.8								
14425.2					√	√	√	3.5								
14441.1					√	√	√	3.6								
14453.9					√	√	√	3.4								
14488.6					√	√	√	3.4								
14882.2						√	√	4.3								
14978.2	√	√	√	16.4	√	√	√	31.8								
14995.7	√	√	√	18.2					√	√	√	3.6				
15002.9													√	√	√	8.3
15007.3	√	√	√	13.3	√	√	√	36.2								
15036.6									√	√		6.8	√	√	√	6.8
15043.7	√	√	√	28.8	√	√	√	39.1					√	√	√	6.8
15066.7	√		√	12.1	√	√	√	28.6								
15079.1	√		√	21.2												
15084.4	√	√	√	23.2												
15105.0						√	√	9.4								

Detected protein ion signals (*m/z*)	MeOH-CAWS			Average S/N	MeOH-CDWS			Average S/N	iPrOH-CAWS			Average S/N	iPrOH-CDWS			Average S/N
	1	2	3		1	2	3		1	2	3		1	2	3	
15123.8	√	√	√	19.9		√	√	17.0					√	√		4.7
15142.2					√	√	√	15.7	√	√		4.7				
15152.1					√	√	√	15.2								
15167.0						√	√	15.4								
15174.1	√			3.4		√	√	9.4								
15183.4	√			3.3	√	√	√	17.4								
15191.9	√			3.8												
15202.2	√			3.5												
15212.1					√	√	√	17.7								
15229.5	√			3.2		√	√	13.4								
15250.7	√			3.2		√	√	10.6								
15267.9	√			4.4	√	√	√	9.3								
15279.2						√	√	9.1								
15285.5	√	√	√	10.3	√	√	√	8.6								
15292.8						√	√	8.9								
15311.3	√			5.6	√	√	√	8.9								
15323.8					√	√	√	9.5								
15329.2	√			5.5	√	√	√	9.2					√	√		3.6
15345.3					√	√	√	9.7								
15354.2	√			5.7	√	√	√	9.2								
15361.4	√			5.3		√	√	8.6								
15377.5	√			5.6		√	√	8.5								
15389.4	√			5.0	√	√	√	7.9								
15415.1	√			5.3												
15427.6	√			5.9												
15434.1	√			5.7												
15442.0					√	√		7.6								
15452.7					√		√	7.7								
15470.7					√	√	√	5.1								
15485.0					√		√	4.2								
15524.1					√	√	√	3.6								

Detected protein ion signals (*m/z*)	MeOH-CAWS			Average S/N	MeOH-CDWS			Average S/N	iPrOH-CAWS			Average S/N	iPrOH-CDWS			Average S/N
	1	2	3		1	2	3		1	2	3		1	2	3	
15562.4						√	√	3.4								
15589.4					√	√	√	4.8								
15627.2					√	√	√	5.0								
15634.4					√	√	√	4.6								
15657.9						√	√	6.3								
15705.6					√	√	√	30.8								
15723.3	√			4.8												
15743.8	√			4.9	√	√	√	20.1								
15760.4	√			5.3												
15777.4						√	√	11.0								
15782.6	√			5.6	√	√	√	10.9								
15792.9	√			5.3	√	√	√	6.9								
15804.5						√	√	8.8								
15827.2					√		√	7.3								
15846.6	√	√	√	5.7	√	√	√	8.9					√	√		3.4
15877.2					√	√	√	6.5								
15911.6					√	√	√	7.1								
15925.1					√		√	6.2								
15946.4					√	√	√	5.1								
15955.5							√	4.7								
15965.2					√	√	√	4.4								
15990.2					√		√	3.6								
16010.2						√	√	3.7								
18618.7					√	√	√	3.1								
18645.4					√	√	√	4.1								
18654.1					√	√	√	4.4								
18677.7	√			3.1												
18694.0	√			3.5	√	√	√	8.1								
18708.6	√			3.7												
18716.9	√			3.2	√	√	√	7.7								
18723.1						√	√	4.9								

Detected protein ion signals (*m/z*)	MeOH-CAWS			Average S/N	MeOH-CDWS			Average S/N	iPrOH-CAWS			Average S/N	iPrOH-CDWS			Average S/N
	1	2	3		1	2	3		1	2	3		1	2	3	
18730.0	√			3.8	√	√	√	4.2								
18752.7	√			3.2												
18768.5	√			3.4	√	√	√	3.6								
18805.6					√	√	√	3.1								
20700.1					√	√	√	3.5								
20716.7					√	√	√	3.0								
20752.4					√	√	√	3.6								
20765.7					√	√	√	11.7								
20774.8					√	√	√	11.0								
20789.4					√		√	4.5								
21162.9					√		√	4.9								
21171.3					√	√	√	4.4								
21190.2					√	√	√	3.8								
21215.4					√	√	√	3.4								
21760.3					√	√	√	3.4								
21780.8					√	√	√	3.8								
21822.0						√	√	7.0								
21874.1					√	√	√	21.6								
21905.0					√	√	√	14.6								
21936.7						√	√	10.8								
21949.9					√	√	√	10.7								
21962.7					√	√	√	11.1								
21971.2					√	√	√	10.9								
22027.6					√	√	√	6.5								
22050.3					√	√	√	4.9								
22073.8						√	√	4.0								
22085.9					√	√	√	3.9								
22114.4					√	√		5.0								
29863.1					√	√	√	4.2								
29882.9					√	√	√	5.4								
29920.1					√	√		8.1								

Detected protein ion signals (*m/z*)	MeOH-CAWS			Average S/N	MeOH-CDWS			Average S/N	iPrOH-CAWS			Average S/N	iPrOH-CDWS			Average S/N
	1	2	3		1	2	3		1	2	3		1	2	3	
29927.6						√	√	3.9								
29937.5					√	√	√	7.6								
29954.9						√	√	7.0								
29976.8					√	√		5.4								
29987.2					√	√		3.9								
30020.0					√	√	√	4.5								
30028.9					√	√	√	4.1								
30046.4					√	√		3.6								
30064.2					√	√	√	4.0								
30076.7					√	√	√	4.3								
30086.6					√	√		4.8								
30102.0					√	√		3.8								
30119.0					√		√	4.2								
30124.0					√	√		3.9								
30138.9					√	√	√	4.3								
30151.4					√	√		4.5								
30161.3					√	√	√	3.5								
30171.3					√	√		3.0								
30188.1					√		√	3.2								
30345.6					√		√	4.1								
30363.4					√			4.3								
30394.8					√		√	4.4								
30464.6					√		√	4.2								
30474.9					√		√	3.8								
30502.0					√		√	3.0								
30581.4					√	√	√	3.6								
30603.9					√	√		3.4								
30620.7					√	√	√	4.6								
30636.5					√	√		5.3								
30650.1					√	√	√	5.2								
30658.7					√	√		4.8								

Detected protein ion signals (*m/z*)	MeOH-CAWS			Average S/N	MeOH-CDWS			Average S/N	iPrOH-CAWS			Average S/N	iPrOH-CDWS			Average S/N
	1	2	3		1	2	3		1	2	3		1	2	3	
30670.2					√	√	√	4.8								
30691.8					√	√	√	4.9								
30704.4					√	√		4.1								
30742.1					√		√	3.5								
30754.7					√	√	√	3.1								
30773.6					√			3.2								
30788.1					√			3.1								
30802.5						√	√	3.3								
30811.4					√	√	√	3.4								
30830.9					√			3.6								
30850.3					√	√		3.5								
30860.3					√		√	3.7								
30876.7					√			3.4								
30898.4					√			3.0								
30920.0					√			3.1								
Number of detected proteins	106	117	113		231	234	229		98	108	99		139	145	139	
Average number of detected proteins		112				231				101				141		

Note: "√" means the protein ion could be detected in three technical duplicates.

Table S4. The protein ion signals detected by (+)MALDI-TOF MS in serial liver parallel tissue sections washed with 70% MeOH, 100% MeOH, MeOH-CDWS, 70% iPrOH, 100% iPrOH, and iPrOH-CDWS for 1.0 min, respectively (biological replicates, n=3).

Detected protein ion signals (*m/z*)	70% MeOH			Average S/N	100% MeOH			Average S/N	MeOH-CDWS			Average S/N	70% iPrOH			Average S/N	100% iPrOH			Average S/N	iPrOH-CDWS			Average S/N
	1	2	3		1	2	3		1	2	3		1	2	3		1	2	3		1	2	3	
2007.6													√	√	√	8.2	√	√	√	9.2		√	√	8.3
2012.8									√	√	√	5.3	√	√	√	6.6	√	√	√	7.4	√		√	8.5
2024.7													√	√		11.3			√	11.9	√	√		9.6
2029.6									√			3.5	√	√		13.4		√		9.6	√		√	8.6
2034.8													√	√	√	23.2		√		11.0	√	√		9.2
2045.7									√			3.4	√	√	√	11.3		√		8.4				
2050.1													√	√	√	25.7	√	√	√	10.1		√	√	13.7
2060.7																		√	√	10.1	√	√		11.1
2069.2																	√	√	√	20.8		√	√	11.3
2073.2																	√	√	√	18.5	√	√	√	11.3
2083.8																					√	√		6.9
2090.3																		√	√	21.6	√	√		5.8
2099.8																						√	√	7.3
2122.6																			√	9.3		√	√	7.1
2126.8									√	√	√	3.5	√		√	8.7	√	√	√	8.7				
2173.2													√	√	√	10.5	√	√	√	10.9		√	√	13.6
2178.1									√	√	√	4.1	√	√	√	10.9	√	√	√	10.3				
2193.9																	√	√	√	3.7		√		3.1
2199.6																						√	√	4.1
2205.8													√	√	√	8.0						√	√	4.5
2210.1																						√	√	5.3
2215.1													√	√	√	7.8	√	√	√	3.0	√		√	5.1
2221.8																					√	√	√	5.9
2225.3													√	√	√	4.0	√	√	√	11.2	√		√	6.1
2233.1													√	√	√	4.2				14.2	√		√	10.2
2247.6													√	√	√	4.8	√	√	√	5.0	√	√		7.6
2252.5																					√		√	6.8

Detected protein ion signals (*m/z*)	70% MeOH			Average S/N	100% MeOH			Average S/N	MeOH-CDWS			Average S/N	70% iPrOH			Average S/N	100% iPrOH			Average S/N	iPrOH-CDWS			Average S/N
	1	2	3		1	2	3		1	2	3		1	2	3		1	2	3		1	2	3	
2256.3													√	√	√	5.4	√	√	√	5.7	√	√		6.8
2260.1													√	√	√	8.6					√		√	6.2
2264.2													√	√	√	8.6	√	√	√	8.6			√	5.9
2270.1													√	√		5.5				6.4	√	√		6.5
2274.4																	√	√	√	7.7	√		√	6.7
2279.9													√	√	√	8.3			√	9.2	√	√	√	5.6
2284.7																	√	√	√	11.4	√		√	7.2
2303.3																	√	√	√	13.2	√	√	√	4.2
2308.1													√	√	√	15.0					√	√		5.9
2313.6													√	√	√	14.8	√	√	√	13.8	√	√		3.7
2320.7																					√	√		3.7
2324.1																	√	√	√	18.8	√		√	6.1
2332.4																	√	√	√	17.6	√	√		5.3
2338.6													√	√		17.3					√	√	√	4.3
2345.7													√	√	√	22.4	√	√	√	22.4				
2349.5									√	√		4.1						√		19.7				
2354.0													√	√	√	19.7	√	√	√	20.7	√		√	6.5
2365.3													√			19.6	√	√	√	19.6	√		√	6.8
2370.1													√	√	√	21.0	√	√		21.0	√		√	6.6
2381.3													√	√	√	20.5	√	√	√	20.5	√	√	√	6.1
2385.5													√	√	√	19.6					√		√	6.3
2397.5													√		√	18.9	√	√	√	18.9	√		√	4.1
2401.7													√	√	√	16.7		√		16.7	√		√	4.8
2407.4														√	√	16.8		√		16.8		√	√	5.5
2423.9														√	√	14.4					√	√	√	6.4
2430.0														√	√	15.8					√	√		3.5
2435.7													√	√		14.3	√	√	√	14.3	√	√		3.6
2448.8									√	√	√	4.3	√	√	√	10.4	√	√		10.4	√		√	3.1
2482.8									√			4.1		√	√	13.5					√	√		3.3

Detected protein ion signals (*m/z*)	70% MeOH			Average S/N	100% MeOH			Average S/N	MeOH-CDWS			Average S/N	70% iPrOH			Average S/N	100% iPrOH			Average S/N	iPrOH-CDWS			Average S/N
	1	2	3		1	2	3		1	2	3		1	2	3		1	2	3		1	2	3	
2487.1																					√	√		4.0
2493.9										√	√	3.4	√	√	√	14.5	√	√	√	14.5	√		√	8.7
2499.0									√	√		4.5	√	√	√	14.5		√		14.5		√	√	4.6
2535.3									√	√	√	4.8	√	√	√	11.1						√	√	4.8
2555.9																						√	√	7.2
2570.4									√	√	√	3.2	√	√	√	19.3						√	√	4.2
2576.1																	√	√	√	19.3		√	√	4.2
2585.9									√	√	√	3.2	√	√	√	11.2	√	√	√	17.8		√	√	4.5
2594.5																		√		16.2		√	√	6.3
2599.5													√	√	√	16.2	√	√		16.2			√	5.9
2609.6									√	√	√	3.2										√	√	3.1
2618.7																					√	√	√	4.7
2627.0													√	√	√	8.7	√	√	√	13.9		√	√	3.9
2633.4													√	√	√	5.5	√	√	√	5.5	√	√	√	4.3
2640.1																						√	√	3.4
2748.2																						√	√	3.7
2775.3													√	√	√	13.6						√	√	5.4
2794.8									√	√	√	3.2					√	√	√	21.5				
2800.0																	√	√	√	9.6				
2804.6																	√	√	√	13.6				
2818.3													√	√	√	23.8	√	√	√	23.8	√	√	√	3.9
2838.2																						√	√	4.2
2843.3													√	√	√	19.7				19.7	√		√	3.9
2859.9																					√		√	4.8
2863.5																	√	√		12.6	√		√	3.1
2869.1																					√	√	√	3.2
2896.5																	√	√	√	6.4				
3034.8									√	√	√	7.9	√	√	√	19.7								
3073.5									√	√	√	5.6	√	√	√	23.3	√	√	√	23.3				

Detected protein ion signals (*m/z*)	70% MeOH			Average S/N	100% MeOH			Average S/N	MeOH-CDWS			Average S/N	70% iPrOH			Average S/N	100% iPrOH			Average S/N	iPrOH-CDWS			Average S/N
	1	2	3		1	2	3		1	2	3		1	2	3		1	2	3		1	2	3	
3100.5													√	√	√	23.8						√	√	4.4
3137.2									√	√	√	5.6					√	√	√	25.3				
3148.8										√	√	5.2					√		√	16.5		√	√	4.1
3161.3									√	√		5.0									√		√	3.1
3165.8																	√		√	12.8	√	√		3.1
3214.4									√	√	√	3.3					√		√	11.0	√	√	√	6.1
3256.2									√	√	√	3.3												
3297.1													√	√	√	4.3					√	√	√	3.3
3348.4									√	√	√	13.8	√	√	√	4.3	√	√	√	4.3	√	√		3.2
3389.8									√	√	√	16.7	√	√	√	3.8						√	√	3.6
3409.5									√		√										√	√	√	3.3
3428.1																					√	√	√	3.4
3446.6																	√	√	√	4.2		√	√	4.3
3456.4									√	√	√	8.6									√	√	√	38.8
3475.4									√	√	√	8.0									√	√	√	4.7
3483.8																	√	√	√	4.4	√		√	4.6
3494.1									√	√	√	5.0									√	√	√	9.3
3537.1									√	√	√	4.5					√	√	√	4.5	√	√	√	4.3
3566.7									√	√	√	3.5					√	√	√	3.2	√	√		3.0
3609.9																	√			3.0				
3789.5																					√	√	√	12.4
3814.4																	√	√	√		√	√	√	5.4
3829.4													√	√	√	5.7	√	√		5.3		√	√	4.3
3883.6									√	√	√	4.2												
4226.4									√	√	√	3.9	√	√	√	4.3	√	√	√	3.2				
4241.4													√	√	√	5.3	√			3.8	√	√	√	3.2
4285.7															√	3.8					√	√	√	4.9
4489.1													√	√		5.4					√	√	√	4.5
4529.2													√	√	√	5.6	√	√	√	4.6	√		√	3.1

Detected protein ion signals (*m/z*)	70% MeOH			Average S/N	100% MeOH			Average S/N	MeOH-CDWS			Average S/N	70% iPrOH			Average S/N	100% iPrOH			Average S/N	iPrOH-CDWS			Average S/N
	1	2	3		1	2	3		1	2	3		1	2	3		1	2	3		1	2	3	
4618.7									√	√	√	25.0	√	√	√	5.2	√	√	√	4.3	√	√	√	9.3
4746.5																	√	√	√	6.8	√	√	√	8.8
4785.5																					√	√	√	3.3
4796.5													√	√	√	4.8					√	√	√	3.2
4948.0																	√	√	√	4.6				
4956.2									√	√	√	3.1					√	√	√	3.8		√	√	3.2
4961.9																					√	√	√	10.8
4967.4																	√	√	√	3.5				
4970.1																	√	√	√	4.4				
4989.9																	√	√	√	4.0				
5000.9																					√	√	√	4.3
5436.3													√		√	4.4		√	√	3.8				
5444.3	√	√	√	27.7	√	√	√	25.7	√	√	√	3.4	√	√	√	14.0	√	√		3.3	√	√		4.7
5460.5													√	√	√	13.8								
5475.8													√	√	√	14.5								
5483.2													√	√	√	4.5								
5486.5													√	√	√	4.6								
5644.1					√	√	√	4.3																
5648.3	√	√	√	3.6																				
5697.6	√																√	√	√	3.4				
5706.2	√	√	√	4.7	√	√	√	6.7																
5751.9					√	√	√	4.8																
6021.3									√	√	√	3.3									√	√		3.3
6063.6	√	√	√	6.3	√	√	√	6.3																
6068.6	√	√	√	6.7														√		3.2	√	√	√	5.8
6169.6									√	√	√							√	√	3.9	√	√	√	4.1
6210.5																					√	√	√	4.7
6223.4									√	√	√	9.8									√	√	√	10.6
6237.9									√	√	√	5.3									√	√	√	3.4

Detected protein ion signals (*m/z*)	70% MeOH			Average S/N	100% MeOH			Average S/N	MeOH-CDWS			Average S/N	70% iPrOH			Average S/N	100% iPrOH			Average S/N	iPrOH-CDWS			Average S/N
	1	2	3		1	2	3		1	2	3		1	2	3		1	2	3		1	2	3	
6243.6	√	√	√	5.7	√	√	√	5.0	√	√	√	4.1									√	√	√	3.3
6251.5									√	√	√	3.9									√	√		3.6
6259.6					√	√	√	3.9	√	√	√	4.0									√	√	√	3.5
6266.1																					√	√		3.1
6274.4									√	√	√	8.9									√	√	√	5.9
6282.2																					√	√	√	3.6
6288.1																					√	√	√	3.7
6314.9									√	√	√	3.4									√	√		3.8
6534.1	√	√	√	5.9																				
6561.5					√	√	√	17.9	√	√	√	3.3									√	√	√	3.6
6565.1													√	√		3.0								
6568.8	√	√	√	17.9									√	√		3.7								
6572.2									√	√	√	16.1			√	3.3					√	√	√	7.0
6584.1																								
6589.5																								
6611.0																					√		√	3.1
6646.5													√	√	√	22.6					√	√	√	14.8
6658.3									√	√	√	4.2									√	√		4.0
6683.8													√	√	√	4.4					√	√	√	4.1
6689.6	√	√	√	17.1	√	√	√	17.1	√	√		3.0									√	√	√	3.3
6809.5					√	√	√	4.0																
6812.1					√	√	√	3.6																
6817.5	√	√	√	8.8																				
6824.9									√	√	√	4.5										√	√	3.4
7003.2						√	√	4.6													√	√	√	3.6
7132.1	√	√	√	7.2	√	√	√	7.2																
7138.3																								
7143.2									√	√	√	3.4										√	√	3.7
7190.2									√	√	√	12.7									√	√		3.2

Detected protein ion signals (*m/z*)	70% MeOH			Average S/N	100% MeOH			Average S/N	MeOH-CDWS			Average S/N	70% iPrOH			Average S/N	100% iPrOH			Average S/N	iPrOH-CDWS			Average S/N
	1	2	3		1	2	3		1	2	3		1	2	3		1	2	3		1	2	3	
7477.6														√	√	3.2								
7480.9															√	3.0								
7482.7					√	√	√	28.2													√	√		3.3
7487.3	√	√	√	28.2									√	√	√	3.7					√	√		3.2
7490.5									√	√	√	8.5	√	√	√	6.8								
7494.8														√	√	3.4					√	√	√	3.1
7505.0									√	√	√	16.6	√	√	√	3.2								
7512.6	√	√	√	6.2																	√	√	√	4.5
7515.1													√	√	√	8.5						√	√	3.8
7520.5									√	√	√	5.0		√	√	6.3								
7526.4									√	√		5.7												
7535.2									√	√		3.1												
7540.4										√	√	3.9												
7594.1	√	√	√	3.6					√	√	√	3.2												
7607.0	√	√	√	3.4					√	√		3.4												
7644.1	√	√	√	3.4																				
7649.9									√	√	√	6.1												
7799.8	√	√	√	21.6																				
7854.3					√	√	√	3.7	√	√	√	5.2												
7862.1	√	√	√	7.7																				
7873.2	√	√	√	7.6	√	√	√	4.0	√	√	√	3.4												
7880.8	√	√	√	4.8	√	√	√	4.1	√	√	√	3.0												
7903.7	√	√	√	8.5																				
7933.6									√	√	√	3.6												
8088.9					√	√	√	4.6																
8367.7	√	√	√	6.9	√	√	√	6.9																
8389.3																					√	√	√	3.4
8408.6									√	√	√	3.3									√		√	3.6
8414.8									√	√	√	3.1												

Detected protein ion signals (*m/z*)	70% MeOH			Average S/N	100% MeOH			Average S/N	MeOH-CDWS			Average S/N	70% iPrOH			Average S/N	100% iPrOH			Average S/N	iPrOH-CDWS			Average S/N
	1	2	3		1	2	3		1	2	3		1	2	3		1	2	3		1	2	3	
8449.5									√	√	√	68.1									√	√	√	31.3
8455.7					√	√	√	3.1																
8462.1	√	√	√	4.3																				
8472.5									√	√	√	11.6												
8488.9									√	√	√	13.0												
8510.6									√	√	√	8.0												
8527.7									√	√	√	6.1												
8532.2										√	√	5.8												
8546.5									√	√	√	4.7												
8550.2										√	√	4.9												
8590.4									√	√		3.3												
8599.3									√	√	√	3.0									√	√		4.6
8690.8	√	√	√	5.9	√	√	√	5.9																
8706.1																					√	√	√	5.1
8712.1	√	√	√	5.1																				
8741.6																					√	√	√	3.1
8754.3																					√		√	3.1
8844.3																					√	√	√	3.1
8920.8	√	√	√	5.2	√	√	√	5.1																
8941.5													√	√	√	5.1					√	√	√	3.4
8954.2									√	√	√	14.2												
8981.6									√	√	√	3.5												
9287.8			√	3.1																	√	√	√	3.5
9595.4																								
9603.1	√	√	√	19.5	√	√	√	13.9	√	√	√	5.8												
9893.2	√	√	√		√	√	√	14.2	√	√	√	6.0									√	√	√	5.1
9905.6									√	√	√	10.9									√	√	√	8.3
9909.0	√	√	√	9.4					√	√	√	23.1									√	√	√	38.8
9931.1									√	√	√	13.2									√		√	3.3

Detected protein ion signals (*m/z*)	70% MeOH			Average S/N	100% MeOH			Average S/N	MeOH-CDWS			Average S/N	70% iPrOH			Average S/N	100% iPrOH			Average S/N	iPrOH-CDWS			Average S/N
	1	2	3		1	2	3		1	2	3		1	2	3		1	2	3		1	2	3	
9945.3									√	√	√	16.1									√	√	√	6.8
9952.8	√	√	√	8.8																				
9969.9																					√		√	4.7
9976.7																					√	√	√	4.3
10012.7									√		√	6.4												
10016.1																						√	√	3.4
10027.5																					√	√	√	3.6
10034.7									√	√	√	4.3												
10057.3																								
10078.1									√			3.3												
10086.5									√		√	3.4												
10114.9									√	√	√	3.9												
10688.7																								
10696.7	√	√	√	3.8																				
10845.1					√	√	√	4.2																
10943.9									√	√	√	5.4												
11299.6					√	√	√	4.6																
11306.8									√	√	√	6.2												
11318.5									√	√	√	5.3												
11343.9									√	√	√	5.7												
11359.7																								
11365.7									√	√	√	4.6												
11382.9									√	√	√	3.7												
11396.5										√	√	3.1												
11648.7					√	√	√	3.8																
11683.2									√	√	√	4.1												
11866.9	√	√	√	4.2																				
12095.0			√	3.2	√	√	√	4.8													√	√		3.1
12103.2	√	√	√	3.6																	√	√		3.6

Detected protein ion signals (*m/z*)	70% MeOH			Average S/N	100% MeOH			Average S/N	MeOH-CDWS			Average S/N	70% iPrOH			Average S/N	100% iPrOH			Average S/N	iPrOH-CDWS			Average S/N
	1	2	3		1	2	3		1	2	3		1	2	3		1	2	3		1	2	3	
12131.1									√	√	√	3.4										√	√	3.3
12167.9																						√	√	4.3
12315.3	√	√	√	4.4																				
12336.0					√	√	√	16.0																
12343.5	√	√	√	4.2																				
12384.7	√	√	√	5.0																				
12397.1	√	√	√	5.2	√	√	√	5.5																
12905.5	√			3.4	√	√	√	5.1																
12910.5	√	√	√	3.8																				
13120.3					√	√	√	5.5																
13129.0	√	√	√	9.9																				
13774.6									√	√	√	7.1												
13795.1										√	√	4.4												
13810.3										√	√	4.3												
13815.5									√	√		4.6												
13835.9									√	√	√	3.3												
13897.1									√	√	√	3.1												
13951.9										√	√	3.9												
13979.3									√	√	√	4.3												
14006.4									√	√	√	9.8												
14021.5									√	√	√	6.7												
14030.1										√	√	7.2												
14046.4									√	√	√	6.3												
14067.0									√		√	6.1												
14086.6									√	√	√	7.5												
14106.5									√	√	√	4.7												
14117.3									√	√	√	5.4												
14140.8									√	√	√	4.5												
14161.0										√	√	6.3												

Detected protein ion signals (*m/z*)	70% MeOH			Average S/N	100% MeOH			Average S/N	MeOH-CDWS			Average S/N	70% iPrOH			Average S/N	100% iPrOH			Average S/N	iPrOH-CDWS			Average S/N
	1	2	3		1	2	3		1	2	3		1	2	3		1	2	3		1	2	3	
14164.2					√	√	√	3.4	√	√	√	6.6												
14181.0	√	√	√	3.6																				
14188.8	√	√	√	5.2																				
14203.3			√	3.6	√	√	√	7.1	√	√	√	5.1												
14244.4					√	√	√	16.3	√	√	√	4.4												
14282.5	√	√	√	25.0	√	√	√	21.3	√	√	√	57.6												
14302.3									√	√	√	8.7												
14324.6	√	√	√	3.6					√	√	√	48.6												
14344.7									√	√	√	38.3												
14361.4									√	√	√	5.9												
14370.7										√	√	5.7												
14381.9					√	√	√	3.8	√	√	√	28.5												
14389.7										√		5.1												
14403.6									√	√		4.4												
14413.0									√	√	√	3.8												
14425.2									√	√	√	3.5												
14441.1					√	√	√	3.7	√	√	√	3.6												
14453.9	√	√	√	3.6					√	√	√	3.4												
14488.6					√	√	√	5.7	√	√	√	3.4												
14836.1	√	√	√	3.4																				
14882.2										√	√	4.3		√	√	5.0								
14945.1					√	√	√	9.2																
14950.3	√	√	√	7.4									√	√	√	6.3								
14978.2									√	√	√	31.8												
15002.9																						√	√	8.3
15007.3									√	√	√	36.2												
15036.6																						√	√	6.8
15043.7									√	√	√	39.1									√		√	6.8
15066.7					√	√	√	11.8	√	√	√	28.6												

Detected protein ion signals (*m/z*)	70% MeOH			Average S/N	100% MeOH			Average S/N	MeOH-CDWS			Average S/N	70% iPrOH			Average S/N	100% iPrOH			Average S/N	iPrOH-CDWS			Average S/N
	1	2	3		1	2	3		1	2	3		1	2	3		1	2	3		1	2	3	
15084.4	√	√	√	10.6																				
15095.7	√	√	√	10.0		√	√	9.4																
15098.7					√	√		9.3																
15105.0	√	√	√	11.1	√	√	√	11.0		√	√	9.4	√	√	√	5.6								
15113.1	√	√	√	10.1	√	√	√	11.5																
15118.3	√	√	√	12.4																				
15123.8					√	√	√	11.3		√	√	17.0												
15130.7	√	√	√	12.4																				
15135.6	√	√	√	10.6																				
15142.2									√	√	√	15.7	√		√	5.4					√	√		4.7
15152.1	√	√	√	10.4					√	√	√	15.2	√	√	√	5.5								
15155.2													√	√	√	5.3								
15167.0	√	√	√	19.2						√	√	15.4	√			5.0								
15174.1										√	√	9.4												
15183.4									√	√	√	17.4												
15207.6										√		9.6												
15212.1	√			5.6	√	√	√	7.5	√	√	√	17.7												
15229.5	√	√	√	7.9	√	√	√	7.4		√	√	13.4												
15237.2	√	√	√	12.5									√	√	√	4.1								
15250.7	√	√	√	10.2	√	√	√	8.8		√	√	10.6	√	√	√	4.9								
15260.3	√	√	√	10.0																				
15267.9	√	√	√	11.5	√	√	√	11.1	√	√	√	9.3	√		√	4.3								
15271.3	√	√	√	10.6									√	√	√	4.2								
15279.2										√	√	9.1												
15285.5	√	√	√	10.9					√	√	√	8.6												
15292.8	√	√	√	9.4						√	√	8.9	√	√	√	3.9								
15305.4	√	√	√	9.3																				
15311.3	√	√	√	9.2	√	√	√	9.2	√	√	√	8.9												
15320.7					√	√	√	9.5	√	√	√	9.5												

Detected protein ion signals (*m/z*)	70% MeOH			Average S/N	100% MeOH			Average S/N	MeOH-CDWS			Average S/N	70% iPrOH			Average S/N	100% iPrOH			Average S/N	iPrOH-CDWS			Average S/N
	1	2	3		1	2	3		1	2	3		1	2	3		1	2	3		1	2	3	
15329.2					√	√	√	9.5	√	√	√	9.2												
15341.7	√	√	√	9.1	√	√	√	9.1													√	√		3.4
15345.3	√	√		10.2	√	√	√	10.2	√	√	√	9.7												
15354.2	√	√	√	10.7					√	√	√	9.2												
15361.4		√	√	10.1	√	√	√	10.1		√	√	8.6												
15367.2	√	√		10.9	√	√	√	10.9																
15377.5	√	√	√	10.5					√		√	8.5												
15389.4	√	√		10.6					√	√	√	7.9												
15434.1	√	√	√	13.2	√	√	√	13.2																
15452.7	√	√	√	11.8					√		√	7.6												
15456.6		√	√	19.3	√	√	√	7.6				7.7												
15470.7					√	√	√	7.3	√	√	√	5.1												
15510.2									√		√	4.2												
15524.1									√	√	√	3.6												
15562.4										√	√	3.2												
15568.6	√	√		20.2																				
15574.5	√	√	√	18.6																				
15589.4									√	√	√	4.8												
15627.2									√	√	√	5.0												
15634.4									√	√	√	4.6												
15657.9										√	√	6.3												
15705.6					√	√	√	22.6	√	√	√	30.8										√	√	3.6
15743.8					√	√	√	26.8	√	√	√	20.1												
15777.4	√	√	√	30.5						√	√	11.0												
15782.6									√	√	√	10.9										√	√	3.6
15792.9										√	√	6.9										√	√	3.4
15804.5										√	√	8.8	√			3.6	√		√	3	√	√		3.8
15827.2									√		√	7.3	√	√	√	3.4						√	√	4.3
15835.4													√	√		3.5					√	√		3.6

Detected protein ion signals (*m/z*)	70% MeOH			Average S/N	100% MeOH			Average S/N	MeOH-CDWS			Average S/N	70% iPrOH			Average S/N	100% iPrOH			Average S/N	iPrOH-CDWS			Average S/N
	1	2	3		1	2	3		1	2	3		1	2	3		1	2	3		1	2	3	
15846.6	√	√	√	36.1	√	√	√	32.1	√	√	√	28.9	√	√	√	3.3					√	√		3.6
15867.5	√	√	√	12.6	√	√	√	12.3	√	√		9.8									√	√	√	4.6
15877.2	√	√		12.6			√	12.6	√	√	√	9.5									√	√		3.0
15889.9	√	√	√	13.1																	√		√	3.6
15911.6									√	√	√	7.1									√	√		3.4
15925.1									√		√	6.2									√		√	3.3
15946.4									√	√	√	5.1									√	√		4.2
15955.5											√	4.7									√		√	4.6
15965.2	√		√	12.7					√	√	√	4.4									√		√	3.8
15990.2									√		√	3.6									√	√		3.6
16010.2					√	√	√	10.8		√	√	3.7									√		√	3.8
18618.7										√	√	3.1									√	√		4.0
18645.4									√	√	√	4.1												
18654.1									√	√	√	4.4												
18685.4									√	√	√	8.1												
18694.0										√	√	7.7												
18723.1									√	√	√	4.9												
18730.0									√	√	√	4.2												
18768.5									√	√	√	3.6												
18805.6									√		√	3.1												
20700.1					√	√	√	3.2	√	√	√	3.2												
20716.7									√	√	√	3.5												
20743.7									√	√		3.1												
20752.4										√	√	3.2												
20765.7									√	√	√	3.6												
20774.8										√	√	11.7												
20789.4									√		√	11.0												
21162.9									√		√	4.5												
21171.3									√	√	√	4.9												

Detected protein ion signals (*m/z*)	70% MeOH			Average S/N	100% MeOH			Average S/N	MeOH-CDWS			Average S/N	70% iPrOH			Average S/N	100% iPrOH			Average S/N	iPrOH-CDWS			Average S/N
	1	2	3		1	2	3		1	2	3		1	2	3		1	2	3		1	2	3	
21190.2									√	√	√	4.4												
21215.4									√	√	√	3.8												
21760.3									√	√	√	3.4												
21780.8									√	√	√	3.8												
21800.7									√	√	√	7.0												
21874.1									√	√	√	21.6												
21905.0									√	√	√	14.6												
21936.7										√	√	10.8												
21949.9									√	√	√	10.7												
21962.7									√	√	√	11.1												
21971.2									√	√	√	10.9												
22027.6									√	√	√	6.5												
22050.3									√	√	√	4.9												
22073.8										√	√	4.0												
22085.9									√	√	√	3.9												
22114.4										√		5.0												
29863.1									√	√	√	4.2												
29882.9									√	√	√	5.4												
29920.1									√	√		8.1												
29927.6									√	√	√	3.9												
29937.5									√	√	√	7.6												
29954.9										√	√	7.0												
29976.8					√	√	√	4.9	√	√		5.4												
30020.0									√	√	√	4.5												
30028.9									√	√	√	4.1												
30046.4									√	√		3.6												
30064.2									√	√	√	4.0												
30076.7									√	√	√	4.3												
30086.6	√	√	√	3.9					√	√	√	4.8												

Detected protein ion signals (*m/z*)	70% MeOH			Average S/N	100% MeOH			Average S/N	MeOH-CDWS			Average S/N	70% iPrOH			Average S/N	100% iPrOH			Average S/N	iPrOH-CDWS			Average S/N
	1	2	3		1	2	3		1	2	3		1	2	3		1	2	3		1	2	3	
30096.8									√	√	√													
30102.0									√	√		3.8												
30119.0									√		√	4.2												
30124.0					√	√	√	3.0	√	√	√	3.9												
30138.9									√	√	√	4.3												
30151.4	√	√		3.2					√	√		4.5												
30161.3									√	√	√	3.5												
30171.3									√	√		3.0												
30178.2		√		3.4																				
30188.1									√		√	3.2												
30257.7	√	√		3.1																				
30335.6									√			4.9												
30345.6						√	√	3.1	√			4.1												
30363.4									√			4.3												
30394.8	√	√		5.9					√			4.4												
30464.6					√	√	√	9.2	√			4.2												
30474.9	√	√	√	10.0		√	√	10.0	√			3.8												
30502.0									√			3.1												
30524.1									√			3.0												
30581.4									√	√	√	3.6												
30603.9					√	√	√	6.8	√	√	√	3.4												
30620.7									√	√	√	4.6												
30636.5									√	√	√	5.3												
30650.1									√	√	√	5.2												
30658.7									√	√	√	4.8												
30670.2									√	√	√	4.8												
30691.8	√	√	√	7.5						√	√	4.9												
30710.4	√	√	√	7.2					√	√		4.1												
30742.1									√		√	3.5												

Detected protein ion signals (*m/z*)	70% MeOH			Average S/N	100% MeOH			Average S/N	MeOH-CDWS			Average S/N	70% iPrOH			Average S/N	100% iPrOH			Average S/N	iPrOH-CDWS			Average S/N
	1	2	3		1	2	3		1	2	3		1	2	3		1	2	3		1	2	3	
30754.7									√	√	√	3.1												
30773.6									√			3.2												
30788.1					√	√	√	4.1	√			3.1												
30802.5										√	√	3.3												
30811.4									√	√	√	3.4												
30830.9									√			3.6												
30850.3	√	√	√	3.9					√	√		3.5												
30860.3									√		√	3.7												
30876.7									√			3.4												
30898.4					√	√	√	3.3	√			3.0												
30920.0	√	√	√	4.0					√			3.1												
Number of detected proteins	93	92	87		70	74	74		231	236	231		87	87	86		67	74	66		139	145	141	
Average of detected proteins	91				73				233				86				69				142			

Note: "√" means the protein ion could be detected in three technical duplicates.

Table S5. The protein ion signals detected by MALDI-MSI in the positive-ion mode in the rat liver tissue sections washed by nine different washing methods, respectively, for the orthogonal array testing.

Detected protein ion signals (*m/z*)	Nine different washing combinations																								
	$a_1b_1c_1$			$a_1b_2c_2$			$a_1b_3c_3$			$a_2b_1c_2$			$a_2b_2c_3$			$a_2b_3c_1$			$a_3b_1c_3$			$a_3b_2c_1$			$a_3b_3c_2$		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
2007.6	√	√	√																								
2012.8				√	√	√	√	√	√																		
2017.3	√	√	√	√	√	√																					
2024.7				√	√	√																					
2029.6				√	√																						
2034.8				√	√	√																					
2041.2				√	√	√																					
2045.7				√	√	√																					
2050.1				√	√																						
2060.7				√	√	√																					
2090.3				√	√	√																					
2126.8				√	√					√	√	√															
2141.6	√	√	√																								
2177.9										√		√															
2184.4										√		√							√	√	√						
2193.9	√	√	√																								
2320.7							√	√	√																		
2324.1				√	√	√																					
2363.3							√	√	√							√	√	√									
2349.5				√	√	√					√	√															
2354.0				√	√	√																					
2365.3	√	√	√																								
2482.8				√	√	√	√	√	√							√	√	√									
2487.1				√	√	√	√	√	√							√	√	√									

Detected protein ion signals (*m/z*)	Nine different washing combinations																								
	$a_1b_1c_1$			$a_1b_2c_2$			$a_1b_3c_3$			$a_2b_1c_2$			$a_2b_2c_3$			$a_2b_3c_1$			$a_3b_1c_3$			$a_3b_2c_1$			$a_3b_3c_2$		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
2493.9																						√	√	√			
2499.0				√		√																			√	√	√
2503.3	√	√	√																								
2509.7	√	√	√	√	√	√																					
2513.7	√	√	√																								
2531.3				√	√	√																					
2542.2							√	√	√							√	√	√									
2548.2				√		√				√	√	√															
2563.2	√	√	√	√		√	√	√	√				√	√	√	√	√	√				√	√	√			
2570.4				√	√	√				√		√							√	√	√				√	√	√
2576.1							√	√	√		√	√															
2585.9	√	√	√	√	√	√	√	√	√	√	√																
2594.5				√	√	√																					
2599.5	√	√	√										√	√	√	√	√	√									
2602.3				√	√	√																			√	√	√
2609.6				√	√	√				√	√	√															
2627.0	√	√	√																								
2788.3							√	√	√				√	√	√	√	√	√									
2794.8				√	√	√				√	√	√													√	√	√
2813.7	√	√	√										√	√	√												
2818.3				√	√	√																					
2824.6				√	√	√																					
2838.2				√	√	√																					
2843.3				√	√	√																					
2849.9				√	√	√																					
3003.1				√	√	√																					

Detected protein ion signals (*m/z*)	Nine different washing combinations																								
	$a_1b_1c_1$			$a_1b_2c_2$			$a_1b_3c_3$			$a_2b_1c_2$			$a_2b_2c_3$			$a_2b_3c_1$			$a_3b_1c_3$			$a_3b_2c_1$			$a_3b_3c_2$		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
3025.5																√	√	√									
3034.8										√	√	√				√	√	√	√	√	√						
3046.1				√	√	√																					
3058.3	√	√	√																								
3073.5																√	√	√									
3210.6							√	√	√				√	√	√	√	√	√									
3214.4				√	√	√				√	√	√													√	√	√
3221.3													√	√	√	√	√	√									
3226.9				√	√	√																			√	√	√
3236.7	√	√	√	√	√	√																					
3240.6				√	√	√							√	√	√	√	√	√									
3246.1				√	√	√																			√	√	√
3252.3							√	√	√				√	√	√	√	√	√									
3256.2										√	√	√										√	√	√			
3261.6				√	√	√																			√	√	√
3273.6				√	√	√										√	√	√									
3290.2				√	√	√	√	√	√							√	√	√									
3297.1				√	√	√																					
3333.2																√	√	√									
3341.4							√	√	√				√	√	√	√	√	√				√	√	√	√	√	√
3348.4				√	√	√				√	√	√													√	√	√
3353.7																			√	√	√						
3363.5	√	√	√	√	√	√																					
3383.7													√	√	√	√	√	√				√	√	√			
3389.8	√	√	√	√	√	√				√	√	√													√	√	√
3396.6																			√	√	√						

Detected protein ion signals (*m/z*)	Nine different washing combinations																										
	$a_1b_1c_1$			$a_1b_2c_2$			$a_1b_3c_3$			$a_2b_1c_2$			$a_2b_2c_3$			$a_2b_3c_1$			$a_3b_1c_3$			$a_3b_2c_1$			$a_3b_3c_2$		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
3409.5	√	√	√	√	√	√							√	√	√	√	√	√									
3420.6																√	√	√									
3428.1	√	√	√	√	√	√										√	√	√							√	√	√
3434.1	√	√	√	√	√	√																					
3446.6	√	√	√	√	√	√	√	√	√										√	√	√						
3458.9	√	√	√	√	√	√	√	√	√				√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
3479.2	√	√	√	√	√	√	√	√	√										√	√	√						
3483.8	√	√	√							√	√	√	√	√	√	√	√	√	√	√	√	√	√	√			
3496.2				√	√	√				√	√	√															
3502.7	√	√	√																√	√	√						
3519.8	√	√	√	√	√	√	√	√	√				√	√	√				√	√	√						
3530.2							√	√	√							√	√	√	√	√	√						
3537.1	√	√	√	√	√	√													√	√	√				√	√	√
3543.4	√	√	√																								
3558.9	√	√	√																								
3566.7													√	√	√	√	√	√				√	√	√			
3576.4				√	√	√				√	√	√							√	√	√						
3588.1	√	√	√																								
3602.4													√	√	√	√	√	√				√	√	√			
3609.9				√	√	√																					
3617.9	√	√	√																								
3630.0	√	√	√																								
3668.2																			√	√	√						
3718.4													√	√	√												
3762.5													√	√	√												
3789.5	√	√	√																								

Detected protein ion signals (*m/z*)	Nine different washing combinations																										
	$a_1b_1c_1$			$a_1b_2c_2$			$a_1b_3c_3$			$a_2b_1c_2$			$a_2b_2c_3$			$a_2b_3c_1$			$a_3b_1c_3$			$a_3b_2c_1$			$a_3b_3c_2$		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
3875.8							√	√	√				√	√	√	√	√	√									
3883.6				√	√	√				√	√	√										√	√	√	√	√	√
3906.7	√	√	√																								
3914.6																√	√	√									
4133.9													√	√	√							√	√	√			
4218.5																√	√	√									
4226.4										√	√	√															
4252.0	√	√	√																								
4529.2													√	√	√	√	√	√				√	√	√			
4567.1																						√	√	√			
4571.8																						√	√	√			
4618.7										√	√	√															
4785.5							√	√	√													√	√	√			
4956.2																						√	√	√			
4961.9																			√	√	√						
4986.7	√	√	√																								
5012.7																√	√	√				√	√	√			
5020.9													√	√	√							√	√	√			
5038.3																						√	√	√			
5053.1				√	√	√	√	√	√				√	√	√	√	√	√				√	√	√	√	√	√
5071.9																						√	√	√	√	√	√
5080.7																						√	√	√	√	√	√
5095.2																						√	√	√			
5125.1																√	√	√				√	√	√			
5130.9				√	√	√										√	√	√									
5165.5	√	√	√													√	√	√									

Detected protein ion signals (*m/z*)	Nine different washing combinations																										
	$a_1b_1c_1$			$a_1b_2c_2$			$a_1b_3c_3$			$a_2b_1c_2$			$a_2b_2c_3$			$a_2b_3c_1$			$a_3b_1c_3$			$a_3b_2c_1$			$a_3b_3c_2$		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
5173.2																						√	√	√			
5203.7	√	√	√																								
5263.5																						√	√	√			
5326.1													√	√	√							√	√	√			
5361.3							√	√	√				√	√	√							√	√	√			
5434.5													√	√	√												
5438.9																						√	√	√			
5443.6										√	√	√													√	√	√
5450.6																			√	√	√						
5476.3	√	√	√																								
5640.2													√	√	√							√	√	√			
5649.8																									√	√	√
5695.9													√	√	√												
5713.2																			√	√	√						
5741.5	√	√	√																								
5817.1																						√	√	√			
5826.5																									√	√	√
6210.5													√	√	√												
6223.4													√	√	√												
6255.7				√	√	√				√	√	√															
6259.6																			√	√	√						
6266.1										√	√	√							√	√	√						
6274.4													√	√	√												
6299.5				√	√	√				√	√	√															
6314.9																			√	√	√						
6350.0							√	√	√																		

Detected protein ion signals (*m/z*)	Nine different washing combinations																								
	$a_1b_1c_1$			$a_1b_2c_2$			$a_1b_3c_3$			$a_2b_1c_2$			$a_2b_2c_3$			$a_2b_3c_1$			$a_3b_1c_3$			$a_3b_2c_1$			$a_3b_3c_2$		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
6573.8	√	√	√				√	√	√	√	√	√															
6590.4																			√	√	√						
6531.2													√	√	√												
6561.5													√	√	√												
6572.2										√	√	√															
6648.4	√	√	√	√	√	√				√	√	√							√	√	√						
6658.3	√	√	√																√	√	√						
6689.6	√	√	√				√	√	√																√	√	√
6811.8	√	√	√				√	√	√				√	√	√												
6822.8							√	√	√																		
6827.2							√	√		√	√	√				√	√	√				√	√	√			
6835.5							√	√	√																		
6969.1							√		√							√	√	√									
6979.1							√	√	√																		
6985.4							√	√	√																√	√	√
7023.1							√	√	√																		
7096.6							√	√	√							√	√	√									
7109.7							√		√							√	√	√				√	√	√			
7116.8							√	√								√	√	√				√	√	√	√	√	√
7131.5							√	√					√	√	√	√	√	√									
7137.2							√	√	√													√	√	√			
7143.2							√	√	√	√	√	√				√	√	√									
7149.9							√	√	√							√	√	√	√	√	√				√	√	√
7156.8							√	√	√																		
7175.3							√	√	√																		
7179.1							√	√	√							√	√	√				√	√	√			

Detected protein ion signals (*m/z*)	Nine different washing combinations																								
	$a_1b_1c_1$			$a_1b_2c_2$			$a_1b_3c_3$			$a_2b_1c_2$			$a_2b_2c_3$			$a_2b_3c_1$			$a_3b_1c_3$			$a_3b_2c_1$			$a_3b_3c_2$		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
7186.1	√	√	√																						√	√	√
7190.2										√	√	√				√	√	√							√	√	√
7197.4																			√	√	√				√	√	√
7211.2							√	√	√							√	√	√									
7223.9				√	√	√																√	√	√			
7232.7							√	√	√																		
7251.9	√	√	√																								
7271.6							√	√	√							√	√	√									
7322.6																√	√	√									
7328.8							√	√	√																		
7411.1							√	√	√							√	√	√									
7423.9							√	√	√																		
7440.2													√	√	√												
7490.5							√	√	√	√	√	√	√	√	√	√	√	√									
7498.2																√	√	√	√	√	√	√	√	√			
7505.0				√	√	√																					
7512.6				√	√	√							√	√	√				√	√	√	√	√	√	√	√	√
7520.5				√	√	√													√	√	√						
7526.4	√	√	√																								
7535.2										√	√	√															
7540.4	√	√	√				√	√	√							√	√	√									
7554.1	√	√	√																						√	√	√
7568.3	√	√	√																						√	√	√
7607.1										√	√	√															
7638.3	√	√	√																								
7650.1	√	√	√							√	√	√															

Detected protein ion signals (*m/z*)	Nine different washing combinations																								
	$a_1b_1c_1$			$a_1b_2c_2$			$a_1b_3c_3$			$a_2b_1c_2$			$a_2b_2c_3$			$a_2b_3c_1$			$a_3b_1c_3$			$a_3b_2c_1$			$a_3b_3c_2$		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
7661.1							√	√	√							√	√	√									
7765.5													√	√	√												
7796.1				√	√	√							√	√	√							√	√	√			
7840.4										√	√	√	√	√	√										√	√	√
7854.3																			√	√	√	√	√	√			
7862.1													√	√	√							√	√	√			
7873.2				√	√	√	√	√	√	√	√	√	√	√	√												
7880.8										√	√																
7888.9											√		√	√	√												
7894.7																									√	√	√
7900.3																									√	√	√
7920.0													√	√	√										√	√	√
7927.6	√	√	√																								
7933.6	√	√	√																√	√	√						
7973.1													√	√	√							√	√	√			
8169.8													√	√	√												
8408.6										√	√	√															
8435.8													√	√	√	√	√	√									
8450.5													√	√	√												
8459.0																			√	√	√						
8463.5										√	√	√															
8488.9										√	√	√															
8500.3	√	√	√																								
8692.3													√	√	√												
8706.1																						√	√	√			
8920.4													√	√	√												

Detected protein ion signals (*m/z*)	Nine different washing combinations																								
	$a_1b_1c_1$			$a_1b_2c_2$			$a_1b_3c_3$			$a_2b_1c_2$			$a_2b_2c_3$			$a_2b_3c_1$			$a_3b_1c_3$			$a_3b_2c_1$			$a_3b_3c_2$		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
8928.6													√	√	√	√	√	√				√	√	√			
8935.0																						√	√	√			
8944.1				√	√	√				√	√	√															
8954.2																			√	√	√				√	√	√
8981.6										√	√	√															
8996.9	√	√	√																								
9036.1	√	√	√																								
9600.4													√	√	√												
9719.3							√	√	√							√	√	√									
9727.0																						√	√	√			
9853.4																√	√	√									
9866.3																√	√	√									
9876.1																√	√	√	√	√	√						
9880.2										√	√	√															
9905.6	√	√	√				√	√	√				√	√	√	√	√	√									
9910.7	√	√	√							√	√	√							√	√	√	√	√	√			
9918.3	√	√	√													√	√	√	√	√	√						
9925.9	√	√	√				√	√	√	√	√	√	√	√	√	√	√	√									
9931.1							√	√	√							√	√	√							√	√	√
9940.9							√		√										√	√	√						
9945.3							√	√	√							√	√	√	√	√	√						
9959.9							√	√	√	√	√	√	√	√	√	√	√	√	√	√	√						
9965.0	√	√					√												√	√	√	√	√	√	√	√	√
9969.9																			√	√	√					√	
9976.7				√	√	√				√	√	√				√	√	√								√	√
9982.3																									√	√	√

Detected protein ion signals (*m/z*)	Nine different washing combinations																										
	$a_1b_1c_1$			$a_1b_2c_2$			$a_1b_3c_3$			$a_2b_1c_2$			$a_2b_2c_3$			$a_2b_3c_1$			$a_3b_1c_3$			$a_3b_2c_1$			$a_3b_3c_2$		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
9987.2	√		√																√	√	√						
10129.1																			√	√	√						
10145.4																			√	√	√						
10176.7	√	√	√																								
10241.7	√	√	√																								
10342.2																						√	√	√			
10475.8													√	√	√												
10679.2													√	√	√	√	√	√									
10689.9																						√	√	√	√	√	√
10744.7																√	√	√									
10786.3													√	√	√	√	√	√									
10839.6																						√	√	√			
10853.4																√	√	√	√	√	√						
10879.8													√	√	√												
10936.2	√	√	√	√	√	√																√	√	√			
10943.9										√	√	√															
10985.9													√	√	√												
10999.6																						√	√	√			
11175.6													√	√	√	√	√	√									
11183.2																						√	√	√			
11199.1																									√	√	√
11207.3													√	√	√												
11213.4													√	√	√												
11239.1													√	√	√												
11249.4																						√	√	√			
11261.0													√	√	√												

Detected protein ion signals (*m/z*)	Nine different washing combinations																								
	$a_1b_1c_1$			$a_1b_2c_2$			$a_1b_3c_3$			$a_2b_1c_2$			$a_2b_2c_3$			$a_2b_3c_1$			$a_3b_1c_3$			$a_3b_2c_1$			$a_3b_3c_2$		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
11272.2													√	√	√												
11283.4													√	√	√												
11293.3													√	√	√				√	√	√	√	√	√			
11301.0																			√	√	√						
11306.8										√	√	√															
11311.2													√	√	√										√	√	√
11319.8										√	√	√							√	√	√						
11325.8													√	√	√												
11333.4																			√	√	√						
11347.5																			√	√	√						
11359.8													√	√	√												
11365.7										√	√	√							√	√	√						
11384.4										√	√	√	√	√	√				√	√	√						
11401.4										√	√	√															
11473.7																			√	√	√						
11485.4										√	√	√							√	√	√						
11550.2													√	√	√												
11609.7													√	√	√	√	√	√									
11619.6													√	√	√	√	√	√				√	√	√			
11633.2													√	√	√												
11683.3										√	√	√															
11715.3							√	√																			
11744.5							√																		√	√	√
11752.6	√	√	√																								
12112.5							√	√	√				√	√	√	√	√	√									
12122.2																						√	√	√			

Detected protein ion signals (*m/z*)	Nine different washing combinations																								
	$a_1b_1c_1$			$a_1b_2c_2$			$a_1b_3c_3$			$a_2b_1c_2$			$a_2b_2c_3$			$a_2b_3c_1$			$a_3b_1c_3$			$a_3b_2c_1$			$a_3b_3c_2$		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
12131.1										√	√	√															
12202.9																									√	√	√
12351.6													√	√	√												
12362.1																						√	√	√			
12368.0				√	√	√																					
12378.2																						√	√	√			
12383.1				√	√	√																					
12412.6													√	√	√												
12427.1													√	√	√												
12441.0													√	√	√				√	√	√						
12490.9				√	√	√																√	√	√			
12497.2				√	√	√																					
13019.1																			√	√	√						
13238.9													√	√	√												
13757.9													√	√	√												
13774.7										√	√	√	√	√	√												
13783.4													√	√	√										√	√	√
13788.2																			√	√	√						
13795.1										√	√	√	√	√	√												
13801.1																			√	√	√						
13810.3										√	√	√							√	√	√						
13815.5										√	√	√															
13820.6																			√	√	√						
13830.8																			√	√	√						
13835.9										√	√	√															
13844.3										√	√	√															

Detected protein ion signals (*m/z*)	Nine different washing combinations																								
	$a_1b_1c_1$			$a_1b_2c_2$			$a_1b_3c_3$			$a_2b_1c_2$			$a_2b_2c_3$			$a_2b_3c_1$			$a_3b_1c_3$			$a_3b_2c_1$			$a_3b_3c_2$		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
13897.1										√	√																
13930.0										√	√		√	√	√												
13936.1										√	√	√															
13943.0										√		√															
13951.9										√	√																
13960.1										√	√	√															
13965.2										√	√	√															
13970.0										√	√								√	√	√						
13976.4													√	√	√				√	√	√						
13979.3										√	√	√															
13990.9																			√	√	√						
13997.6													√	√	√				√	√	√						
14006.4										√	√	√	√	√	√												
14011.0													√	√	√												
14017.4										√	√	√	√	√	√				√	√	√						
14021.5													√	√	√												
14030.1										√	√	√															
14050.4										√	√	√							√	√	√						
14058.0																			√	√	√						
14062.1													√	√	√												
14067.0										√	√	√															
14081.4																			√	√	√						
14086.6										√	√	√															
14106.5																			√	√	√						
14117.3										√	√	√							√	√	√						
14140.8							√	√	√	√	√	√	√	√	√	√	√	√	√	√	√						

Detected protein ion signals (*m/z*)	Nine different washing combinations																										
	$a_1b_1c_1$			$a_1b_2c_2$			$a_1b_3c_3$			$a_2b_1c_2$			$a_2b_2c_3$			$a_2b_3c_1$			$a_3b_1c_3$			$a_3b_2c_1$			$a_3b_3c_2$		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
14150.0										√	√	√	√	√	√												
14161.0										√	√	√															
14164.2										√	√	√	√	√	√												
14174.4																√	√	√									
14181.0													√	√	√				√	√	√				√	√	√
14186.1													√	√	√	√	√	√	√	√	√						
14196.6								√	√	√	√	√													√	√	√
14203.3																			√	√	√				√		
14222.5								√	√	√	√	√	√	√	√										√		√
14226.1								√	√							√	√	√	√	√	√						
14230.8																√	√	√	√	√	√	√	√	√	√	√	√
14236.3																						√	√	√			
14240.4																			√	√	√						
14244.4	√	√	√							√	√	√															
14259.9																			√	√	√				√	√	√
14262.3								√	√										√	√	√						
14265.1																√	√	√									
14275.6																						√	√	√			
14283.1							√	√	√	√	√	√	√	√	√	√	√	√									
14295.6							√	√	√				√	√	√	√	√	√	√	√	√				√	√	√
14306.6										√	√	√										√	√	√			
14310.2							√	√	√				√	√	√							√	√	√			
14318.1							√	√	√				√	√	√												
14324.6										√	√	√				√	√	√									
14328.2	√	√	√				√	√	√																√	√	√
14339.2				√	√	√													√	√	√	√	√	√			

Detected protein ion signals (*m/z*)	Nine different washing combinations																										
	$a_1b_1c_1$			$a_1b_2c_2$			$a_1b_3c_3$			$a_2b_1c_2$			$a_2b_2c_3$			$a_2b_3c_1$			$a_3b_1c_3$			$a_3b_2c_1$			$a_3b_3c_2$		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
14344.7																√	√	√									
14350.7							√	√	√				√	√	√	√	√	√							√	√	√
14357.0																									√	√	√
14361.4							√	√	√	√	√	√							√	√	√	√	√	√			
14367.3	√	√	√	√	√	√																					
14370.7							√	√	√	√	√	√							√	√	√				√	√	√
14381.9										√	√	√							√	√	√				√	√	√
14389.7																√	√	√									
14398.5																√	√	√									
14403.6	√	√	√							√	√	√															
14410.4	√		√													√	√	√							√	√	√
14415.8	√	√	√							√	√	√															
14420.1	√	√																							√		
14426.1										√	√	√				√	√	√				√	√	√	√		√
14433.2																									√	√	√
14441.1										√	√	√				√	√	√									
14450.5										√	√	√				√	√	√									
14455.6							√	√	√							√	√	√									
14467.7																						√	√	√	√	√	√
14488.6										√	√	√				√	√	√									
14490.5																									√	√	√
14510.5																			√	√	√				√	√	√
14569.9	√	√	√																								
14817.1				√	√	√																					
14830.5													√	√	√												
14844.2				√	√	√																√	√	√			

Detected protein ion signals (*m/z*)	Nine different washing combinations																										
	$a_1b_1c_1$			$a_1b_2c_2$			$a_1b_3c_3$			$a_2b_1c_2$			$a_2b_2c_3$			$a_2b_3c_1$			$a_3b_1c_3$			$a_3b_2c_1$			$a_3b_3c_2$		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
14856.3													√	√	√												
14864.0				√	√	√							√	√	√												
14870.4													√	√	√	√	√	√									
14878.1				√	√	√																					
14882.2										√	√	√	√	√	√	√	√	√									
14892.7													√	√	√	√	√	√									
14903.1				√	√	√							√	√	√							√	√				
14917.5				√	√	√																√		√			
14926.2				√	√	√																	√		√	√	√
14933.5																			√	√	√	√	√	√			
14940.7							√	√	√				√	√	√				√	√	√	√	√				
14959.1	√	√	√													√	√	√				√	√				
14970.0				√	√	√																√	√	√			
14978.2										√	√	√															
14995.7	√	√	√																√	√	√				√	√	√
15007.3	√		√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√				√	√				
15024.4		√	√				√	√	√	√	√	√	√	√	√	√		√	√	√	√	√	√	√	√	√	√
15027.2							√	√	√							√	√	√									
15036.6				√	√	√	√	√	√				√	√	√	√	√					√		√			
15043.7							√	√	√	√	√	√	√	√	√	√	√	√				√		√			
15048.3				√	√	√	√	√	√							√	√	√				√	√	√			
15055.0							√	√	√							√		√									
15061.4				√	√	√																√	√	√	√	√	√
15079.1				√	√	√							√	√	√				√	√	√				√	√	√
15084.4				√	√	√										√	√	√	√	√	√						
15090.1													√	√	√	√	√	√	√	√	√	√	√	√	√	√	√

Detected protein ion signals (*m/z*)	Nine different washing combinations																										
	$a_1b_1c_1$			$a_1b_2c_2$			$a_1b_3c_3$			$a_2b_1c_2$			$a_2b_2c_3$			$a_2b_3c_1$			$a_3b_1c_3$			$a_3b_2c_1$			$a_3b_3c_2$		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
15096.7	√	√	√	√	√	√							√	√	√	√	√	√				√	√	√	√	√	√
15109.0				√	√	√										√	√		√	√	√						
15116.1				√	√	√							√	√	√	√		√				√	√	√			
15123.8	√	√	√	√	√	√				√	√	√	√	√	√	√	√					√	√	√			
15142.2				√	√	√				√	√	√					√	√	√	√	√	√	√	√			
15152.1				√	√	√													√			√	√	√			
15160.2										√	√	√				√	√	√	√	√	√						
15167.0				√	√	√				√	√	√	√	√	√		√	√	√	√	√						
15174.1																√	√		√	√	√						
15179.0				√	√	√							√	√	√				√	√		√	√	√			
15183.4				√	√	√				√	√	√							√	√	√						
15191.9													√	√	√												
15196.7	√	√	√													√	√	√	√	√	√	√	√	√			
15202.2																			√	√	√				√	√	√
15207.6				√	√	√				√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
15212.1	√	√	√																								
15229.5	√	√	√	√	√	√																√	√	√			
15232.0										√	√	√	√	√	√				√	√	√						
15240.0	√	√	√	√	√	√							√	√	√												
15248.7	√	√	√	√	√	√				√	√	√							√	√	√	√	√	√			
15254.8													√	√	√												
15259.2													√	√	√				√	√	√						
15267.9				√	√	√				√	√	√							√	√	√						
15270.2	√	√	√							√	√	√	√	√	√												
15279.2	√	√	√							√	√	√							√	√	√						
15285.5	√	√	√							√	√	√	√	√	√												

Detected protein ion signals (*m/z*)	Nine different washing combinations																								
	$a_1b_1c_1$			$a_1b_2c_2$			$a_1b_3c_3$			$a_2b_1c_2$			$a_2b_2c_3$			$a_2b_3c_1$			$a_3b_1c_3$			$a_3b_2c_1$			$a_3b_3c_2$		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
15292.8				√	√	√				√	√	√	√	√	√												
15299.4	√	√	√										√	√	√							√	√	√			
15304.0	√	√	√	√	√	√													√	√	√						
15311.3										√	√	√							√	√	√	√	√	√			
15314.9				√	√	√				√	√	√	√	√	√				√	√	√						
15323.8				√	√	√				√	√	√	√	√	√				√		√						
15329.2													√	√	√				√	√	√						
15341.7	√	√	√							√	√	√	√	√	√				√		√	√	√	√			
15345.3							√	√	√				√	√	√				√	√	√	√	√	√			
15354.2										√	√	√							√	√	√	√	√	√			
15361.4	√	√	√							√	√	√	√	√	√				√	√							
15367.1										√	√	√	√	√	√				√	√	√						
15374.6	√	√	√																								
15382.8				√	√	√				√	√	√	√	√	√				√	√	√						
15388.1	√	√	√	√	√	√				√	√	√							√	√	√						
15397.3				√	√	√							√	√	√												
15404.3										√	√	√	√	√	√												
15415.1													√	√	√				√	√	√						
15420.0	√	√	√	√	√	√													√	√	√						
15427.6	√	√	√																√	√	√						
15434.1	√	√	√	√	√	√																					
15438.3				√	√	√							√	√	√												
15442.0	√	√	√	√	√	√													√	√	√						
15452.7	√	√	√																								
15463.4	√		√																								
15470.7	√	√	√																								

Detected protein ion signals (*m/z*)	Nine different washing combinations																										
	$a_1b_1c_1$			$a_1b_2c_2$			$a_1b_3c_3$			$a_2b_1c_2$			$a_2b_2c_3$			$a_2b_3c_1$			$a_3b_1c_3$			$a_3b_2c_1$			$a_3b_3c_2$		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
15475.1	√	√	√							√	√	√															
15491.0	√	√	√							√	√	√															
15503.9	√	√	√																								
15522.9	√	√	√																								
15526.4	√	√	√							√	√	√															
15538.3	√	√	√																								
15548.8	√	√	√																								
15556.7	√	√											√	√	√												
15562.4	√		√	√	√	√				√	√	√	√	√	√												
15569.4	√	√												√	√							√	√	√			
15574.5				√	√	√							√	√	√												
15589.4	√	√	√	√	√	√							√	√	√												
15598.4				√	√	√								√	√							√	√	√			
15601.2				√	√	√							√	√	√							√	√	√			
15607.6				√	√	√								√	√							√	√	√			
15615.2				√	√	√							√	√	√												
15621.2				√	√	√																√	√	√			
15627.2										√	√	√	√	√	√							√	√	√			
15634.4				√	√	√				√	√	√	√	√	√												
15643.7				√	√	√				√	√	√	√	√	√												
15657.9										√	√	√															
15667.6				√	√	√																					
15683.4							√	√	√				√	√	√										√	√	√
15688.3	√	√	√													√	√	√									
15698.0	√	√	√	√	√	√																√	√	√			
15705.6										√	√	√															

Detected protein ion signals (*m/z*)	Nine different washing combinations																										
	$a_1b_1c_1$			$a_1b_2c_2$			$a_1b_3c_3$			$a_2b_1c_2$			$a_2b_2c_3$			$a_2b_3c_1$			$a_3b_1c_3$			$a_3b_2c_1$			$a_3b_3c_2$		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
15711.3	√	√	√													√	√	√									
15723.3	√	√	√				√	√	√				√	√	√	√	√	√	√	√	√				√	√	√
15730.2	√	√	√																								
15738.7	√	√	√	√	√	√																√	√	√			
15743.8										√	√	√				√	√	√									
15747.3	√	√	√																	√	√						
15760.4													√	√		√	√	√	√	√		√	√	√			
15770.2							√	√	√	√	√	√	√	√	√					√	√	√	√	√			
15777.4										√	√	√										√	√	√			
15782.6				√	√	√				√	√	√							√	√	√	√	√	√			
15792.9																			√	√	√	√	√	√	√	√	√
15799.3	√	√	√										√		√							√	√	√			
15804.5				√	√	√				√	√	√	√			√	√	√	√	√	√				√	√	√
15809.8													√	√	√							√	√	√			
15814.1																						√	√	√	√	√	√
15827.2										√	√	√	√	√	√												
15830.1				√	√	√				√	√	√															
15835.4	√	√	√							√	√	√										√	√	√	√	√	√
15843.0													√	√	√							√	√	√			
15848.1										√	√	√										√	√	√			
15854.0				√	√	√																√	√	√			
15863.3				√	√	√							√	√								√	√	√	√	√	√
15867.5	√	√	√	√	√	√				√	√	√	√	√	√				√	√	√	√	√	√			
15877.2	√	√	√	√	√	√				√	√	√	√	√								√	√	√			
15882.7										√	√	√										√	√	√			
15889.9													√		√							√	√	√			

Detected protein ion signals (*m/z*)	Nine different washing combinations																								
	$a_1b_1c_1$			$a_1b_2c_2$			$a_1b_3c_3$			$a_2b_1c_2$			$a_2b_2c_3$			$a_2b_3c_1$			$a_3b_1c_3$			$a_3b_2c_1$			$a_3b_3c_2$		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
15892.3													√	√	√												
15904.0				√	√	√							√	√								√	√	√			
15911.6	√	√	√							√	√	√	√	√	√							√	√	√			
15918.2	√	√	√	√	√	√							√	√								√	√	√			
15925.1										√	√	√	√	√	√							√	√	√			
15932.0	√	√	√																			√	√	√			
15940.2													√	√	√												
15946.4	√	√	√	√	√	√																√	√	√			
15955.5	√	√	√	√	√	√				√	√	√										√	√	√			
15960.0	√	√	√	√	√	√				√	√	√	√	√	√							√	√	√			
15965.2										√	√	√															
15970.1	√	√	√																								
15977.2	√	√	√																								
15983.4	√	√	√																			√	√	√			
15990.2				√	√	√							√	√	√												
16000.0	√	√	√																								
16008.4	√	√	√	√	√	√				√	√	√										√	√	√			
16016.1	√	√	√																								
16022.3	√	√	√																								
16028.0	√	√	√	√	√	√							√	√	√							√	√	√			
16041.2	√	√	√	√	√	√							√	√	√							√	√	√			
16046.8	√	√	√										√	√													
16054.0													√		√							√	√	√			
16062.3	√	√	√										√		√												
16068.8																						√	√	√			
16074.7																						√	√	√			

Detected protein ion signals (*m/z*)	Nine different washing combinations																								
	$a_1b_1c_1$			$a_1b_2c_2$			$a_1b_3c_3$			$a_2b_1c_2$			$a_2b_2c_3$			$a_2b_3c_1$			$a_3b_1c_3$			$a_3b_2c_1$			$a_3b_3c_2$		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
16081.5													√	√	√												
16093.9													√	√	√												
18618.7										√	√	√															
18645.4										√	√	√															
18654.1										√	√	√							√	√	√						
18669.2																			√	√	√						
18677.1																			√	√	√						
18685.6										√	√	√							√	√	√						
18694.0										√	√	√							√	√	√						
18708.6																			√	√	√						
18716.9																			√	√	√						
18723.1										√	√	√															
18730.0										√	√	√							√	√	√						
18738.9										√	√	√							√	√	√						
18752.7	√	√	√																								
18757.2	√	√	√							√	√	√							√	√	√						
18768.5	√	√	√							√	√	√							√	√	√						
18798.7	√	√	√																								
18805.6										√	√	√															
18812.0	√	√	√							√	√	√															
20700.1										√	√	√															
20709.7										√	√	√															
20716.7										√	√	√															
20720.8										√	√	√															
20733.0										√	√	√															
20752.4										√	√	√															

Detected protein ion signals (*m/z*)	Nine different washing combinations																								
	$a_1b_1c_1$			$a_1b_2c_2$			$a_1b_3c_3$			$a_2b_1c_2$			$a_2b_2c_3$			$a_2b_3c_1$			$a_3b_1c_3$			$a_3b_2c_1$			$a_3b_3c_2$		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
20765.7										√	√	√															
20789.4										√	√	√															
21162.9										√	√	√															
21190.2										√	√	√															
21215.4										√	√	√															
21879.4										√	√	√															
22027.6										√	√	√															
22050.3										√	√	√															
22060.9										√	√	√															
22073.8										√	√	√															
22085.9										√	√	√					√	√									
23400.4																√	√	√									
23439.1																√											
23451.9																√	√	√									
23463.5																√	√	√									
23496.2																									√	√	√
29370.9										√	√	√															
29392.2										√	√	√															
29421.8										√	√	√															
29843.2							√	√	√																		
29853.1										√	√	√															
29863.1							√	√	√																		
29882.9										√	√	√															
29903.5							√	√	√																		
29920.1							√	√	√	√	√	√															
29927.6										√	√	√															

Detected protein ion signals (*m/z*)	Nine different washing combinations																									
	$a_1b_1c_1$			$a_1b_2c_2$			$a_1b_3c_3$			$a_2b_1c_2$			$a_2b_2c_3$			$a_2b_3c_1$			$a_3b_1c_3$			$a_3b_2c_1$			$a_3b_3c_2$		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
29933.1							√	√	√													√	√	√			
29940.0										√	√	√															
29950.9																						√	√	√			
29957.3										√	√	√										√	√	√			
29969.8																						√	√	√			
29976.8	√	√	√																								
29987.2	√	√	√																			√	√	√			
30014.5	√	√	√							√	√	√										√	√	√			
30019.5										√	√	√										√	√	√			
30022.3	√	√	√							√	√	√															
30066.7										√	√	√															
30076.7	√	√	√																								
30084.1	√	√	√							√	√																
30096.8	√	√	√	√	√	√				√	√	√															
30101.6	√	√	√	√	√	√				√	√	√															
30123.9	√	√	√	√	√	√				√	√	√															
30138.9	√	√	√	√	√	√				√	√	√															
30161.3	√	√	√							√	√	√															
30168.0	√	√	√																								
30177.7	√	√	√																								
30402.1	√	√	√							√	√	√															
30418.7	√	√	√																								
30452.6				√	√	√																					
30470.3				√	√	√																					
30495.2										√	√	√															
30517.5	√	√	√	√	√	√																					

Detected protein ion signals (*m/z*)	Nine different washing combinations																								
	$a_1b_1c_1$			$a_1b_2c_2$			$a_1b_3c_3$			$a_2b_1c_2$			$a_2b_2c_3$			$a_2b_3c_1$			$a_3b_1c_3$			$a_3b_2c_1$			$a_3b_3c_2$		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
30613.0				√	√	√				√	√	√															
30620.7										√	√	√															
30636.5				√	√	√				√	√	√															
30650.1										√	√	√										√	√	√			
30658.7	√	√	√	√	√	√				√	√	√															
30676.2	√	√	√	√	√	√																					
30691.8	√	√	√							√	√	√										√	√	√			
30704.4	√	√	√	√	√	√				√	√	√															
30708.5	√	√	√																			√	√	√			
30722.0	√	√	√							√	√	√										√	√	√			
30731.3	√	√	√																								
30737.3	√	√	√	√	√	√																					
30742.1	√	√	√							√	√	√															
30747.4	√	√	√																								
30754.7										√	√	√															
30764.0	√	√	√																								
30773.6	√	√	√																								
30788.1	√	√	√							√	√	√															
30802.5	√	√	√	√	√	√				√	√	√															
30808.2	√	√	√																								
30837.8	√	√	√	√	√	√				√	√	√															
30850.3	√	√	√																								
30860.3	√	√	√							√	√	√															
30876.7	√	√	√																								
30898.4	√	√	√																								
30904.0	√	√	√																								

Detected protein ion signals (*m/z*)	Nine different washing combinations																										
	$a_1b_1c_1$			$a_1b_2c_2$			$a_1b_3c_3$			$a_2b_1c_2$			$a_2b_2c_3$			$a_2b_3c_1$			$a_3b_1c_3$			$a_3b_2c_1$			$a_3b_3c_2$		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
30910.0	√	√	√																								
30931.4	√	√	√																								
30976.9	√	√	√																								
30987.1	√	√	√																								
31003.1	√	√	√																								
31017.6	√	√	√																								
31026.3	√	√	√																								
31061.3	√	√	√																								
31326.4				√	√	√																					
31381.1				√	√	√																					
31466.4	√	√	√																								
Total Number of detected proteins	192	189	188	163	160	160	92	91	90	215	214	210	184	181	181	125	124	123	142	141	140	151	147	149	81	79	80
Average of detected proteins	190			161			91			213			182			124			141			149			80		

Note: "√" means the protein ion could be detected in three technical duplicates.

Table S6. The protein ion signals detected by (+)MALDI-TOF MS in serial liver parallel tissue sections washed by optimal MeOH-CDWS with -80℃, -20℃, and 25℃, respectively (biological replicates, n=3).

Detected protein ion signals (*m/z*)	-80℃ MeOH-CDWS			-20℃ MeOH-CDWS			25℃ MeOH-CDWS		
	1	2	3	1	2	3	1	2	3
2063.0								√	√
2126.8							√	√	√
2139.8							√	√	√
2177.9							√	√	√
2191.6							√	√	√
2363.3									√
2448.8							√	√	√
2483.9									√
2503.3							√	√	√
2513.7									√
2548.2							√	√	√
2563.2							√	√	√
2570.4							√	√	
2581.4							√	√	√
2602.3							√	√	√
2624.4							√	√	√
2794.9							√	√	√
3037.7							√	√	√
3216.8							√	√	√
3252.3							√	√	√
3276.6								√	√
3350.0							√	√	√
3363.5							√	√	√
3389.8							√	√	√
3409.5							√	√	√
3428.6							√	√	√
3434.1									√
3448.5								√	√
3451.4							√		√
3458.8							√	√	√
3479.2							√	√	√
3488.8							√		√
3496.2							√	√	√
3517.2							√	√	√
3565.2							√	√	
3576.4							√	√	√
3617.9							√	√	√
3883.2		√	√				√	√	√
4226.4							√	√	√
4248.3	√								√
4523.8							√	√	√
4620.9							√	√	
4894.3		√	√		√	√	√	√	√

Detected protein ion signals (*m/z*)	-80℃ MeOH-CDWS			-20℃ MeOH-CDWS			25℃ MeOH-CDWS		
	1	2	3	1	2	3	1	2	3
4948.6	√			√			√	√	√
4956.2							√	√	
4964.2							√	√	√
4986.7									√
5017.4							√	√	√
5311.6									√
5315.2							√		√
5321.6						√	√		√
5368.9	√		√						
5378.8									√
5422.0		√	√	√	√	√	√		√
5437.4		√	√		√	√	√		√
5444.3		√	√				√	√	
5454.2		√	√	√	√	√	√	√	√
5459.1		√	√	√	√	√	√	√	√
5468.6	√		√	√	√	√			
5476.1	√	√	√				√	√	√
5482.4	√	√	√				√		
5490.8	√	√							
5508.1	√	√					√	√	
5512.9							√	√	
5531.5							√	√	
5536.1				√	√	√			
5641.3							√	√	
5646.7					√	√			
5660.7					√	√			
5710.5				√	√				
5718.4				√		√			
5745.4							√	√	
5748.4	√	√	√						
5753.3							√	√	
5797.9				√		√		√	√
6037.4	√	√	√						
6062.8		√	√						
6067.1	√	√	√						
6093.3				√		√			
6117.4	√	√					√	√	√
6122.2				√	√			√	√
6129.2								√	√
6176.2	√	√	√		√	√			
6180.6	√	√	√						
6228.5				√	√				
6240.8				√		√	√	√	
6245.6							√	√	
6255.7							√	√	
6260.2							√	√	√

Detected protein ion signals (*m/z*)	-80℃ MeOH-CDWS			-20℃ MeOH-CDWS			25℃ MeOH-CDWS		
	1	2	3	1	2	3	1	2	3
6266.1								√	
6275.7							√	√	
6296.9				√		√	√		√
6349.5								√	√
6466.1	√	√	√						
6516.1	√	√	√						
6526.5	√	√	√					√	√
6532.3							√		
6546.9				√	√				
6561.1				√		√	√	√	
6564.8	√	√	√	√		√			
6578.0				√		√			
6581.6							√	√	
6596.7				√		√			
6604.1							√	√	
6613.5				√		√	√	√	
6686.3				√	√			√	√
6737.6	√	√	√						
6809.6				√	√		√	√	
6860.2					√	√			
7128.7					√	√			
7134.1		√	√						
7178.1	√	√						√	√
7299.1	√	√	√	√	√				
7339.9					√	√			
7390.5								√	√
7396.0				√	√				
7482.6					√	√			
7488.2	√		√						
7493.3								√	√
7503.6								√	√
7508.5							√	√	
7513.4							√		√
7531.3				√		√	√	√	
7541.8								√	√
7550.5							√	√	
7554.6							√	√	
7584.6									√
7588.4								√	√
7634.1				√	√				
7638.5		√	√						
7682.4	√	√							
7739.9		√	√						
7766.5					√	√			
7771.4	√	√	√						
7774.8								√	√

Detected protein ion signals (*m/z*)	-80℃ MeOH-CDWS			-20℃ MeOH-CDWS			25℃ MeOH-CDWS		
	1	2	3	1	2	3	1	2	3
7778.3		√	√		√	√	√		√
7791.4					√	√	√		√
7795.9	√	√	√						
7800.2							√		√
7810.4				√		√	√		√
7819.3								√	√
7825.4	√	√	√				√	√	√
7830.2	√	√	√					√	√
7836.6	√	√	√	√	√	√	√	√	√
7843.2	√	√	√					√	√
7850.6				√	√	√		√	√
7856.6							√	√	√
7865.1								√	√
7871.2								√	√
7874.4	√	√	√	√	√	√			
7877.7	√	√	√					√	√
7884.7	√	√		√	√			√	
7887.5	√	√	√					√	
7894.4				√	√		√	√	
7898.3	√	√	√				√	√	
7903.4	√	√	√					√	√
7907.2	√	√	√					√	√
7915.7	√	√	√	√	√			√	√
7919.5				√		√		√	√
7925.3	√	√					√	√	
7931.9							√	√	
7936.8								√	√
7941.8								√	√
7945.6	√		√						
7950.3							√	√	
7954.7				√		√		√	
7957.2									
7962.9				√		√			
7966.2							√	√	
8082.9							√	√	
8087.6								√	√
8134.3								√	
8359.3							√	√	√
8406.3				√	√				
8431.8								√	√
8455.9				√	√				
8459.8	√	√	√						
8463.5							√	√	
8469.7					√	√			
8475.8	√	√							
8499.0				√		√			

Detected protein ion signals (*m/z*)	-80℃ MeOH-CDWS			-20℃ MeOH-CDWS			25℃ MeOH-CDWS		
	1	2	3	1	2	3	1	2	3
8505.2	√		√						
8515.2				√					
8520.9	√	√							
8580.2	√	√							
8627.6	√	√							
8687.7								√	√
8692.8	√		√						
8708.1					√	√			
8731.8				√	√				
8737.2	√		√						
8754.6	√		√						
8916.9				√	√				
8920.7				√	√				
8944.6	√		√				√	√	√
8960.9				√	√				
8964.2							√	√	
8969.4	√	√							
9283.2					√	√			
9302.3				√		√			
9308.2		√	√						
9323.7				√	√				
9342.8				√	√			√	√
9350.1	√		√						
9389.5	√	√							
9395.7					√	√			
9437.3				√	√				
9580.7								√	√
9593.4							√		√
9598.3		√	√						
9607.1					√	√			
9611.6		√	√				√		√
9616.3	√	√	√						
9621.5							√	√	
9634.5				√		√			
9639.5									
9652.3				√		√			
9868.6							√	√	
9880.9				√	√				
9925.9							√	√	
9941.6							√		√
9946.6							√		
9951.5							√		√
9980.9							√	√	
9990.5								√	√
10685.7					√	√			
10721.6				√		√			

Detected protein ion signals (*m/z*)	-80℃ MeOH-CDWS			-20℃ MeOH-CDWS			25℃ MeOH-CDWS		
	1	2	3	1	2	3	1	2	3
10838.6							√		
10842.6									√
10877.2								√	√
11301.2							√	√	
11302.2						√			
11307.9		√	√				√		
11333.6								√	√
11340.8	√	√							
11648.3							√	√	
11680.7							√	√	
11711.0		√	√						
11741.9	√	√							
11860.2				√		√			
11865.6		√	√						
11888.9				√					
11895.4	√								√
12097.6							√		√
12124.1								√	√
12320.8	√		√						
12329.4	√		√						
12333.1					√	√			
12339.4				√	√				
12347.8								√	
12360.2				√		√			
12376.2					√	√			
12392.8			√						
12400.3		√							√
12418.9				√					
12422.7					√	√			
12427.4								√	√
12432.5				√	√				
12438.3				√		√			
12460.4		√	√						
12541.8	√		√						
12548.2		√	√						
12884.1		√							
12903.5				√		√			
12929.9								√	√
12934.5	√		√						
13010.5	√	√							
13034.3								√	√
13117.9				√	√				
13141.3				√	√				
14119.8					√	√			
14127.8		√	√						
14137.2				√		√			

Detected protein ion signals (*m/z*)	-80℃ MeOH-CDWS			-20℃ MeOH-CDWS			25℃ MeOH-CDWS		
	1	2	3	1	2	3	1	2	3
14145.3					√	√			
14187.3							√		√
14195.6								√	√
14201.0					√		√		√
14206.1								√	√
14212.7								√	√
14218.1							√	√	
14220.7	√	√	√						
14237.7	√	√			√	√			
14244.9	√		√						
14254.8								√	√
14264.6				√	√	√	√		√
14271.5							√	√	
14278.4									
14284.3				√		√			
14291.6					√	√			
14300.7				√		√			
14306.6							√		√
14312.8							√		√
14317.2							√	√	
14322.6				√	√	√			
14329.2								√	√
14338.7				√	√				
14354.9								√	
14449.3								√	√
14464.8								√	
14818.7							√		√
14830.5							√	√	
14834.8							√		√
14842.6							√	√	√
14845.0							√	√	
14849.5							√	√	
14856.7							√		√
14867.8							√	√	
14873.1							√		√
14887.1							√	√	
14897.1							√	√	
14900.1								√	√
14904.3					√	√	√	√	
14913.7								√	√
14918.9	√		√						
14930.3				√		√			
14942.3							√		√
14948.4	√	√	√						
14955.7				√	√				
14962.9									

Detected protein ion signals (*m/z*)	-80℃ MeOH-CDWS			-20℃ MeOH-CDWS			25℃ MeOH-CDWS		
	1	2	3	1	2	3	1	2	3
14980.4									√
14992.8								√	
14996.7							√		√
15001.6						√			√
15007.4				√	√			√	√
15013.5					√	√	√		
15017.5				√					√
15022.8					√	√			√
15028.1				√				√	√
15033.4				√	√	√	√	√	
15039.8								√	√
15044.4				√	√		√		√
15051.9							√	√	
15057.7							√	√	√
15066.9					√	√	√	√	√
15077.6				√	√	√	√	√	√
15082.3								√	
15086.4									√
15091.1								√	√
15096.4							√	√	
15104.3		√	√		√	√		√	√
15109.4				√		√	√		√
15117.4				√				√	√
15121.8							√	√	
15127.1					√	√	√	√	√
15134.2					√			√	√
15139.5				√	√	√			
15148.4							√	√	
15151.0				√					√
15155.5	√		√		√	√			
15162.9	√		√		√	√	√	√	
15166.8				√		√		√	√
15177.4				√	√			√	
15182.7							√		
15187.4							√		√
15201.4							√	√	
15210.9								√	
15215.8									√
15221.2							√		
15226.5							√	√	
15233.6							√		√
15242.8								√	√
15246.1							√		√
15255.3							√	√	√
15262.1								√	√
15269.3							√	√	√

Detected protein ion signals (*m/z*)	-80℃ MeOH-CDWS			-20℃ MeOH-CDWS			25℃ MeOH-CDWS		
	1	2	3	1	2	3	1	2	3
15276.3							√	√	√
15281.6							√	√	√
15285.2								√	√
15294.1							√	√	
15299.5							√		
15308.4							√	√	√
15313.7							√		
15320.9							√	√	√
15331.2								√	
15335.1							√		
15339.7							√		√
15349.4								√	√
15354.8									√
15359.6							√	√	√
15364.9							√	√	
15369.1							√	√	√
15378.8							√	√	√
15385.1								√	√
15389.7				√	√	√		√	√
15395.9							√	√	
15400.4				√	√	√	√	√	√
15412.8								√	√
15416.4				√	√	√		√	
15428.6							√	√	√
15439.5							√	√	√
15451.3				√	√	√	√	√	√
15457.1								√	√
15462.1				√		√	√	√	√
15469.2	√	√			√	√	√	√	√
15476.4		√	√	√					√
15479.1	√	√	√	√	√	√	√	√	√
15485.2	√							√	
15490.7	√			√	√	√			√
15494.6		√	√					√	√
15498.2	√	√						√	√
15505.4	√	√	√					√	√
15511.9								√	√
15517.1					√	√			
15521.5	√	√					√	√	√
15529.9								√	√
15539.1								√	
15563.7					√	√	√		√
15577.8	√							√	
15593.4									√
15598.5									√
15602.0								√	

Detected protein ion signals (*m/z*)	-80℃ MeOH-CDWS			-20℃ MeOH-CDWS			25℃ MeOH-CDWS		
	1	2	3	1	2	3	1	2	3
15627.3									√
15632.2	√	√	√				√		√
15636.3								√	√
15640.7					√	√	√		√
15647.2							√	√	√
15659.8						√	√		√
15665.2					√	√	√		√
15674.2				√			√	√	√
15682.4					√			√	√
15686.8				√		√	√		
15690.5								√	√
15694.9							√		√
15699.7	√	√	√		√	√	√	√	√
15706.9				√	√				
15710.9							√		
15714.7		√	√					√	
15723.2					√	√	√		√
15726.7	√	√	√				√		√
15731.6		√	√	√	√		√	√	√
15736.8	√	√	√					√	
15739.2				√	√	√	√	√	√
15745.7	√	√	√		√			√	√
15748.8				√	√		√	√	
15767.6					√	√			
15773.8	√	√	√	√	√	√	√		√
15781.5	√	√	√		√	√	√	√	√
15784.4							√		
15787.8				√		√	√	√	√
15795.5				√	√		√	√	
15804.6				√	√				
15811.6									√
15820.7							√	√	√
15827.9							√	√	
15833.4				√	√	√	√		√
15840.5								√	√
15844.1				√	√	√			√
15853.2							√	√	√
15869.7	√	√	√	√	√	√	√		
15876.7		√	√	√		√	√	√	√
15886.0								√	√
15894.1					√	√	√	√	√
15901.9				√		√			
15909.1			√	√	√	√	√	√	√
15916.9		√	√	√			√	√	√
15928.9	√	√	√				√		√
15938.6				√			√	√	

Detected protein ion signals (*m/z*)	-80℃ MeOH-CDWS			-20℃ MeOH-CDWS			25℃ MeOH-CDWS		
	1	2	3	1	2	3	1	2	3
15946.4					√	√	√	√	√
15952.6	√	√		√			√	√	√
15962.3							√	√	√
15969.6					√		√	√	√
15975.8								√	√
15980.6				√	√	√		√	√
15985.4							√	√	√
15993.3				√	√	√			
15997.7				√	√	√		√	√
16004.6				√			√		√
16012.0								√	
16029.1								√	√
16035.7								√	
16191.1	√		√						
17346.4	√		√						
17353.9							√	√	√
17357.1	√	√		√	√			√	√
17360.9	√	√	√	√	√	√	√		√
17370.4		√	√	√		√	√		√
17399.5				√		√	√	√	
17404.4				√	√				
17410.7				√	√				
17431.4					√	√			
17442.9				√	√				
17770.2	√	√	√	√	√				
17777.1	√	√	√					√	√
17792.8					√	√			
17803.7	√		√						
17808.2	√	√	√						
17817.1								√	√
17829.5				√	√				
17835.2					√	√			
17852.5				√		√			
20614.6	√		√						
20634.7	√	√	√						
20639.6					√	√			
20653.2				√	√	√			
23367.7							√		√
23374.0							√		√
23384.9				√		√			
23390.4				√		√			
23394.9							√		√
25663.2	√	√							
25671.7	√	√							
29170.1							√		√
29192.4							√		√

Detected protein ion signals (*m/z*)	-80℃ MeOH-CDWS			-20℃ MeOH-CDWS			25℃ MeOH-CDWS		
	1	2	3	1	2	3	1	2	3
29775.5							√		
29800.4							√		
29820.3							√		
29832.8							√		
29842.8							√		
29855.3							√		
29867.8							√		
29897.4							√		
29915.3							√		
29917.7							√		
29937.8							√		
30000.3							√		
30096.8								√	√
30395.4								√	√
30403.0								√	√
30418.0								√	
30422.4							√		
30437.5							√		
30452.6							√		
30455.1							√		
30475.3							√		
30495.5							√		√
30500.8							√		√
30510.7							√		
30518.3							√		√
30528.0							√		
30541.0							√		
30551.1							√		
30558.7							√		
30568.8							√		
30578.9							√		
30581.4							√	√	
30594.0							√		√
30626.9							√		√
30639.6							√	√	
30679.5							√	√	
30695.3							√		√
30742.1							√	√	
30757.5							√	√	
30788.8							√	√	
30802.5							√		
30812.5							√		√
30822.1							√	√	
30860.4							√	√	
30880.9							√	√	
31096.1							√		√

Detected protein ion signals (*m/z*)	-80℃ MeOH-CDWS			-20℃ MeOH-CDWS			25℃ MeOH-CDWS		
	1	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**
31104.4							√		√
31114.5							√	√	√
31137.5							√	√	√
31150.3							√	√	√
31175.8							√	√	√
31178.3							√	√	√
31181.8							√	√	√
31575.8							√		√
31590.6							√		√
31605.1							√		√
31626.2									√
31644.4									√
31665.3									√
31683.0									√
Number of detected proteins	126	125	122	98	101	99	270	274	272
Average of detected proteins		124			99			273	

Note: "√" means the protein ion could be detected in three technical duplicates.

Table S7. Comparison of the detectable proteins in the rat liver tissue sections washed by MeOH-CDWS with seven different washing rotation frequencies (*i.e.*, 1 rpm, 3 rpm, 5 rpm, 7 rpm, 9 rpm, 11 rpm, 13 rpm) at 25°C by (+)MALDI-TOF MS using SA as the matrix (biological replicates, n=3).

Detected protein ion signals (*m/z*)	Washing rotation frequency																				
	1 rpm			3 rpm			5 rpm			7 rpm			9 rpm			11 rpm			13 rpm		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
2126.8				√	√	√	√	√	√	√	√	√	√	√	√	√	√	√			
2139.8				√		√	√	√	√	√		√	√	√	√	√	√	√			
2448.8					√	√	√	√	√		√	√	√	√	√	√	√	√			
2503.3				√	√		√	√	√	√	√		√	√	√	√		√			
2548.2				√	√	√	√	√	√	√		√	√	√	√	√	√	√			
2563.2					√	√	√	√	√		√	√	√	√	√	√	√	√			
2570.4				√	√		√	√		√	√		√	√		√	√		√	√	√
2581.4					√	√	√	√	√		√	√	√	√	√	√	√	√	√	√	
3037.7				√	√		√	√	√	√	√		√	√	√	√		√	√	√	
3216.8					√	√	√	√	√		√	√	√	√	√	√	√	√	√		
3252.3				√	√		√	√		√	√		√	√		√	√			√	√
3276.6					√	√		√	√		√	√		√	√		√	√	√	√	
3350.0				√	√		√	√		√			√	√		√	√				
3363.5				√	√	√	√	√	√	√	√	√	√	√	√	√	√	√			
3389.8					√	√	√	√	√		√	√	√	√	√	√		√	√	√	
3409.5	√	√	√		√	√	√	√	√		√	√	√	√	√	√	√	√	√		√
3428.6	√	√	√	√	√	√	√	√	√	√		√	√	√	√	√	√	√	√	√	
3448.5	√	√			√	√		√	√		√	√		√	√		√	√		√	√
3455.1		√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	
3479.2	√	√			√	√	√	√	√		√	√	√	√	√	√	√	√	√		
3496.2	√		√	√	√	√	√	√	√	√	√	√	√	√	√		√	√	√	√	√
3517.2	√	√	√	√		√	√	√	√	√	√	√	√	√	√	√	√	√		√	√
3565.2	√	√	√	√	√		√	√		√	√		√	√		√			√		√
3576.4	√		√	√		√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	
3617.9	√	√		√	√		√	√		√	√		√	√		√	√			√	√
3883.2		√	√	√	√		√	√		√	√		√	√		√	√		√	√	
3906.7	√	√		√	√	√			√	√	√	√			√			√	√		√
4193.5	√		√		√	√			√		√	√			√			√	√	√	√
4226.4	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
4248.3	√	√	√			√			√			√			√			√	√	√	√
4523.8	√	√	√	√	√		√	√		√	√		√	√		√	√		√	√	√
4620.9	√	√	√	√	√		√	√		√	√		√	√		√	√				
4894.3				√	√	√	√	√	√	√	√	√	√	√	√	√	√	√			
5010.3										√	√	√	√	√		√	√	√	√		√
5015.1				√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
5315.3					√	√	√	√	√	√	√	√	√	√	√						√
5319.6				√	√	√	√	√	√	√	√	√		√	√				√	√	
5422.0				√		√	√	√	√		√	√								√	√
5437.4	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
5444.3	√	√	√	√	√																
5459.1	√	√	√	√	√	√							√	√	√						

Detected protein ion signals (*m/z*)	Washing rotation frequency																				
	1 rpm			3 rpm			5 rpm			7 rpm			9 rpm			11 rpm			13 rpm		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
5468.6	√	√	√													√	√	√		√	√
5476.1	√	√		√	√	√															
5536.1	√		√	√		√				√	√	√	√	√	√	√		√		√	√
5643.0	√	√	√	√	√	√	√	√	√				√	√	√	√					
5653.9	√	√	√		√	√			√							√	√	√	√	√	
5658.2	√	√	√	√	√	√	√	√	√		√	√				√	√	√			√
5663.9	√	√	√				√	√	√	√	√	√	√	√	√				√		√
5696.1	√		√	√	√	√	√	√	√	√	√	√	√	√	√				√		√
5700.2	√	√	√													√	√	√	√	√	
5741.5	√	√	√	√		√	√	√	√	√	√	√					√		√	√	√
5745.4	√	√		√	√	√	√	√	√				√	√	√						
6037.4	√	√	√	√	√	√				√	√	√									
6054.5							√	√	√												
6062.8					√	√															
6067.1				√	√	√															
6093.3				√	√	√															
6159.4									√							√	√	√	√	√	√
6195.4																√	√				
6199.4																	√			√	√
6208.8	√						√	√	√	√	√	√	√	√			√				
6212.8	√	√	√													√		√	√		√
6222.7	√																				√
6228.5									√								√		√	√	
6236.8							√	√	√			√	√	√	√	√	√	√	√		√
6240.8				√	√		√	√	√										√	√	
6245.6	√		√	√	√		√	√	√							√	√	√	√	√	√
6255.7	√	√	√														√				
6260.2		√	√				√	√	√	√	√	√	√	√		√	√	√	√		√
6266.1	√	√	√																		
6275.7	√	√	√																		
6282.9			√													√	√	√		√	√
6296.9				√	√	√	√	√	√												
6526.5	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√		√
6532.3				√																	
6557.9	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
6624.1																	√				
6630.7	√	√	√							√	√	√	√			√	√	√			
6634.3							√	√	√				√								
6680.3	√															√	√				
6686.3		√	√	√		√	√	√	√	√	√	√	√	√	√		√	√	√		√
6802.2										√	√										
6806.4				√		√				√	√	√	√	√	√				√		√
6809.6	√	√	√	√	√	√	√	√	√	√	√		√	√	√	√	√	√			
7299.1				√	√	√															
7339.9				√		√															

Detected protein ion signals (*m/z*)	Washing rotation frequency																				
	1 rpm			3 rpm			5 rpm			7 rpm			9 rpm			11 rpm			13 rpm		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
7467.4							√		√												
7476.8	√	√	√	√	√	√				√	√	√	√	√	√	√	√	√	√	√	√
7482.6		√		√		√	√	√	√												
7493.3	√				√	√															
7497.7		√		√	√																
7503.6	√			√	√	√															
7508.5				√						√	√	√		√	√	√	√	√	√	√	
7518.2							√									√	√	√	√	√	
7526.4													√	√							
7578.6				√	√	√				√	√	√									
7584.6				√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	
7588.4						√	√	√	√			√							√	√	
7624.0										√						√		√			
7628.1				√	√					√	√	√				√			√	√	√
7634.1				√	√									√	√	√	√	√	√		√
7638.5					√	√	√	√	√	√	√	√				√	√	√			
7646.5						√	√	√	√	√											
7749.3				√	√	√				√	√										
7756.1								√	√				√	√		√	√	√	√		
7762.2							√	√	√	√	√	√					√		√	√	√
7766.5							√	√	√	√			√	√	√						
7774.8																√		√		√	
7787.6	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
7791.4				√		√															
7810.4				√		√	√	√	√											√	√
7825.4				√	√	√	√	√	√	√	√	√									
7830.2	√	√	√		√	√		√	√	√	√	√	√	√	√	√	√	√	√		
7843.2					√	√	√	√	√					√	√		√	√			
7856.6	√			√		√	√	√	√	√	√	√		√	√	√		√			
7865.1							√	√	√	√	√	√		√	√						
7871.2								√		√	√	√		√	√						
7887.5				√	√	√		√		√	√	√				√	√	√	√	√	√
7894.4	√	√			√	√	√	√	√	√				√	√				√	√	√
7903.4	√	√			√	√	√	√	√	√			√	√							
7911.8	√	√	√			√	√		√	√							√	√			
7915.7				√	√	√	√	√					√		√						
8051.5							√	√	√							√	√	√			
8082.9	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
8087.6										√	√	√								√	√
8092.1					√		√	√	√	√	√	√	√	√	√						
8098.6	√	√	√								√										
8112.2	√	√	√							√	√		√								
8317.0	√	√					√	√	√												
8359.3	√	√	√	√	√	√		√	√	√	√	√	√	√	√	√	√	√	√	√	√
8425.8																√	√	√			

Detected protein ion signals (*m/z*)	Washing rotation frequency																				
	1 rpm			3 rpm			5 rpm			7 rpm			9 rpm			11 rpm			13 rpm		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
8469.7										√	√	√									
8520.9																	√	√			
8525.9																√	√				
8529.5																	√	√			
8539.1			√				√	√	√	√	√	√	√	√		√	√				
8572.7										√	√	√						√	√	√	√
8683.8				√	√	√	√	√	√	√	√	√	√	√	√	√		√	√	√	√
8702.0											√										
8916.9		√	√				√	√	√				√	√		√	√	√			
8920.7				√	√	√															
8954.1																√	√	√			
8960.9							√	√	√												
9297.0	√	√	√	√	√	√		√	√	√	√	√	√	√	√				√	√	√
9376.1		√	√				√	√	√												
9580.7						√															
9589.2	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
9593.4				√		√															
9607.1				√	√	√	√	√	√	√	√	√	√	√	√			√	√	√	√
9611.6				√		√															
9842.6							√	√	√				√	√		√					
9861.9													√	√	√			√			
9868.6				√												√	√	√	√	√	√
9880.9	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
9902.2															√	√					
9906.9																√	√	√			
9917.6							√	√	√					√	√						
9925.9					√			√	√												
9935.0							√	√	√				√	√	√						
9941.6				√		√													√	√	√
9946.6	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
10087.3							√	√	√										√	√	√
10111.5	√		√													√	√	√			
10221.3	√	√	√				√		√			√									√
10460.3	√	√	√				√					√				√		√			
10685.7							√	√	√							√			√		
10838.6	√						√	√	√							√	√	√			√
10842.6			√													√	√	√	√	√	√
10877.2		√																	√	√	√
11226.6							√	√	√										√		
11236.9								√								√	√	√	√	√	√
11241.2															√		√				
11247.9										√	√	√	√	√	√	√	√	√			
11254.0											√		√	√					√	√	√
11264.4				√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
11280.6												√									

Detected protein ion signals (*m/z*)	Washing rotation frequency																				
	1 rpm			3 rpm			5 rpm			7 rpm			9 rpm			11 rpm			13 rpm		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
11293.0							√	√	√	√	√	√	√	√	√						
11298.9	√	√	√										√		√				√	√	√
11302.2	√		√																		
11307.9				√		√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
11321.7	√	√	√							√	√	√									
11340.8		√					√	√	√			√									
11350.5			√	√		√	√		√											√	
11642.8							√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
12083.1		√																			
12087.7	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√		√	
12092.8			√													√		√			
12097.6				√	√	√															
12295.1							√	√	√												
12314.2							√		√												
12320.8						√															
12329.4	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√		√	√	√	√	√
12333.1				√		√															
12392.8		√	√	√	√	√	√	√	√	√	√	√	√	√	√		√	√	√	√	√
12418.9							√		√				√	√							
12469.2							√	√	√												
12589.6				√		√				√											
12595.4				√		√															
12872.1										√	√	√									
13112.2				√	√	√	√	√	√	√	√	√	√	√	√		√	√	√	√	√
13421.6				√	√	√	√	√	√	√	√	√	√	√	√						
13705.6							√		√							√	√	√			
13722.1							√	√	√	√	√	√	√	√	√	√	√	√			
13738.8										√	√	√	√								
13749.6										√		√	√	√	√	√	√	√			
13798.0										√	√	√									
13905.0										√	√	√									
13924.8								√													
13934.9							√	√	√												
13938.7							√	√	√												
13947.0		√	√	√	√	√				√	√	√	√	√	√						
13954.1		√	√				√	√	√				√	√		√	√	√			
13978.6							√	√	√												
13982.5									√												
13992.1						√	√	√	√	√	√	√	√	√	√	√	√				
14018.1							√		√												
14032.4							√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
14112.0							√	√	√	√		√	√		√	√	√	√	√	√	√
14195.6	√	√	√			√				√	√	√	√	√	√	√	√		√	√	√
14206.1					√					√	√	√	√	√	√	√	√	√			
14212.7					√								√	√	√						

Detected protein ion signals (*m/z*)	Washing rotation frequency																				
	1 rpm			3 rpm			5 rpm			7 rpm			9 rpm			11 rpm			13 rpm		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
14218.1					√								√		√						
14237.7	√	√	√	√		√	√	√					√	√	√	√	√	√	√		√
14254.8	√	√	√		√											√	√	√	√	√	
14264.6	√	√		√	√					√	√	√				√	√	√	√		√
14271.5	√	√	√		√					√		√	√	√	√	√		√	√	√	
14322.6				√	√					√	√	√	√		√	√	√	√	√	√	
14329.2					√					√	√	√	√	√	√	√	√	√	√	√	√
14354.9					√					√	√	√	√	√	√	√	√	√	√	√	√
14368.8							√	√	√	√		√				√	√	√	√	√	√
14449.3						√				√	√	√	√	√	√						
14464.8	√	√	√		√					√	√	√	√	√	√	√		√			
14584.2	√	√			√					√	√	√			√		√	√			
14797.6	√	√	√							√	√	√	√	√		√	√				
14804.3										√		√				√	√			√	√
14811.3										√	√	√	√	√	√				√	√	√
14818.7	√	√	√		√		√	√	√	√	√	√	√	√	√	√	√	√			
14820.3	√	√	√						√				√	√		√	√	√	√	√	√
14825.3	√	√						√					√	√	√				√	√	√
14830.1	√	√	√		√							√	√	√	√		√		√	√	√
14835.2						√			√		√	√	√	√	√	√					
14840.3				√	√	√	√		√	√		√				√	√	√			
14845.7										√		√		√		√	√		√	√	√
14850.3				√	√	√	√	√	√		√	√	√								
14854.9						√	√	√	√	√	√	√	√	√	√	√		√	√	√	√
14861.2				√	√	√	√	√	√	√		√		√	√	√	√			√	√
14868.8					√	√						√	√	√	√	√	√			√	√
14873.0	√		√		√	√							√	√	√	√	√	√			
14887.1	√			√	√	√													√	√	√
14889.8	√	√	√							√							√				
14894.6	√		√	√	√		√			√	√	√	√	√	√	√	√	√	√	√	√
14909.7	√	√		√	√	√									√	√		√			
14937.6	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
14942.3				√	√	√										√	√	√	√	√	√
14980.4						√										√	√	√	√	√	√
14992.8							√	√	√				√						√	√	√
15007.4				√	√	√	√	√	√				√	√	√		√		√	√	√
15013.5				√	√		√	√	√				√		√	√					
15044.4										√	√		√	√	√	√	√	√			
15051.9				√	√								√	√	√						
15057.7			√	√	√	√							√	√	√	√	√				
15066.9	√	√	√	√	√	√							√		√						
15078.9	√	√	√				√	√	√	√	√		√	√	√	√	√	√	√	√	√
15082.3	√	√			√	√	√	√	√				√	√	√	√	√	√			
15086.0	√	√	√			√	√	√	√	√	√		√	√	√	√	√			√	√
15091.1					√	√							√	√	√						

Detected protein ion signals (*m/z*)	Washing rotation frequency																				
	1 rpm			3 rpm			5 rpm			7 rpm			9 rpm			11 rpm			13 rpm		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
15092.4	√	√	√	√		√				√	√	√	√		√	√	√			√	√
15096.4				√	√					√	√	√	√	√	√						
15100.1		√	√		√	√	√	√	√	√		√	√	√	√	√	√	√	√		
15104.3					√	√	√	√		√	√	√				√	√	√	√	√	√
15109.4					√	√	√	√	√	√	√	√				√		√	√	√	√
15114.4																√	√	√	√	√	√
15121.8	√	√	√	√	√	√	√									√	√	√	√	√	√
15127.1				√	√	√				√	√	√						√		√	√
15134.2					√	√															
15139.5		√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√			
15148.4		√	√	√		√	√		√								√	√	√	√	√
15151.0	√	√				√															
15155.5			√	√	√	√				√	√	√				√	√	√		√	√
15162.9	√	√	√	√	√	√	√	√	√				√	√	√		√	√	√	√	√
15166.8	√	√	√		√	√	√	√	√												
15182.7	√	√	√	√	√	√	√	√	√							√	√	√	√		√
15187.4	√	√	√	√		√	√	√	√							√	√				
15201.4				√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
15205.7				√	√	√	√	√	√				√	√	√	√	√	√	√	√	√
15210.9				√	√	√	√		√	√	√	√				√	√		√	√	√
15215.8					√	√		√				√			√	√	√		√	√	√
15220.9							√			√	√				√	√	√		√	√	√
15226.5				√	√	√			√	√	√	√		√				√			
15233.6		√	√			√	√	√	√	√	√	√	√	√		√	√		√	√	√
15237.5		√	√	√	√	√							√	√	√	√		√	√	√	
15246.1		√	√	√	√	√				√	√	√	√	√	√	√	√	√	√		√
15255.3				√	√	√							√	√	√	√	√		√	√	√
15262.1				√	√	√										√			√		√
15269.3				√		√					√								√	√	
15276.3					√		√	√	√												
15281.6				√	√	√								√						√	√
15285.2	√				√	√				√			√	√	√	√	√	√			
15294.1				√	√	√	√	√	√			√	√	√		√		√			
15303.2				√			√	√	√				√	√	√	√	√	√	√	√	√
15308.4				√	√	√					√	√		√	√	√	√	√	√	√	√
15320.9				√	√	√				√	√	√				√	√	√	√	√	√
15321.0		√	√				√							√		√	√	√	√	√	√
15331.2				√	√	√		√	√	√	√	√	√		√	√	√	√			
15339.7	√	√	√	√		√	√	√	√												
15345.3					√					√	√	√	√		√						
15349.4	√	√	√		√	√								√							
15354.8	√	√	√				√	√	√	√	√	√	√		√						
15359.6					√					√	√	√	√	√	√				√	√	√
15364.9			√				√			√	√	√									
15369.1				√				√	√	√	√			√	√		√	√	√	√	√

Detected protein ion signals (*m/z*)	Washing rotation frequency																				
	1 rpm			3 rpm			5 rpm			7 rpm			9 rpm			11 rpm			13 rpm		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
15378.8				√	√	√				√	√	√			√	√	√	√	√	√	
15385.1				√	√	√												√	√	√	√
15389.7					√								√	√						√	√
15395.9	√	√	√	√		√				√										√	√
15400.4						√	√	√	√	√	√	√	√	√	√	√	√		√	√	
15408.1	√			√	√					√			√	√	√						
15412.8					√	√															
15416.4					√		√	√	√	√	√	√					√	√			
15419.1				√	√					√	√					√		√		√	√
15423.3				√	√	√			√	√	√	√	√	√	√		√	√		√	√
15428.6				√		√	√	√		√	√			√	√	√		√			
15435.7				√	√	√	√	√	√		√	√			√				√	√	
15441.6		√		√	√	√				√	√	√	√			√	√	√	√		√
15447.1							√	√	√	√	√	√	√	√	√	√	√	√			
15451.3			√	√	√	√				√	√	√	√	√	√				√	√	√
15457.1	√	√		√		√	√	√	√										√		√
15460.5				√		√	√	√	√				√	√	√	√	√	√			
15464.9	√			√	√		√	√	√	√	√	√	√	√	√				√	√	√
15469.2			√		√	√	√	√			√								√	√	√
15474.5		√		√		√	√	√	√	√	√		√	√	√	√	√	√			
15479.1				√	√	√															
15485.2	√					√				√	√	√	√		√			√	√	√	√
15490.7				√	√	√	√	√	√	√	√	√	√	√	√	√			√	√	√
15494.6	√	√	√		√	√	√	√	√	√		√	√		√		√	√			
15498.2	√				√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
15505.4										√	√	√	√	√	√		√	√			
15511.9	√	√	√				√	√	√				√	√	√						
15517.1						√	√	√	√				√	√	√	√		√	√	√	√
15521.5	√			√	√	√													√	√	√
15529.9	√	√			√	√	√	√	√												√
15534.8	√						√	√	√												
15539.1					√	√	√	√	√	√	√	√	√	√	√	√	√		√	√	√
15558.2	√	√	√	√	√	√	√	√	√	√	√	√	√		√	√		√	√	√	√
15577.8	√	√	√		√		√	√	√	√	√	√	√	√	√						
15593.4	√		√			√	√	√	√	√	√	√	√	√	√		√	√	√	√	√
15598.5						√							√		√		√	√	√	√	√
15619.5	√	√	√										√	√	√		√	√	√	√	√
15627.3						√	√	√	√	√	√	√	√	√	√		√	√	√	√	√
15632.2				√		√	√	√	√	√	√	√	√	√	√		√	√			
15636.3					√	√	√	√	√	√	√	√	√		√						
15640.7				√		√	√	√	√	√	√	√	√	√	√			√	√	√	√
15647.2				√	√	√		√	√	√	√	√	√	√	√	√		√	√	√	√
15650.8			√					√	√	√	√	√	√	√	√		√	√	√	√	√
15659.8				√		√							√	√	√	√		√	√	√	√
15665.2						√							√		√	√		√		√	√

Detected protein ion signals (*m/z*)	Washing rotation frequency																				
	1 rpm			3 rpm			5 rpm			7 rpm			9 rpm			11 rpm			13 rpm		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
15674.2				√	√	√	√	√	√	√	√	√	√	√	√					√	√
15682.4					√	√				√	√	√	√	√	√	√		√			
15686.8				√					√	√	√	√	√		√						
15690.5					√	√			√	√	√		√	√	√		√	√			
15694.9				√		√	√	√	√				√	√	√	√	√	√			
15699.7				√	√	√	√	√	√	√			√	√	√	√		√	√	√	
15706.9	√		√	√	√			√										√	√		√
15710.9		√	√	√									√	√	√						
15714.7			√		√					√	√	√			√	√	√	√	√	√	√
15723.2				√	√	√	√	√	√	√	√	√	√	√	√	√	√	√			
15727.9				√		√	√	√		√	√	√									
15731.6				√	√	√				√	√	√									
15736.8				√	√	√	√	√	√	√	√	√	√	√	√			√	√	√	
15739.2				√	√	√	√	√	√	√	√	√									
15745.7		√	√		√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
15748.8				√	√					√	√	√									
15760.9		√	√	√		√	√		√	√	√	√	√	√	√	√		√	√		√
15770.9				√	√			√	√							√	√	√			
15773.8	√	√		√	√												√				
15781.5				√		√	√			√	√	√	√	√	√	√	√	√	√	√	
15787.8		√		√			√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
15795.5				√	√					√	√	√	√	√	√	√	√	√	√	√	√
15811.6						√	√	√	√	√	√	√	√	√	√	√	√	√		√	√
15820.7				√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	
15827.9				√	√		√	√	√							√	√	√	√	√	√
15833.4				√		√	√	√	√	√						√					
15840.5					√	√	√	√	√	√	√	√	√	√	√						
15844.1				√		√	√	√	√												
15853.2	√	√		√	√	√							√	√	√	√	√				
15859.8				√	√	√	√	√					√	√	√						
15864.8						√		√		√	√	√	√	√	√					√	√
15876.2				√	√	√							√	√	√	√	√	√	√	√	√
15881.6					√	√	√	√	√		√	√							√	√	√
15886.0					√		√	√	√	√	√	√		√	√		√	√	√	√	√
15894.1				√			√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
15901.9					√		√	√	√												
15905.2				√	√	√	√	√	√	√	√	√									
15909.1				√	√	√															
15916.9				√	√	√	√	√	√	√											
15920.9			√	√						√		√							√	√	
15928.9				√	√	√							√	√	√						
15934.6					√	√											√		√	√	
15938.6				√		√	√	√	√	√					√						
15946.4				√	√	√				√	√		√	√	√						
15952.6				√	√	√	√	√	√												

Detected protein ion signals (*m/z*)	Washing rotation frequency																				
	1 rpm			3 rpm			5 rpm			7 rpm			9 rpm			11 rpm			13 rpm		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
15962.3				√	√	√	√	√	√	√	√	√									
15969.6			√	√	√	√	√	√	√												
15980.6					√	√	√	√	√	√	√	√				√		√		√	√
15985.4				√	√	√				√	√	√									
15989.3							√	√	√	√	√	√									
15997.7				√	√	√	√	√	√												
16004.6				√	√	√			√	√	√	√					√				
16012.0					√					√	√	√								√	√
16029.1					√	√						√									
16047.9													√	√							
16058.3						√						√									
16091.6										√	√	√									
16097.2							√	√	√		√										
16116.6											√										
16123.3										√		√									
16129.4										√	√	√			√						
16134.0									√			√									
16143.8						√				√	√	√									
16155.5				√	√	√	√	√		√	√	√				√	√	√	√	√	√
16160.5							√	√	√	√	√	√									
16169.9						√	√	√	√	√	√	√		√	√	√	√	√	√	√	√
16173.3				√	√		√	√	√				√								
16182.9										√	√	√									
16187.9										√	√	√									
16211.7								√													
25132.5					√					√	√	√									
25143.9								√	√	√	√	√									
25149.1				√	√		√	√	√	√	√	√		√							
25158.2					√	√	√		√												
25167.0				√	√	√				√	√	√									
25172.1											√	√									
25182.1							√														
25416.1				√																	
25656.2					√																
25682.7										√	√	√									
25687.7												√									
25693.6				√		√				√	√	√									
25707.7							√	√	√		√	√			√						
25713.9					√	√				√	√	√									
25722.1								√	√												
25729.3									√	√		√									
25739.1					√			√	√												
25762.7				√																	
29096.0				√	√																
29116.4							√	√	√				√	√							

Detected protein ion signals (*m/z*)	Washing rotation frequency																				
	1 rpm			3 rpm			5 rpm			7 rpm			9 rpm			11 rpm			13 rpm		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
29127.5				√			√	√	√					√	√		√	√			
29135.1				√	√	√		√						√		√	√	√			
29141.9									√						√						
29147.2					√		√	√	√	√	√	√									
29154.3				√	√		√	√	√			√			√						
29159.9										√			√	√							
29165.3							√		√										√	√	√
29170.1				√				√			√										
29192.4				√																	
29311.7							√	√	√	√	√	√									
29322.6							√	√	√	√	√	√									
29332.6							√	√	√	√	√	√									
29353.8							√	√	√												
29369.5								√	√												
29486.9							√														
29503.8							√	√													
29535.9							√														
29755.0					√																
29769.8				√			√	√												√	
29778.9				√		√	√	√													
29800.4				√																	
29804.3										√	√	√									
29810.8					√		√	√	√	√			√	√						√	√
29820.3				√	√		√	√	√												
29832.8				√			√	√	√												
29835.1				√																	
29841.0				√		√	√	√	√	√	√	√									
29850.1				√			√	√		√									√		
29855.3				√			√	√	√												
29861.8				√	√	√						√	√	√			√	√			
29867.8				√	√	√	√	√	√		√		√	√	√	√	√	√	√		√
29878.3							√	√	√	√	√	√									
29893.0								√	√	√	√	√									
29897.4							√	√		√	√	√			√						
29907.2																√		√			
29945.2				√																	
29965.5						√															
29982.2					√																
30000.3				√									√	√	√						
30024.8							√	√	√												
30033.0					√						√						√	√			
30046.0							√	√	√												
30075.2							√						√	√	√	√	√				
30083.8							√	√	√												
30096.8					√	√	√					√							√	√	√

Detected protein ion signals (*m/z*)	Washing rotation frequency																				
	1 rpm			3 rpm			5 rpm			7 rpm			9 rpm			11 rpm			13 rpm		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
30105.1							√	√	√												
30112.1											√	√									
30137.8						√															
30283.0									√												
30299.9											√	√		√							
30338.7					√																
30341.1							√	√	√												
30348.1										√			√	√	√	√	√				
30356.5							√		√												
30368.8							√	√	√												
30380.7							√	√					√	√	√	√	√				
30391.2										√	√	√	√	√	√	√	√				
30395.4					√	√	√	√	√												
30403.0					√	√															
30409.2								√	√												
30414.3										√	√	√									
30418.0				√	√			√													
30422.4				√																	
30424.5				√	√		√	√					√	√					√	√	√
30434.5				√				√					√	√	√			√			
30440.6				√	√	√				√	√		√	√	√						
30447.0				√	√	√	√	√	√	√	√	√							√	√	√
30456.7				√	√	√							√	√	√	√	√	√			
30465.3				√			√														
30470.2				√	√	√	√	√	√									√			
30475.3				√																	
30478.6				√	√				√		√	√	√	√	√	√	√			√	√
30483.9						√													√	√	√
30487.1				√		√							√	√	√		√	√			
30492.7					√					√	√	√	√			√					
30495.5				√		√	√	√	√					√	√						
30500.8							√	√								√		√	√	√	√
30510.7				√	√	√	√	√	√								√	√			
30516.1				√		√													√		
30558.7				√			√														
30568.8				√																	
30578.9				√																	
30581.4				√	√	√															
30594.0				√																	
30607.2										√											
30614.2					√																
30626.9				√																	
30639.6				√																	
30653.0							√			√	√	√									
30674.5													√	√	√				√		√

Detected protein ion signals (*m/z*)	Washing rotation frequency																				
	1 rpm			3 rpm			5 rpm			7 rpm			9 rpm			11 rpm			13 rpm		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
30679.5				√						√	√										
30682.3																√					
30688.1				√																	
30695.3				√				√													
30699.4													√								
30705.9				√																	
30728.0																	√				
30742.1				√	√																
30757.5				√	√																
30761.2								√	√												
30788.8				√	√	√															
30802.5				√	√	√															
30812.5				√	√	√															
30822.1				√	√																
30837.2				√	√	√															
30848.2												√									
30860.4				√		√				√	√										
30866.3					√	√				√		√	√	√	√				√		√
30880.9				√								√									
30932.2										√	√	√									
30941.0							√	√	√												
30958.2										√											
30975.7							√			√		√									
30985.6												√						√			
30993.5										√	√	√	√								
31008.7				√			√			√	√	√	√	√	√						
31017.2					√					√	√										
31023.6							√		√				√	√							
31031.0				√	√	√		√	√	√	√	√	√	√	√						
31039.9							√	√	√	√	√	√	√	√	√						
31046.3						√		√				√	√	√							
31053.1								√	√						√						
31061.3						√											√				
31065.0				√	√	√		√	√	√	√	√	√	√							
31069.9								√	√					√	√						
31077.9						√	√	√	√												
31081.0				√						√	√	√	√			√	√	√			√
31089.1							√		√										√	√	√
31096.1				√	√	√	√	√				√				√					
31104.4				√	√	√															
31114.5				√	√	√															
31115.1							√	√	√	√	√	√	√	√	√	√	√	√			
31124.6																			√	√	
31137.5				√	√	√															
31150.3				√	√	√															

Detected protein ion signals (*m/z*)	Washing rotation frequency																				
	1 rpm			3 rpm			5 rpm			7 rpm			9 rpm			11 rpm			13 rpm		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
31181.8				√	√	√															
31214.8					√								√	√	√						
31226.6							√	√													
31234.6											√										
31247.0										√		√									
31258.8										√	√	√									
31285.2				√		√															
31291.1					√					√											
31299.5										√	√	√									
31318.5							√					√									
31331.8										√											
31359.3								√													
31397.8					√					√	√	√									
31443.9										√	√										
31478.2												√									
31561.3											√										
31605.1				√		√															
31626.2				√		√															
31644.4				√		√															
Number of detected proteins	120	121	118	271	269	272	273	270	273	266	257	261	233	231	226	209	212	208	183	184	180
Average number of detected proteins	120			270			272			261			230			209			182		

Note: "√" means the protein ion could be detected in three technical duplicates.

Table S8. Overview of the commonly used washing strategies for the enhancement of protein *in situ* detection in biological tissues by MALDI-MS.

No.	Washing solvents	Tissue sections	Thickness (μm)	Washing strategies	Washing time	Matrix	Mass range (Da)	Number of detected proteins	Refs.
1		Rat liver	12	70% EtOH, 70% EtOH, followed by 100% EtOH	30 s and 60 s	SA	2,000-20,000	--	5
2		Rat liver	12	70% EtOH followed by 90% EtOH/9% acetic acid	30 s each	SA	2,000-20,000	124	6
3		Rat brain	12	70% EtOH followed by 100% EtOH wash	30 s each	SA	2,000-25,000	102	7
4		Rat kidney	12	70% EtOH for 30 s, 90% EtOH for 30 s, followed by wash with ammonium acetate buffer wash	30 s each	SA	2,000-20,000	81	8
5	EtOH	Rat liver	12	70% EtOH for 30 s, 100% EtOH for 30 s, deionized water [a], 70% EtOH for 30s; 100% EtOH for 30 s	30 s each	CHCA	800-3,000	--	5
6		Rat kidney and liver	12	70% EtOH for 30 s, 100% EtOH for 30 s, Formate buffer [b] for 90 s, DI water for 5 s	5 s, 30 s, 90 s	SA	2,000-20,000	122	8
7		Mouse pancreas	10	70% EtOH followed by 100% EtOH wash	60 s each	SA	2,500-2,5000	119	9
8		Mice	10	70% EtOH for 60 s, 100% EtOH for 60 s, 100% $CHCl_3$ for 10 s	10 s, 60 s	CHCA	--	--	10
9		Rat liver	12	70% EtOH for 30 s, 100% EtOH for 30 s, Carnoy's fluid [c] for 90 s, 100% EtOH for 30 s,	30 s, 120 s	SA	2,000-25,000	--	11
10	iProH	Rat liver	12	70% iProH followed by 95% iProH	30 s each	SA	2,000-20,000	129	6
11		Rat kidney	12	70% iProH, 100% iProH, and pure water	60 s	SA	2,500-20,000	--	12
12	MeOH	Rat liver	12	70% MeOH followed by 95% MeOH	30 s each	SA	2,000-20,000	115	6

No.	Washing solvents	Tissue sections	Thickness (μm)	Washing strategies	Washing time	Matrix	Mass range (Da)	Number of detected proteins	Refs.
13	ACN	Rat liver	12	100% ACN	60 s	SA	2,000-20,000	85	[6]
14	$CHCl_3$	Rat liver	12	100% $CHCl_3$	60 s	SA	2,000-20,000	106	[13]
15	Toluene	Rat liver	12	100% toluene	60 s	SA	2,000-20,000	93	[6]
16	Hexane	Rat liver	12	100% hexane	60 s	SA	2,000-20,000	101	[6]
17	Xylene	Rat liver	12	100% xylene	60 s	SA	2,000-30,000	113	[6]
18	Water	Rat liver	12	100% water	60 s	SA	2,000-20,000	96	[6]
19	*t*-BME	Rat liver	12	100% *tert*-butyl methyl ether	60 s	SA	2,000-20,000	100	[6]

Note: EtOH, ethanol; iPrOH, isopropanol; ACN, acetonitrile; $CHCl_3$, chloroform; *t*-BME, *tert*-butyl methyl ether; SA, sinapinic acid; TFA, trifluoroacetic acid; CHCA, α-cyano-4hydroxycinnamic acid.

[a] Dip-washing in two different jars (five short dips each).

[b] Carnoy's fluid (6:3:1 EtOH: $CHCl_3$: acetic acid).

[c] Ammonium formate 500 mM in 10% ACN and 0.1% TFA.

Table S9. Proteins *in situ* detected by (+)MALDI-MS in the rat liver tissue sections washed with iPrOH-CAWS (*i.e.*, 70% and 95% iPrOH washing), Carnoy's washing (*i.e.*, EtOH/$CHCl_3$/acetic acid (6:3:1, v/v/v) washing), EtOH-CAWS (*i.e.*, 70% EtOH followed by 90% EtOH/9% acetic acid washing), and optimal MeOH-CDWS (*i.e.*, 100%, 95%, and 70% MeOH washing), respectively (biological replicates, n=3).

Detected protein ion signals (*m/z*)	iPrOH-CAWS			Carnoy's Wash			EtOH-CAWS			MeOH-CDWS		
	1	2	3	1	2	3	1	2	3	1	2	3
2063.0											√	√
2126.8										√	√	√
2139.8												√
2177.9										√	√	
2191.6			√									√
2363.3	√	√	√									√
2448.8										√		√
2483.9	√	√	√									√
2492.4	√	√	√									
2503.3										√		√
2531.3			√									
2542.2	√	√	√									
2548.2										√	√	√
2563.2	√	√	√									√
2570.4										√	√	√
2581.4		√	√							√	√	√
2602.3	√	√	√							√		√
2788.3	√	√	√									
2794.9										√	√	√
2850.0												√
2999.1			√									
3030.3	√	√	√									
3037.7	√	√	√							√	√	√
3216.8										√	√	√
3252.3		√	√							√	√	√
3276.6	√		√								√	√
3290.2	√		√									
3341.4	√		√									
3350.0	√									√	√	√
3363.5			√									
3383.7	√	√	√							√	√	√
3404.3	√	√	√									
3409.5	√		√							√	√	√
3420.6	√	√	√							√	√	√

Detected protein ion signals (*m/z*)	iPrOH-CAWS			Carnoy's Wash			EtOH-CAWS			MeOH-CDWS		
	1	2	3	1	2	3	1	2	3	1	2	3
3448.5	√	√	√							√	√	√
3451.4										√		√
3458.8										√	√	√
3479.2										√	√	√
3496.2										√	√	√
3517.2												√
3530.2	√	√	√									
3539.7												√
3558.9	√	√	√									
3565.2	√	√								√	√	√
3576.4												√
3588.1			√									√
3602.4	√	√	√									√
3875.8	√	√	√									√
3883.2										√	√	√
3914.6	√	√	√									
4226.4										√	√	√
4523.8	√	√	√							√	√	√
4620.9										√	√	√
4899.1				√	√	√						
4956.2										√	√	√
4964.2	√	√	√							√	√	√
5053.1	√	√	√				√	√	√			
5137.8								√	√			
5165.5	√	√	√				√	√	√			
5325.4				√	√	√						
5363.8	√	√	√		√							
5441.5				√	√	√	√	√	√	√	√	√
5456.5				√	√	√						
5462.1				√	√	√		√	√			
5479.3				√	√	√	√	√	√			
5646.7				√	√	√						
5666.7				√	√	√						
5702.7				√	√	√	√	√	√			
5749.7				√	√	√	√	√				
6075.1				√	√	√	√		√			
6207.2										√	√	√
6223.5					√	√		√	√	√	√	√
6245.6				√	√	√	√	√		√	√	√

Detected protein ion signals (*m/z*)	iPrOH-CAWS			Carnoy's Wash			EtOH-CAWS			MeOH-CDWS		
	1	2	3	1	2	3	1	2	3	1	2	3
6255.7					√	√		√	√	√	√	√
6260.2					√	√	√	√	√	√	√	√
6275.7							√		√	√	√	√
6299.5											√	√
6312.4							√	√	√	√	√	√
6533.8					√	√	√		√			
6538.7				√	√	√	√					
6567.2				√	√	√	√	√	√			
6573.8										√	√	√
6648.4							√		√	√	√	√
6686.9							√		√	√	√	√
6692.4				√	√	√						
6811.8			√	√	√	√						
6822.8	√	√	√						√	√	√	√
6835.5		√	√				√	√	√			
6969.1		√	√						√			
7096.6	√		√					√	√			
7109.7	√		√									
7142.7	√	√	√				√	√	√	√	√	√
7149.9	√		√									
7175.3		√	√				√	√	√			
7179.1	√	√						√	√			
7186.1		√	√					√	√	√	√	√
7211.2		√	√				√	√	√			
7232.7								√	√			
7271.6									√			
7305.4				√	√	√	√		√			
7322.6	√	√	√									
7328.8	√	√	√									
7347.5				√	√	√		√	√			
7411.1	√	√	√				√		√			
7440.2								√	√			
7488.1	√	√	√	√	√	√			√	√	√	√
7498.2	√	√	√		√	√						
7505.1								√	√	√	√	√
7520.3							√		√	√	√	√
7523.9								√	√	√	√	√
7526.9								√	√	√	√	√
7535.3									√			

Detected protein ion signals (*m/z*)	iPrOH-CAWS			Carnoy's Wash			EtOH-CAWS			MeOH-CDWS		
	1	2	3	1	2	3	1	2	3	1	2	3
7540.4							√	√	√	√	√	√
7546.7	√	√	√					√	√			√
7585.5							√	√	√			√
7601.7												√
7607.1										√	√	√
7650.1	√	√						√	√	√	√	√
7661.1		√	√				√	√	√			
7775.5				√	√	√						
7783.7				√	√	√						
7796.1			√	√	√	√						
7808.3				√	√	√	√	√				
7824.3				√	√	√						
7840.4			√	√	√	√	√	√	√			
7846.0				√	√	√		√				
7854.3				√	√	√	√	√		√	√	√
7868.9				√	√	√		√	√			
7873.3	√	√	√	√	√	√	√		√	√	√	√
7880.7										√	√	√
7900.3							√		√			
7920.0				√	√	√		√	√	√	√	√
7934.1										√	√	√
8091.5				√	√	√	√	√	√			
8365.9				√	√	√			√			
8409.5								√	√	√	√	√
8435.8	√	√	√									
8463.5				√	√	√	√		√	√	√	√
8472.5							√		√	√	√	
8488.9									√	√	√	√
8510.6							√		√	√	√	√
8528.2								√	√	√	√	√
8539.7							√	√		√	√	√
8546.5										√	√	√
8550.1							√	√		√	√	
8583.4							√		√		√	
8590.5										√	√	
8599.5												
8928.6	√	√	√					√	√	√	√	
8944.1							√	√		√	√	√
8981.7									√	√	√	√

Detected protein ion signals (*m/z*)	iPrOH-CAWS			Carnoy's Wash			EtOH-CAWS			MeOH-CDWS		
	1	2	3	1	2	3	1	2	3	1	2	3
8996.9								√	√			
9168.2				√	√							
9309.4				√	√	√						
9323.1				√	√	√						
9507.6				√	√	√						
9509.0				√		√						
9583.5				√	√	√						
9602.2				√	√	√						
9617.3				√	√	√						
9620.2				√	√	√						
9719.3	√	√	√									
9853.4	√		√									
9866.3	√		√									
9876.1		√								√	√	√
9890.1	√	√	√	√	√	√						
9909.3	√	√	√							√	√	√
9925.9										√	√	√
9932.8	√	√	√							√	√	√
9940.9		√										
9945.3	√	√										
9949.7										√	√	√
9959.9	√	√	√	√	√	√						
9976.7	√	√	√							√	√	√
10016.6										√	√	√
10034.7										√	√	√
10070.0												√
10115.6										√	√	√
10234.6				√	√	√						
10263.0											√	√
10679.2	√	√	√									
10693.7				√	√	√						
10697.1				√	√							
10744.7	√	√	√	√								
10786.0			√	√								
10839.0			√	√								
10849.4				√	√	√						
10853.4	√	√	√	√	√	√						
10879.8	√	√	√	√	√							
10912.0			√	√	√							

Detected protein ion signals (*m/z*)	iPrOH-CAWS			Carnoy's Wash			EtOH-CAWS			MeOH-CDWS		
	1	2	3	1	2	3	1	2	3	1	2	3
10944.1				√	√	√				√	√	√
11175.6	√	√	√	√	√	√						
11253.3				√	√	√						
11268.5				√		√						
11280.5				√	√	√						
11306.8				√	√	√				√	√	
11315.9				√	√	√						
11319.8				√	√	√				√	√	
11335.4				√	√	√						
11347.5				√	√	√				√	√	
11359.8				√	√	√				√		
11365.7				√	√	√				√	√	
11378.5				√	√	√				√	√	
11384.4			√	√						√	√	
11389.6			√	√	√	√				√	√	√
11619.6	√	√	√									
11633.2	√		√									
11655.4				√	√	√						
11659.2				√		√						
12103.7				√	√	√				√	√	√
12112.5	√	√	√									
12131.1										√	√	√
12406.5				√	√	√						
12414.7				√	√	√						
13129.4				√	√	√						
13738.0				√	√							
13757.9											√	√
13774.7										√	√	
13795.1										√	√	√
13810.2								√	√	√	√	√
13815.5										√	√	
13836.4								√	√	√	√	
13844.3										√	√	
13897.0								√	√	√	√	
13936.2										√	√	
13943.0								√	√	√	√	
13955.6							√			√	√	
13960.1									√	√	√	
13965.2										√	√	

Detected protein ion signals (*m/z*)	iPrOH-CAWS			Carnoy's Wash			EtOH-CAWS			MeOH-CDWS		
	1	2	3	1	2	3	1	2	3	1	2	3
13978.9							√	√	√	√	√	
14006.6									√	√	√	
14021.5							√	√		√	√	
14030.1										√	√	
14046.7										√	√	
14067.4							√	√		√	√	
14086.8										√	√	
14108.6			√				√	√	√			
14117.4							√	√		√	√	
14130.9				√	√	√	√		√			
14135.5					√	√		√	√			
14140.9	√	√	√				√	√	√	√	√	
14150.0									√			
14160.9							√	√		√	√	√
14165.5							√		√	√	√	
14172.3	√	√	√				√	√				
14180.9		√					√	√				
14186.1		√					√	√	√			
14205.2	√	√		√	√	√	√		√	√	√	
14218.2	√	√	√		√	√	√	√		√	√	√
14230.8	√	√					√		√			
14246.4				√	√	√	√	√		√	√	√
14265.1	√	√	√	√	√	√	√		√			
14284.7	√	√		√	√	√	√		√	√	√	√
14295.6	√	√										
14306.6		√	√				√		√	√	√	√
14322.3	√	√	√							√	√	√
14339.6	√	√	√							√	√	√
14350.7	√	√	√									
14362.0										√	√	√
14372.4										√	√	√
14382.8	√	√					√		√	√	√	
14393.1	√	√	√									
14398.5							√	√	√			
14403.6							√	√		√	√	√
14415.8	√	√					√	√	√	√	√	
14426.1	√	√	√				√	√	√	√	√	√
14438.9	√	√								√	√	√
14450.5	√	√	√				√	√		√	√	√

Detected protein ion signals (*m/z*)	iPrOH-CAWS			Carnoy's Wash			EtOH-CAWS			MeOH-CDWS		
	1	2	3	1	2	3	1	2	3	1	2	3
14455.6	√	√	√				√	√		√	√	√
14467.7	√	√	√									
14488.6	√		√				√	√	√	√	√	√
14490.5	√	√	√				√					
14510.5	√		√				√	√	√			
14870.4	√		√		√	√						
14882.2	√	√		√	√	√				√	√	√
14892.7	√	√	√	√	√	√					√	√
14903.1	√	√		√							√	√
14959.1	√	√	√	√	√	√						
14978.4										√	√	√
14986.8	√	√	√									
15007.3	√	√		√	√	√				√	√	
15023.2	√	√	√									
15027.2	√	√		√	√	√						
15048.3	√	√					√	√				
15061.7				√	√	√	√		√			
15067.8				√	√	√		√	√	√	√	√
15075.9	√	√		√	√	√						
15091.2	√	√	√	√	√	√	√	√				
15100.1	√	√		√	√	√	√	√			√	√
15109.0	√	√		√	√	√						
15116.1	√	√		√	√	√						
15124.3				√	√	√				√	√	√
15131.8		√	√				√	√				
15142.4			√	√	√	√	√		√	√	√	
15150.2				√	√	√						
15156.3							√	√	√	√	√	
15160.1	√	√	√	√	√	√	√					
15172.5	√	√			√	√	√	√	√	√	√	
15175.0					√	√	√	√				
15183.5				√	√	√	√	√	√	√	√	
15190.8	√	√	√	√	√	√	√	√			√	√
15208.7				√	√	√	√	√	√	√	√	
15217.2				√	√	√	√		√			
15223.8				√	√	√	√	√				
15229.4							√	√	√	√	√	
15237.2				√	√	√						
15240.0					√	√		√	√	√		√

Detected protein ion signals (*m/z*)	iPrOH-CAWS			Carnoy's Wash			EtOH-CAWS			MeOH-CDWS		
	1	2	3	1	2	3	1	2	3	1	2	3
15255.0				√	√	√						
15262.1				√	√	√						
15268.5										√	√	
15270.2							√	√	√	√		√
15279.2				√	√	√	√			√		
15285.2				√	√	√	√	√		√	√	√
15292.9				√		√	√	√		√	√	√
15306.6				√	√	√						
15309.5				√		√	√	√	√	√	√	√
15314.3				√	√	√	√	√		√	√	
15325.3				√	√	√	√	√		√	√	√
15332.8				√	√	√	√	√		√	√	
15344.8				√	√	√	√	√	√	√	√	√
15354.2				√	√	√	√	√		√	√	√
15361.4				√	√	√	√	√	√	√	√	√
15374.6										√		√
15382.8				√	√	√				√		√
15388.1				√	√		√	√	√	√	√	√
15397.7				√	√	√						
15403.2				√	√	√	√	√	√	√	√	√
15412.0				√	√	√						
15417.4				√	√	√						
15445.2				√	√	√						
15452.7					√	√	√	√	√		√	√
15463.4				√	√	√						
15470.7				√	√		√	√	√	√	√	√
15475.1				√	√	√						
15481.8				√	√	√	√		√	√		√
15513.9				√	√	√						√
15526.4				√	√	√	√	√	√	√	√	√
15562.4							√	√	√	√	√	√
15574.6				√	√	√		√	√		√	√
15589.4								√	√		√	√
15627.2							√	√	√	√	√	√
15634.5								√	√	√	√	√
15657.9				√	√	√	√	√		√	√	√
15674.2				√		√						
15683.4	√	√	√	√		√						
15686.8				√	√	√						

Detected protein ion signals (*m/z*)	iPrOH-CAWS			Carnoy's Wash			EtOH-CAWS			MeOH-CDWS		
	1	2	3	1	2	3	1	2	3	1	2	3
15705.6	√			√	√	√	√	√		√	√	√
15712.4				√	√	√						
15719.3	√	√	√	√	√	√						
15732.0					√	√						
15743.3	√	√	√	√	√	√	√	√	√	√	√	√
15757.0			√		√	√	√	√				
15767.7	√	√	√		√	√	√		√	√	√	
15777.4							√	√	√	√	√	√
15782.8				√	√	√	√	√		√	√	√
15789.8				√	√	√						
15793.5				√	√	√	√	√	√			
15799.3							√	√				
15804.5							√	√		√	√	√
15809.8				√	√	√	√	√				
15820.7				√	√	√		√	√			
15827.9				√	√	√		√	√	√	√	
15830.1							√	√		√	√	√
15836.9									√	√	√	√
15848.1							√	√	√	√	√	
15853.3				√		√						
15869.7				√	√	√	√	√	√	√	√	
15877.2						√				√	√	√
15882.7				√		√		√	√	√		
15898.7				√	√	√						
15911.7				√	√	√				√	√	√
15925.1										√	√	√
15940.2							√	√	√			
15946.7							√	√	√	√	√	√
15955.5										√	√	√
15966.2										√	√	√
16008.4										√	√	√
16013.9										√	√	
16102.9										√		√
18645.6										√	√	√
18654.1										√	√	√
18685.4										√	√	√
18689.6										√	√	√
18723.1										√	√	√
18731.0										√	√	√

Detected protein ion signals (*m/z*)	iPrOH-CAWS			Carnoy's Wash			EtOH-CAWS			MeOH-CDWS		
	1	2	3	1	2	3	1	2	3	1	2	3
18738.9										√	√	√
18750.8										√	√	√
18768.5										√	√	√
18780.4										√	√	√
18806.1										√	√	√
18812.0										√		√
18950.1												√
18962.2										√		√
19849.2										√		√
20700.1										√	√	√
20716.7										√	√	√
20720.8										√	√	√
20733.3										√	√	√
20752.8										√	√	√
20774.8										√	√	
20789.4										√	√	
21162.9										√	√	
21190.2										√	√	
21215.4										√	√	
21781.5										√	√	
21791.8										√	√	
21800.7										√	√	
21822.0										√	√	
21874.1										√	√	√
21879.4										√	√	√
21903.9										√	√	√
21949.9										√	√	√
21952.0										√	√	
21964.8										√	√	√
21971.2										√	√	√
21973.3										√	√	√
22028.9										√	√	√
22050.3										√	√	√
22061.0										√	√	√
22071.7										√	√	√
22086.6										√	√	
23439.1	√	√	√									
23445.2	√	√	√									
23451.9	√	√	√									

Detected protein ion signals (*m/z*)	iPrOH-CAWS			Carnoy's Wash			EtOH-CAWS			MeOH-CDWS		
	1	2	3	1	2	3	1	2	3	1	2	3
23463.5	√	√	√									
25724.2				√	√	√						
25734.8				√	√	√						
25762.3				√	√	√						
25773.9				√	√	√						
25776.5				√	√	√						
25788.1				√	√	√						
25790.4				√	√	√						
25799.7				√	√	√						
25811.3				√	√	√						
29421.8											√	√
29843.2										√		√
29853.1										√	√	√
29863.1										√	√	√
29882.9										√	√	√
29903.5										√	√	√
29920.1										√	√	√
29922.6										√	√	√
29927.6											√	√
29940.0										√	√	√
29950.9											√	√
29957.3										√	√	√
29969.8											√	√
29976.7										√	√	√
30014.5											√	√
30019.5										√	√	√
30022.0										√	√	√
30028.9										√	√	√
30039.4											√	√
30046.2										√	√	√
30061.8										√	√	√
30066.7										√	√	√
30084.1										√	√	√
30089.1										√	√	√
30096.8										√		√
30101.6										√		√
30123.9										√	√	√
30138.9										√	√	√
30151.4											√	√

Detected protein ion signals (*m/z*)	iPrOH-CAWS			Carnoy's Wash			EtOH-CAWS			MeOH-CDWS		
	1	2	3	1	2	3	1	2	3	1	2	3
30161.3										√	√	√
30188.8										√	√	√
30279.8												√
30402.1												√
30418.7												√
30452.6												√
30470.3												√
30495.2												√
30517.5												√
30581.4										√	√	√
30604.3										√	√	√
30618.6										√	√	√
30636.2										√	√	√
30646.8										√	√	√
30651.6											√	√
30658.7										√	√	√
30671.2										√	√	√
30678.2											√	√
30691.2										√	√	√
30704.4										√	√	√
30708.0												√
30719.5										√		√
30731.3												√
30742.1										√	√	√
30757.5										√	√	√
30788.8										√	√	√
30802.5										√	√	√
30812.5										√	√	√
30860.4										√		√
30896.8												√
30910.0												√
30931.4												√
30944.3												√
30965.6											√	√
30976.9											√	√
30987.1											√	√
31042.0										√		√
31061.3										√		√
31094.4												√

Detected protein ion signals (*m/z*)	iPrOH-CAWS			Carnoy's Wash			EtOH-CAWS			MeOH-CDWS		
	1	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**
31119.7										√		√
31160.1												√
31181.8										√		√
31531.1												√
31552.4												√
31575.8												√
31590.6												√
31605.1												√
31626.2												√
31644.4												√
31665.3												√
31683.0												√
31701.6												√
31711.7												√
31728.2												√
Number of detected proteins	114	112	116	159	161	163	121	123	123	270	270	275
Average number of detected proteins		114			161			121			272	

Note: "√" means the protein ion could be detected in three technical duplicates.

Table S10. Comparison of protein *in situ* detected by (+)MALDI-MS in the rat brain tissue sections washed with MeOH-CAWS and optimal MeOH-DAWS, respectively (biological replicates, n=3).

Detected protein ion signals (*m/z*)	MeOH-CAWS				MeOH-CDWS				Ratio of (S/N_{CDWS})/ (S/N_{CAWS})
	1	2	3	Average S/N	1	2	3	Average S/N	
3355.3	√	√	√	3.85					
3461.6						√	√	5.53	2.68
3498.8		√	√	3.01	√	√		4.94	1.64
4025.9					√	√		4.20	
4208.3	√	√	√	5.64					
4225.5	√	√	√	4.91					
4247.6	√	√	√	6.64					
4252.2					√	√	√	4.38	
4535.5	√	√		3.10					
4682.9	√			3.07					
4735.0	√	√		3.29					
4742.9	√	√	√	4.83					
4795.7	√	√		3.03			√	4.55	1.50
4810.5	√	√	√	8.99	√	√	√	19.22	2.14
4815.2					√	√	√	6.20	
4846.6	√			3.43	√		√	5.70	1.66
4886.3	√	√	√	5.30	√	√	√	7.22	1.36
4908.1							√	3.31	
4940.4	√	√	√	3.98	√	√	√	12.65	3.17
4944.6					√	√	√	5.80	
4966.5	√	√	√	9.35	√	√	√	52.37	5.60
4972.8	√	√	√	3.03	√	√	√	4.22	1.39
4980.9		√	√	3.34			√	12.45	3.73
4987.5		√		3.80	√	√	√	9.03	2.38
4992.9	√	√		3.16	√		√	4.98	1.58
4999.5	√	√	√	3.55			√	9.31	2.62
5156.0					√	√	√	6.19	
5696.0	√	√		3.11	√	√		5.77	2.73
6708.2		√	√	3.28		√	√	4.76	2.08

Detected protein ion signals (*m/z*)	MeOH-CAWS				MeOH-CDWS				Ratio of (S/N_{CDWS})/ (S/N_{CAWS})
	1	2	3	Average S/N	1	2	3	Average S/N	
6225.5	√	√	√	3.22					
6271.4	√	√	√	3.18	√	√	√	10.95	3.44
6648.7	√	√	√	5.37	√	√	√	10.62	1.98
6656.9							√	3.38	
6662.6							√	3.57	2.33
6672.5	√	√	√	3.94	√	√	√	7.92	2.01
6682.8	√	√		3.11	√			5.13	1.65
6686.3	√	√	√	3.12		√	√	4.27	1.36
6691.7	√	√	√	4.55	√	√	√	6.29	1.77
6701.8	√	√		3.59	√	√		5.45	1.52
6714.0	√	√	√	18.22	√	√	√	38.06	2.09
6718.5	√	√	√	9.77	√	√	√	16.43	1.68
6727.3	√		√	24.98	√	√	√	49.15	1.97
6739.7	√	√	√	3.78	√		√	5.62	1.49
6746.5	√	√	√	4.88			√	7.49	1.53
6756.1	√	√	√	4.96	√	√	√	11.0	2.22
6762.0	√	√	√	3.17		√		13.59	4.29
6774.9		√	√	3.56	√	√		8.89	2.50
6783.3	√		√	3.24	√	√		5.36	1.65
6789.3	√		√	4.22	√	√		5.67	1.34
6796.9					√	√	√	4.28	
6803.3	√	√	√	3.50					
6810.4	√		√	3.92	√	√	√	6.92	1.77
6919.9	√		√	3.85	√	√	√	6.86	1.78
6930.2	√	√	√	3.49	√	√	√	6.47	1.85
6935.2	√		√	3.11	√		√	6.78	2.18
7038.9	√	√	√	3.69	√	√	√	4.94	1.34
7058.7	√	√	√	3.42	√	√	√	7.11	2.08
7066.2	√	√	√	3.64	√		√	7.70	2.11
7074.0					√	√	√	4.52	
7078.3					√	√	√	4.52	

Detected protein ion signals (*m/z*)	MeOH-CAWS				MeOH-CDWS				Ratio of (S/N_{CDWS})/ (S/N_{CAWS})
	1	2	3	Average S/N	1	2	3	Average S/N	
7090.3					√		√	4.73	
7098.3					√		√	3.81	
7103.3							√	4.02	
7527.6					√	√		4.40	
7544.2	√		√	3.62				4.97	1.38
7580.9	√	√	√	12.7	√	√	√	21.7	1.71
7585.8						√		5.16	
7592.2	√		√	3.64		√		6.66	1.83
8451.5	√		√	3.24		√	√	4.32	1.33
8468.2	√	√	√	3.03	√	√		5.82	1.92
8476.4	√		√	3.51					
8488.2	√		√	3.66					
8497.8	√		√	5.21					
8524.5	√	√	√	4.45					
8542.6					√	√	√	7.88	
8558.3	√	√	√	3.58	√	√	√	16.57	4.62
8567.3	√	√	√	4.52	√	√	√	7.74	1.71
8573.8	√	√	√	3.08	√	√		4.22	1.37
8579.3	√	√	√	4.03	√		√	11.94	2.96
8593.3	√	√	√	4.12	√		√	6.48	1.57
8603.0	√	√	√	3.22	√	√	√	6.19	1.92
8616.7						√	√	4.34	
8648.3	√	√	√	4.36	√	√	√	2.21	1.97
8657.7	√		√	3.19	√		√	4.63	1.45
8764.6						√		3.29	
8936.7						√	√	3.80	
9286.4		√	√	5.71					
9291.6					√	√	√	3.19	
9329.0					√	√	√	3.80	
9344.9	√	√	√	4.58	√	√	√	6.58	1.44
9368.4	√	√	√	3.79	√	√	√	5.79	1.53

Detected protein ion signals (*m/z*)	MeOH-CAWS				MeOH-CDWS				Ratio of (S/N_{CDWS})/ (S/N_{CAWS})
	1	2	3	Average S/N	1	2	3	Average S/N	
9376.4					√	√	√	4.21	
9404.9							√	3.48	
9890.3	√	√	√	3.21	√	√	√	10.29	3.21
9926.3	√	√	√	5.17					
9943.5	√	√	√	6.31					
9957.1	√		√	3.20	√		√	4.80	1.50
9967.9							√	3.82	
9979.6					√	√		3.82	
9991.6	√	√	√	6.56	√	√	√	14.44	2.20
10019.6		√	√	4.52	√	√	√	6.82	1.51
10036.4					√	√	√	4.08	
10238.0						√		3.29	
10255.0	√	√	√	3.57					
10944.6		√	√	8.33					
11259.0						√		4.59	
11278.9					√	√	√	3.50	
11298.7	√	√	√	3.19			√	4.17	1.31
11304.7						√	√	3.92	
11319.1					√	√	√	5.96	
11329.4						√		4.55	
11346.1					√	√		5.41	
11361.0	√	√	√	3.72	√	√	√	6.51	1.75
11834.4					√	√	√	5.14	
12133.5	√	√	√	3.56	√	√	√	4.55	1.28
12148.7	√	√	√	3.47	√	√	√	8.05	2.32
12167.9					√	√	√	4.82	
12368.4	√	√	√	3.11	√	√	√	6.01	1.93
13787.1						√	√	3.49	
13803.3						√	√	4.49	
13813.9						√	√	4.87	
13819.3						√	√	4.13	

Detected protein ion signals (*m/z*)	MeOH-CAWS				MeOH-CDWS				Ratio of (S/N_{CDWS})/ (S/N_{CAWS})
	1	2	3	Average S/N	1	2	3	Average S/N	
13849.9						√	√	4.65	
13858.9						√		3.85	
13866.8						√	√	4.03	
13874.8					√		√	3.77	
13883.0					√	√	√	3.31	
13900.9	√	√	√	3.72	√			6.70	11.0
13907.1					√	√	√	3.00	2.21
13924.8					√		√	3.78	
13929.5					√	√	√	4.53	
13936.6							√	4.06	
13943.4					√	√	√	4.20	
13962.2					√	√	√	5.43	
13990.0					√	√	√	6.74	
13999.8	√	√	√	4.54	√		√	10.63	2.34
14005.1					√	√	√	9.07	
14009.2					√			12.24	
14020.3	√	√	√	5.95	√			10.61	1.78
14029.1					√	√	√	12.74	
14037.4	√	√		4.62	√			13.09	2.83
14044.2	√	√	√	7.24	√	√	√	16.34	2.26
14053.5	√	√	√	6.11	√	√	√	15.47	2.53
14063.1	√	√		4.74	√		√	14.29	3.02
14068.9	√	√	√	4.34	√	√		11.66	2.69
14080.8	√	√	√	5.98					
14089.5	√	√		4.30	√	√	√	21.69	
14096.1	√	√	√	4.84					
14105.5		√	√	6.50	√	√	√	19.15	2.95
14126.7	√	√	√	6.70	√	√	√	27.89	4.16
14137.0	√	√		6.27		√	√	25.61	4.08
14146.7						√	√	13.62	
14154.3	√	√	√	6.23	√		√	12.29	1.97

Detected protein ion signals (*m/z*)	MeOH-CAWS				MeOH-CDWS				Ratio of (S/N_{CDWS})/ (S/N_{CAWS})
	1	2	3	Average S/N	1	2	3	Average S/N	
14159.3					√	√		9.70	2.25
14166.8		√	√	6.28		√	√	20.09	3.20
14172.3	√	√		4.29	√		√	10.44	2.43
14183.5	√	√		4.67	√	√		16.14	3.46
14189.0		√	√	3.68	√	√		6.22	1.69
14193.4	√	√	√	4.19	√	√	√	12.99	3.10
14203.8	√	√		4.44	√	√	√	12.05	2.71
14211.0	√	√		3.96	√	√	√	11.44	2.89
14224.9	√	√		3.60		√		11.03	3.07
14239.8		√	√	3.10	√	√	√	5.23	1.69
14253.4					√		√	8.14	
14260.6					√	√	√	9.14	
14270.8					√	√		9.17	
14281.3					√	√	√	7.11	
14288.2					√	√	√	4.67	
14295.3					√	√		6.69	
14309.2					√	√	√	4.03	
14316.6					√	√		5.91	
14320.5					√		√	5.91	
14329.6					√	√		7.55	
14334.6					√		√	5.90	
14342.7					√	√		6.92	
14352.2					√	√	√	5.59	
14359.1					√			5.02	
14370.6					√	√	√	6.88	
14381.2					√		√	5.22	
14386.8					√			5.17	
14399.0					√			5.78	
14409.3					√	√	√	5.92	
14422.9					√	√		4.70	
14428.3					√	√	√	5.67	

Detected protein ion signals (*m/z*)	MeOH-CAWS				MeOH-CDWS				Ratio of (S/N_{CDWS})/ (S/N_{CAWS})
	1	2	3	Average S/N	1	2	3	Average S/N	
14435.3						√	√	4.35	
14443.7					√	√		3.32	
14459.3					√	√	√	4.15	
14464.8					√	√	√	4.10	
14478.5					√		√	4.11	
14516.9					√		√	4.73	
14527.8					√	√	√	4.04	
14558.7					√			4.36	
14570.5					√		√	5.34	
14580.9					√		√	5.39	
14590.5					√			4.10	
15823.2					√	√		3.75	
15853.9					√	√		4.52	
17153.0					√	√	√	4.34	
18349.1					√	√		3.29	
18387.9					√	√	√	4.31	
18398.2	√	√	√	3.36	√		√	6.29	1.87
18408.2					√		√	7.36	
18427.9					√		√	6.31	
18439.9					√		√	4.24	
18458.7					√		√	3.42	
18463.5					√		√	3.65	
20763.7					√	√		3.42	
21175.4					√	√	√	3.23	
21847.8						√		3.33	
21881.9		√	√	3.10	√	√	√	4.76	1.54
21901.8					√	√	√	7.66	
21912.3					√	√	√	7.03	
21923.9					√	√	√	6.17	
21933.3					√	√	√	6.54	
21938.2					√		√	6.60	

Detected protein ion signals (*m/z*)	MeOH-CAWS				MeOH-CDWS				Ratio of (S/N_{CDWS})/ (S/N_{CAWS})
	1	2	3	Average S/N	1	2	3	Average S/N	
21944.7					√	√		4.28	
21955.3					√		√	5.69	
21961.8							√	5.64	
21970.3						√		5.31	
21991.8						√	√	6.41	
21998.9						√	√	5.03	
22006.8						√	√	7.05	
22026.2					√		√	7.65	
22044.1					√	√	√	4.96	
22054.2						√	√	6.54	
22079.8						√	√	5.67	
22088.6						√	√	5.62	
22101.7						√	√	5.46	
22107.8						√	√	5.17	
22118.5						√	√	4.66	
22140.1					√			4.08	
22146.5					√		√	3.50	
22155.3					√	√	√	3.33	
22163.7					√	√	√	4.05	
22170.2					√	√	√	3.59	
22199.6						√	√	5.57	
22206.8						√	√	4.85	
22215.4						√	√	4.43	
22239.1						√	√	5.24	
22250.3						√	√	3.56	
22260.7						√	√	3.46	
22265.7						√	√	4.04	
22303.9					√	√	√	4.09	
22402.6					√	√	√	3.17	
22440.2						√		3.37	
22454.9						√		3.27	

Detected protein ion signals (*m/z*)	MeOH-CAWS				MeOH-CDWS				Ratio of (S/N_{CDWS})/ (S/N_{CAWS})
	1	2	3	Average S/N	1	2	3	Average S/N	
22477.5						√		3.95	
22487.9						√	√	5.39	
22507.4					√			3.47	
22519.3					√	√		4.93	
22528.4					√	√		3.53	
22537.8					√	√		4.22	
22547.4					√	√		4.61	
22569.8					√	√		3.86	
22577.1					√	√	√	3.89	
22589.9						√	√	4.31	
22618.2						√	√	3.99	
22637.2						√	√	3.89	
22663.2						√		3.30	
26129.3							√	4.19	
26385.3						√	√	4.15	
27330.2						√		3.34	
27373.5						√		3.01	
28108.3					√	√	√	3.10	
28127.4					√			3.26	
28142.4					√			3.49	
28152.3					√			3.45	
28185.9					√	√	√	3.61	
28223.0					√			3.94	
28232.0					√	√		4.56	
28246.6					√	√		4.83	
28256.8					√	√		4.52	
28271.4					√	√		4.20	
28280.7					√	√		3.96	
28303.9					√	√		4.08	
28337.0					√			3.57	
28355.5					√			3.37	

Detected protein ion signals (*m/z*)	MeOH-CAWS				MeOH-CDWS				Ratio of (S/N_{CDWS})/ (S/N_{CAWS})
	1	2	3	Average S/N	1	2	3	Average S/N	
28369.0					√			3.41	
Number of detected proteins	94	91	88		185	188	181		
Average number of detected proteins		91				185			

Note: "√" means the protein ion could be detected.

Table S11. Comparison of protein *in situ* detected by (+)MALDI-MS in the germinating Chinese-yew seed tissue sections washed with MeOH-CAWS and optimal MeOH-CDWS, respectively (biological replicates, n=3).

Detected protein ion signals (*m/z*)	MeOH-CAWS				MeOH-CDWS				Ratio of (S/N_{CDWS})/ (S/N_{CAWS})
	1	2	3	Average S/N	1	2	3	Average S/N	
2184.1					√			3.71	
2194.5					√			3.12	
2207.7		√		4.76					
2212.9					√	√	√	17.60	
2228.1					√	√		5.13	
2250.8					√		√	4.29	
2321.8		√		4.47	√	√	√	18.44	4.12
2366.3					√		√	5.74	
2713.0						√	√	4.23	
2807.6						√		3.18	
2829.2		√		3.46	√	√	√	8.89	2.57
2844.8	√			3.28					
2854.3					√	√		3.49	
2931.5			√	3.09					
2939.9	√	√		3.45					
2947.1						√	√	3.71	
3041.9					√	√	√	3.33	
3067.6						√	√	3.22	
3109.5					√	√	√	11.43	
3122.4					√	√	√	6.79	
3153.2	√	√	√	37.91	√	√	√	61.69	1.63
3160.5		√		4.80					
3167.3					√	√	√	33.52	
3182.5		√		9.65	√	√	√	17.28	1.80
3191.1					√	√	√	134.16	
3205.5					√	√	√	7.31	
3213.5					√	√	√	6.51	
3220.4						√	√	3.43	
3229.5		√		4.93	√	√		14.94	3.28

Detected protein ion signals (*m/z*)	MeOH-CAWS				MeOH-CDWS				Ratio of (S/N_{CDWS})/ (S/N_{CAWS})
	1	2	3	Average S/N	1	2	3	Average S/N	
3141.3	√	√		8.69	√	√	√	30.76	3.54
3252.0					√	√	√	8.79	
3292.6	√		√	7.86	√	√	√	14.52	1.85
3306.5					√	√		3.18	
3311.4					√	√		3.44	
3325.0					√	√	√	4.16	
3365.6					√	√	√	9.35	
3701.4					√	√	√	4.28	
4382.3		√	√	5.81					
4406.2					√		√	3.55	
4487.1					√	√		3.99	
4503.4					√	√		4.76	
4519.3					√	√		3.32	
4544.8					√	√		4.01	
4823.2	√	√		4.08	√	√	√	7.61	1.86
4841.6					√	√	√	15.81	
4860.1							√	4.66	
4860.4						√		4.71	
4964.2					√	√	√	13.78	
5014.1					√	√		4.09	
5122.3	√	√		4.07	√	√	√	17.09	4.20
5221.7							√	9.69	
5247.0	√			3.07					
5365.4					√	√	√	3.33	
5402.9							√	3.12	
5493.1							√	21.03	
5518.4					√	√		5.92	
5530.5							√	3.56	
5562.1					√	√	√	3.80	
5612.0					√	√	√	16.21	
5619.3					√	√		24.34	

Detected protein ion signals (*m/z*)	MeOH-CAWS				MeOH-CDWS				Ratio of (S/N_{CDWS})/ (S/N_{CAWS})
	1	2	3	Average S/N	1	2	3	Average S/N	
5650.9	√	√		3.37	√		√	7.01	2.08
5656.3					√	√	√	9.62	
5667.1		√	√	3.84		√	√	8.06	2.10
5688.2	√	√	√	9.73		√	√	11.47	1.17
5707.3	√	√	√	5.21		√	√	25.45	4.89
5716.1					√	√		6.72	
5727.1	√	√	√	14.24					
5739.4					√	√	√	24.34	
5747.4	√	√	√	11.61					
5757.5	√	√	√	6.59	√	√	√	15.84	2.44
5775.9	√	√	√	3.72	√	√	√	7.72	2.74
5784.8	√	√	√	4.98					
5800.7	√	√	√	4.00	√	√	√	7.25	1.82
5812.7	√	√	√	3.20	√	√		3.66	1.14
5819.6	√	√	√	3.50		√	√	4.17	1.18
5836.7	√	√	√	6.13	√	√	√	7.47	1.21
5851.2	√	√	√	12.33	√	√	√	33.90	2.74
5874.4	√	√	√	13.21	√	√		16.27	1.23
5889.1	√	√		14.37	√	√	√	57.88	4.28
5894.4					√	√	√	71.70	
5904.4	√	√	√	10.54					
5912.1					√	√	√	8.28	
5918.5	√	√	√	7.16	√	√		18.54	2.59
5932.5	√	√	√	4.83	√	√	√	14.86	3.79
5946.3	√			4.01	√		√	4.82	1.23
5954.2		√		4.06		√		4.21	1.03
5957.2	√		√	6.06					
5970.6					√		√	3.87	
5991.6						√	√	5.07	
6002.9					√	√		9.89	
6023.1					√			3.07	

Detected protein ion signals (*m/z*)	MeOH-CAWS				MeOH-CDWS				Ratio of (S/N_{CDWS})/ (S/N_{CAWS})
	1	2	3	Average S/N	1	2	3	Average S/N	
6033.0					√	√		6.07	
6049.3					√	√		10.95	
6069.8					√	√	√	3.83	
6075.1					√	√	√	3.10	
6244.8		√	√	3.72	√		√	8.79	2.36
6287.3		√	√	3.75	√		√	4.03	1.76
6305.4					√	√	√	23.08	
6324.0							√	3.80	
6343.8					√	√	√	6.52	
6380.7					√	√	√	4.05	
6394.6					√	√	√	5.24	
6462.0							√	4.79	
6479.8		√	√	4.45	√	√		6.14	1.38
6555.7						√	√	3.46	
6624.7					√	√		6.68	
6658.8					√	√	√	4.01	
6766.9					√	√		11.28	
7312.9					√	√	√	10.58	
7401.0	√	√	√	4.11		√	√	5.07	1.23
7599.8	√	√	√	8.05					
7637.6	√	√	√	8.91					
7696.1	√	√	√	3.39	√	√	√	14.25	4.23
7828.1						√	√	4.50	
7849.5					√	√	√	3.65	
7916.3							√	3.07	
8380.2	√	√	√	19.59					
8395.4	√	√	√	9.56					
8401.1					√	√	√	25.51	
8421.6	√	√	√	7.32	√	√	√	13.11	1.79
8437.9		√		3.48	√	√	√	6.50	1.87
8453.5	√	√	√	3.79	√	√	√	4.13	1.90

Detected protein ion signals (*m/z*)	MeOH-CAWS				MeOH-CDWS				Ratio of (S/N_{CDWS})/ (S/N_{CAWS})
	1	2	3	Average S/N	1	2	3	Average S/N	
8498.2	√	√	√	3.07	√		√	4.52	1.47
8519.8		√		4.96	√	√	√	8.99	1.81
8546.2	√		√	5.04	√	√	√	14.91	2.97
8562.2	√	√	√	5.84					
8574.1	√	√	√	7.21					
8581.4					√	√	√	16.92	
8594.3	√	√	√	3.55	√	√	√	14.46	4.79
8610.2	√	√	√	3.34	√	√		4.83	1.45
8620.8					√		√	3.94	
8633.5							√	3.88	
9453.2		√	√	3.23			√	3.98	1.23
9493.1		√	√	6.91	√	√	√	9.35	1.35
9548.1	√	√	√	15.24					
9563.6	√	√	√	16.33					
9578.9		√		18.77	√	√	√	100.36	5.35
9586.8	√		√	6.03			√	7.21	1.19
9595.8	√	√	√	7.31		√		8.01	2.00
9611.7	√	√	√	7.33					
9624.9					√	√	√	26.40	
9635.5		√	√	7.93	√	√		13.68	1.73
9657.1	√	√	√	34.10					
9672.3	√	√	√	16.61	√	√	√	105.26	6.34
9694.5	√	√	√	9.82					
9722.5	√	√		5.91	√	√	√	34.68	5.86
9741.6		√		3.20					
9756.3	√	√	√		√	√	√	7.96	
9780.5		√	√	3.98		√	√	4.94	1.24
9939.0	√		√	3.57			√	5.53	1.54
9976.1	√	√		3.63	√	√		4.64	3.50
10261.0	√		√	3.29	√	√	√	4.17	2.15
10294.0	√		√	3.29	√	√	√	4.20	1.28

Detected protein ion signals (*m/z*)	MeOH-CAWS				MeOH-CDWS				Ratio of (S/N_{CDWS})/ (S/N_{CAWS})
	1	2	3	Average S/N	1	2	3	Average S/N	
10343.8	√	√	√	3.55	√	√	√	7.58	2.13
10476.7	√		√	3.00			√	4.92	1.64
10517.8	√		√	3.67			√	4.60	1.25
11408.5	√		√	3.45			√	5.43	1.57
11544.3	√	√	√	3.48	√	√	√	4.72	1.36
11775.1	√			3.57	√	√	√	5.62	1.57
11977.6					√	√		5.58	
12595.3					√	√	√	5.13	
12924.1	√		√	3.60		√	√	9.07	2.52
12957.6							√	4.90	
13316.4					√	√	√	11.11	
13368.3					√	√	√	4.54	
13378.3					√			4.84	
13381.7					√	√	√	4.57	
15160.8					√			3.06	
15191.3					√		√	3.46	
15208.9					√		√	3.70	
15258.8					√	√	√	4.50	
15269.5					√		√	4.24	
15278.4					√		√	4.57	
15296.3					√		√	5.52	
15310.5	√	√	√	6.16					
15348.1	√			5.97	√	√	√	12.60	2.11
15354.5			√	6.23	√	√		7.87	1.26
15369.6			√	5.07					
15377.4	√		√	4.85	√	√	√	10.94	2.26
15390.5	√			3.36		√		4.43	1.32
15401.8	√		√	4.31	√	√	√	8.79	2.39
15414.4	√		√	4.26					
15434.1			√	3.68					
15446.7	√		√	3.24					

Detected protein ion signals (*m/z*)	MeOH-CAWS				MeOH-CDWS				Ratio of (S/N_{CDWS})/ (S/N_{CAWS})
	1	2	3	Average S/N	1	2	3	Average S/N	
15458.7	√			3.08					
15461.0			√	3.86					
15483.8	√		√	4.18					
15491.6			√	5.45					
15532.9					√	√	√	5.05	1.45
15694.0						√	√	8.63	
15735.2					√	√	√	5.43	
15760.2					√	√	√	5.49	
15796.8					√	√	√	3.53	
15818.9					√	√		3.23	
16197.6					√			4.87	
16217.1					√	√		3.33	
16238.9					√	√		3.01	
16511.3							√	3.34	
16520.6							√	3.16	
16915.6					√	√	√	3.27	
18038.0							√	3.87	
17228.5					√	√	√	4.06	
17239.2					√	√	√	3.40	
18200.9					√		√	3.09	
18372.8					√		√	3.20	
18391.4					√	√		3.64	
19010.7							√	3.25	
19076.8							√	5.14	
19264.8						√	√	3.02	
Number of detected proteins	72	69	73		134	132	135		
Average number of detected proteins		71				134			

Note: "√" means the protein ion could be detected in three technical duplicates.

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