Supplementary information
Chemical Profiling of Surviving Cancer Cells Using TOF-SIMS and MCR Analysis Discriminates Cell Components

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Supplementary methods

Seahorse assay

The oxygen consumption rate (OCR) of untreated and cisplatin-surviving cancer cells was measured using the Seahorse XF Analyzer (Agilent, USA). Cells were seeded in Seahorse XF 96-well plates at 20,000–30,000 cells per well, incubated overnight in growth medium, and then switched to serum-free Seahorse XF Assay Medium for 1 hour at 37°C without CO_2. Basal OCR was recorded, followed by sequential injections of oligomycin (2–5 µM) to assess ATP-linked respiration, FCCP (2 µM) for maximal respiratory capacity, and rotenone/antimycin A (1 µM each) for non-mitochondrial OCR. Data were collected with Seahorse XF Wave software to calculate basal OCR, ATP-linked OCR, maximal OCR, non-mitochondrial OCR, and spare respiratory capacity (maximal OCR minus basal OCR). Statistical analyses were performed to compare untreated and surviving cells' OCR profiles.

Supplementary tables

Table S1. The 20 highest loadings from 6 factors of MCR analysis from negative ion image of the mixed untreated and surviving cancer cells sample.

	Factor 1		**Factor 2**		**Factor 3**	
No.	*m/z*	Loadings (a.u.)	*m/z*	Loadings (a.u.)	*m/z*	Loadings (a.u.)
1	158.9893	0.013	72.0769	0.007	45.0188	0.017
2	78.9967	0.012	141.1099	0.007	100.0768	0.015
3	62.9890	0.012	42.0166	0.007	71.0272	0.015
4	180.9790	0.011	82.1128	0.007	73.0107	0.015
5	260.9582	0.011	155.1281	0.006	87.0422	0.014
6	198.9893	0.009	101.1138	0.006	59.0514	0.014
7	97.0071	0.009	129.1533	0.006	55.0436	0.013
8	276.9321	0.009	130.0964	0.006	43.0448	0.012
9	134.1003	0.008	115.0858	0.006	69.0114	0.012
10	90.0480	0.007	26.0065	0.006	41.0072	0.010
11	182.9677	0.006	156.1238	0.006	99.0467	0.009
12	127.9952	0.006	145.1199	0.006	113.0648	0.008
13	214.9729	0.006	56.0740	0.005	85.0715	0.008
14	164.9651	0.006	167.1374	0.005	83.0463	0.008
15	117.0783	0.006	40.0394	0.005	71.0633	0.008
16	102.9953	0.006	170.1455	0.005	57.0166	0.007
17	80.9959	0.005	142.1141	0.005	115.0293	0.007
18	244.9407	0.005	44.0353	0.005	58.0233	0.007
19	95.9871	0.005	70.0596	0.005	53.0132	0.007
20	223.9682	0.005	71.0633	0.005	112.0628	0.006
	Factor 4		**Factor 5**		**Factor 6**	
No.	*m/z*	Loadings (a.u.)	*m/z*	Loadings (a.u.)	*m/z*	Loadings (a.u.)
1	101.9657	0.024	42.0166	0.007	15.9896	0.035
2	177.9684	0.023	26.0065	0.007	75.9933	0.031
3	114.9687	0.022	91.0235	0.006	77.0053	0.030
4	129.1081	0.022	40.0394	0.006	59.9875	0.028
5	163.9661	0.021	44.0353	0.005	136.9862	0.023
6	133.9950	0.020	41.0306	0.005	135.9761	0.023
7	156.9615	0.020	71.0274	0.005	178.9643	0.018
8	63.9772	0.020	107.0234	0.005	17.0024	0.017
9	121.9908	0.019	25.0095	0.005	60.9861	0.016
10	94.9498	0.018	65.0401	0.005	118.9819	0.015
11	92.9474	0.017	47.0181	0.005	196.9755	0.015
12	64.9571	0.017	71.0633	0.004	77.9858	0.015

13	85.9663	0.015	50.0270	0.004	137.9746	0.013
14	54.9660	0.014	98.0475	0.004	238.9548	0.009
15	55.9844	0.013	66.0745	0.004	31.9942	0.009
16	43.0189	0.011	97.0818	0.004	119.9681	0.008
17	54.0089	0.011	73.0747	0.004	58.9806	0.008
18	132.9109	0.010	54.0603	0.004	61.9717	0.007
19	46.9703	0.010	66.1011	0.004	179.9646	0.007
20	73.9871	0.008	82.0615	0.004	256.9491	0.007

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Supplementary Discussion

Seahorse assay

Figure S5 shows the oxygen consumption rate (OCR) of untreated and cisplatin-surviving cancer cells over time, measured by Seahorse assay. The assay includes two main conditions: baseline OCR (cells in media without inhibitors) and OCR after etomoxir treatment to inhibit fatty acid oxidation. When cells are treated with etomoxir, OCR typically decreases if the cells are relying on fatty acids as a primary fuel source. From Figure S5, untreated cells exhibited no difference between media and etomoxir condition, while the surviving cells increase OCR in the etomoxir condition. An increased OCR with etomoxir treatment could indicate that the inhibition of fatty acid oxidation could induce a stress response, activating pathways that increase mitochondrial respiration as a protective mechanism. This response might lead to a temporary boost in OCR due to cellular attempts to adapt to the metabolic restriction. The Seahorse assay consists of sequential additions of compounds to assess mitochondrial function: (A) Basal respiration measures natural OCR; (B) Oligomycin inhibits ATP synthase, with the drop in OCR reflecting ATP-linked respiration; (C) FCCP uncouples oxidative phosphorylation, with the increase in OCR indicating maximal respiratory capacity; and (D) Rotenone and Antimycin A inhibit mitochondrial respiration, with the remaining OCR representing non-mitochondrial oxygen consumption. From Figure S5, the higher oxygen consumption rate in the surviving cells compared to the untreated cells indicates a metabolic adaptation characterized by increased mitochondrial activity. This enhancement likely supports greater energy production and contributes to the cells' resistance and resilience to stress.

Figure S1. The overlay spectra of untreated cancer cells (blue) and surviving cancer cells (pink) in positive ion mode in the range of (**A**) *m/z* 100-225, (**B**) *m/z* 225-350, (**C**) *m/z* 350-475 and (**D**) *m/z* 475-600.

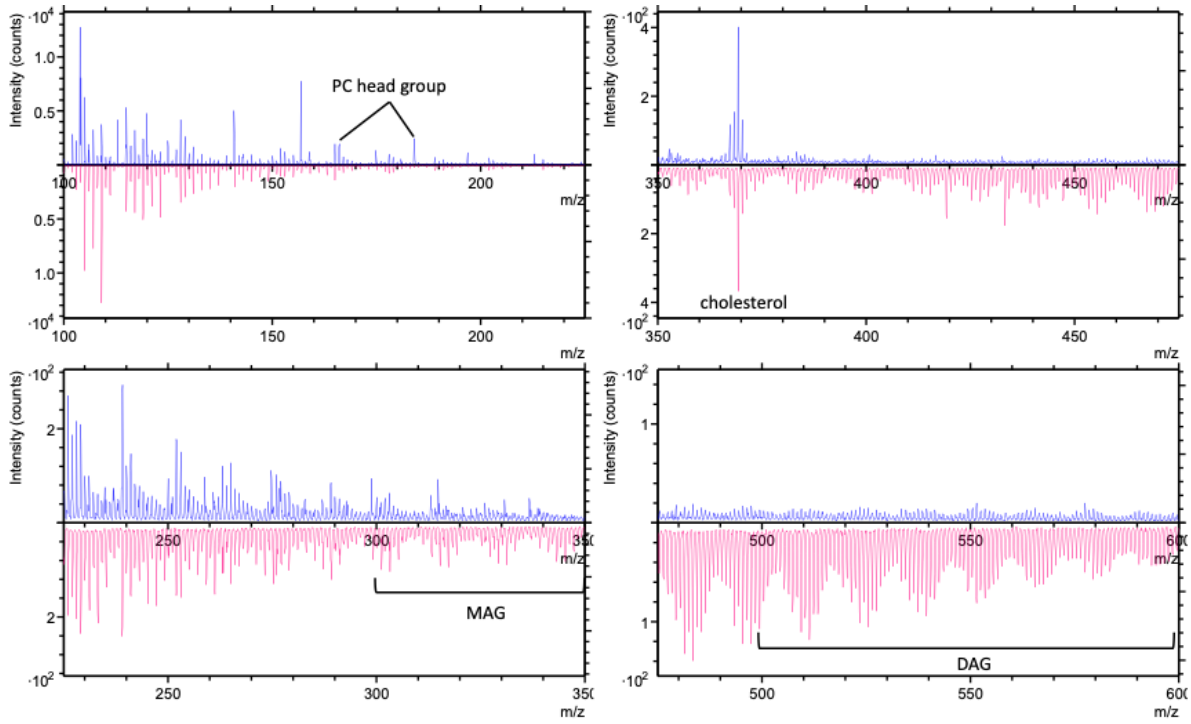


Figure S2. The overlay spectra of untreated cancer cells (blue) and surviving cancer cells (pink) in negative ion mode in the range of (**A**) *m/z* 100-225, (**B**) *m/z* 225-350, (**C**) *m/z* 350-475 and (**D**) *m/z* 475-600.

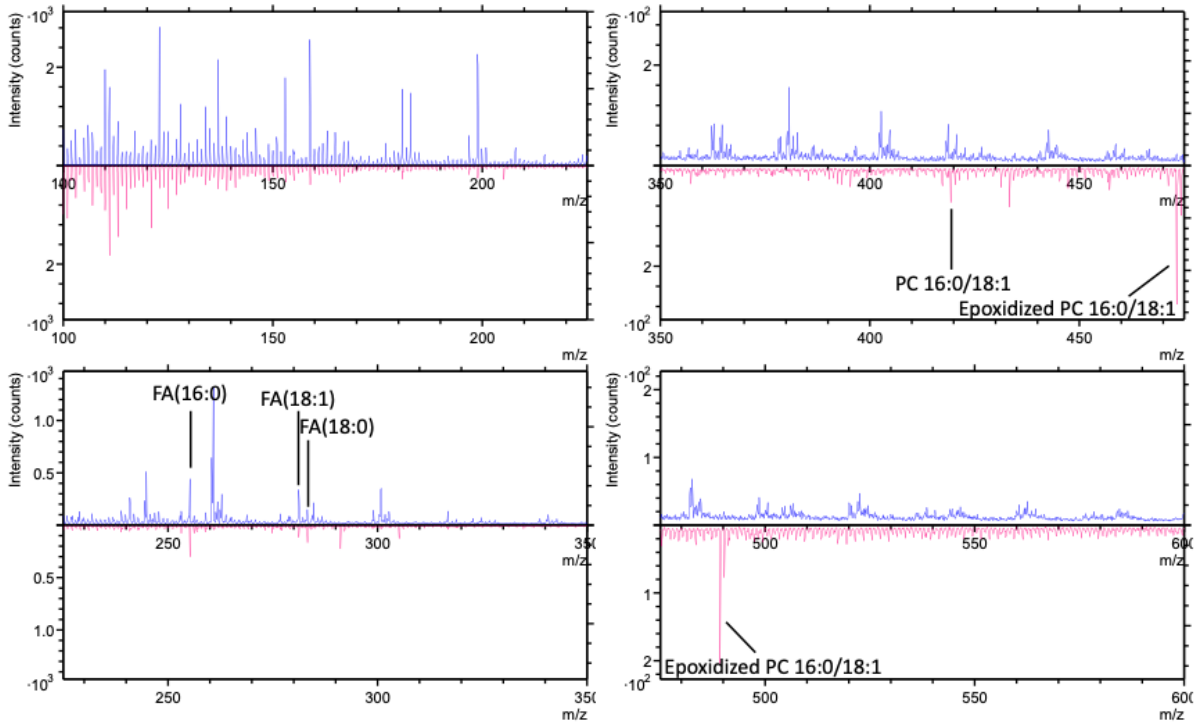


Figure S3. The loading spectra from 6 factors of PCA analysis from negative ion image of the mixed untreated and surviving cancer cells sample from Figure. 6. (**A**) factor 1 and (**B**) factor 2.

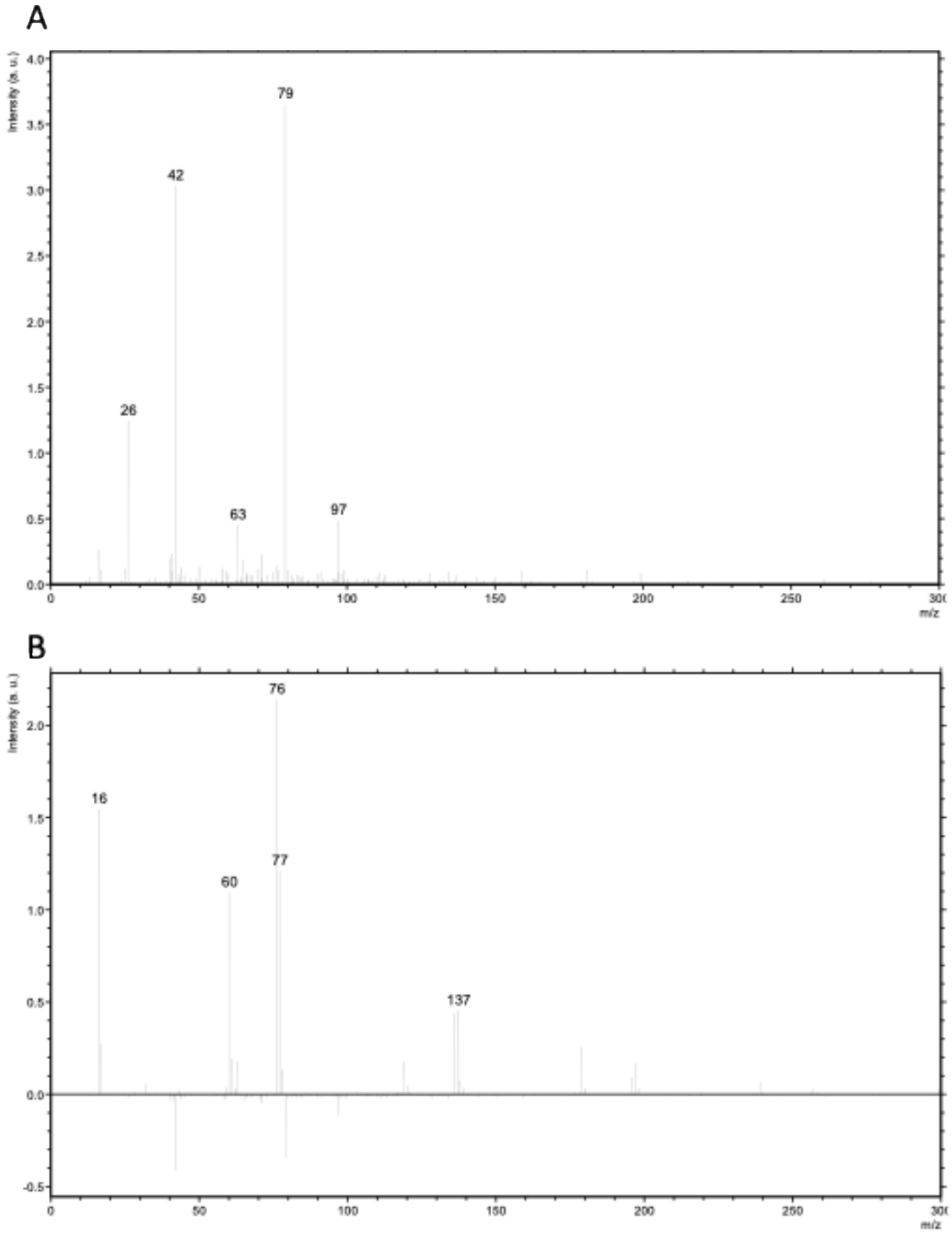


Figure S4. The loading spectra from 6 factors of PCA analysis from negative ion image of the mixed untreated and surviving cancer cells sample from Figure. 6. (**A**) factor 3 and (**B**) factor 4.

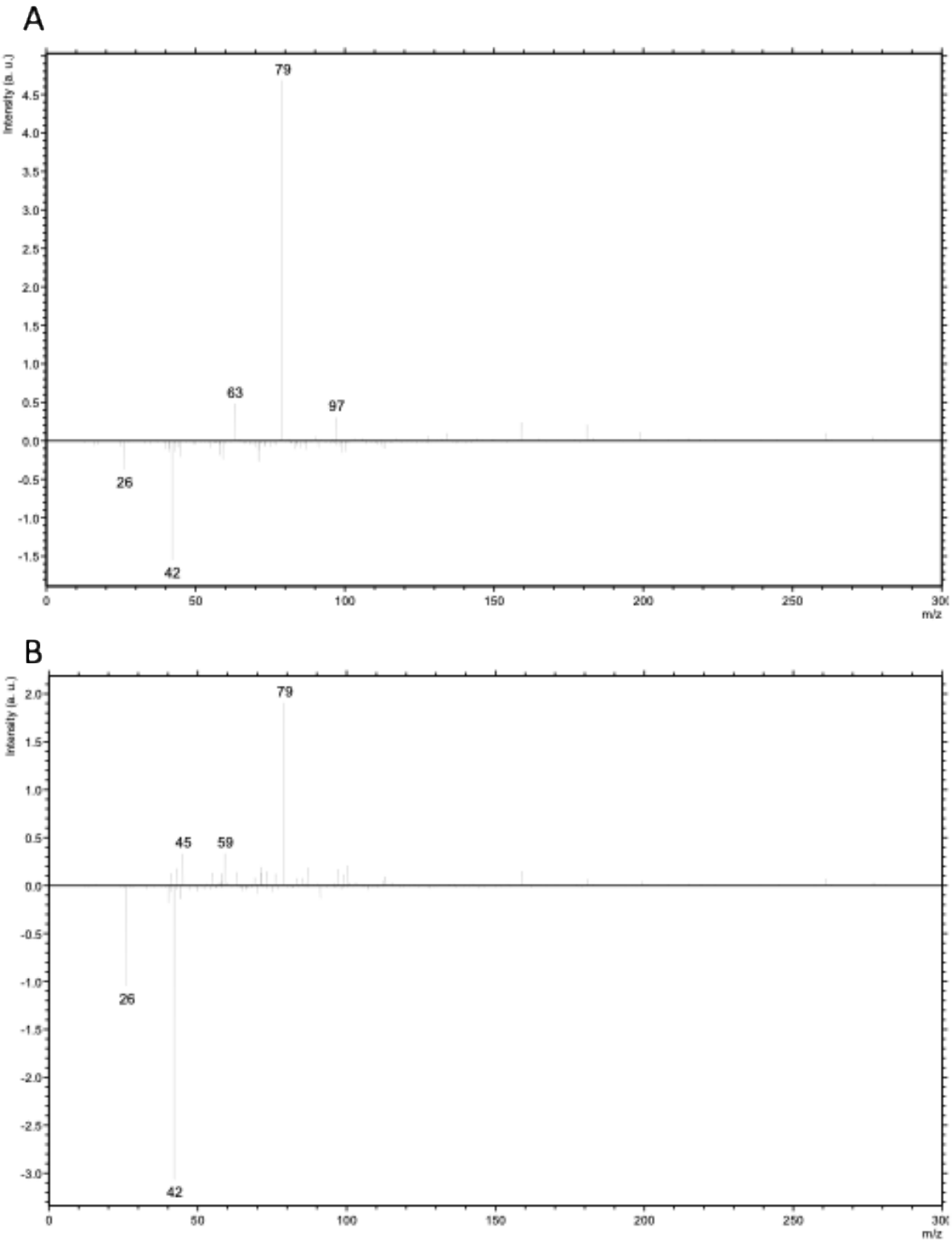


Figure S5. The loading spectra from 6 factors of PCA analysis from negative ion image of the mixed untreated and surviving cancer cells sample from Figure. 6. (**A**) factor 5 and (**B**) factor 6.

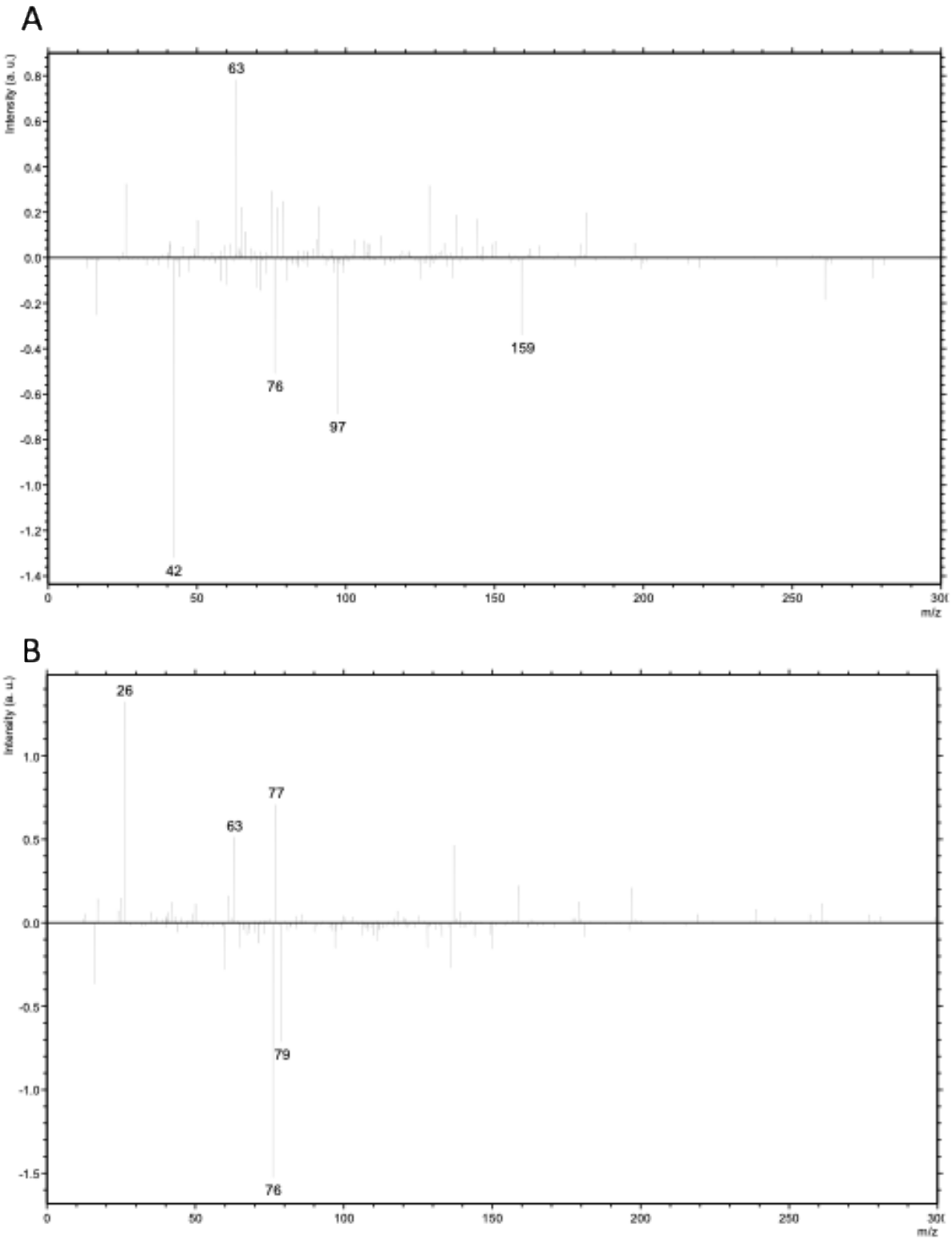


Figure S6. The loading spectra from 6 factors of MCR analysis from negative ion image of the mixed untreated and surviving cancer cells sample from Figure. 7. (**A**) factor 1 and (**B**) factor 2.

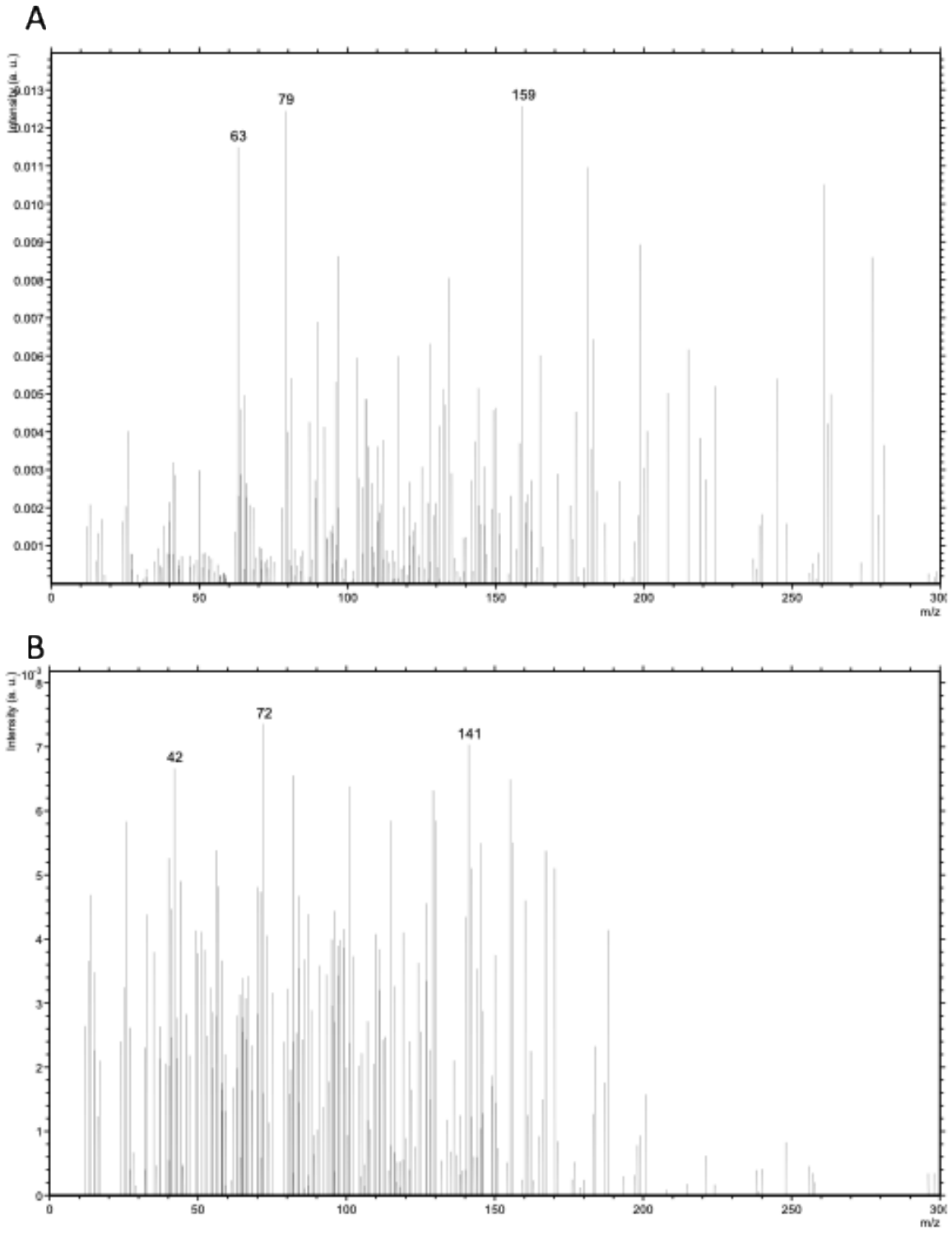


Figure S7. The loading spectra from 6 factors of MCR analysis from negative ion image of the mixed untreated and surviving cancer cells sample from Figure. 7. (**A**) factor 3 and (**B**) factor 4.

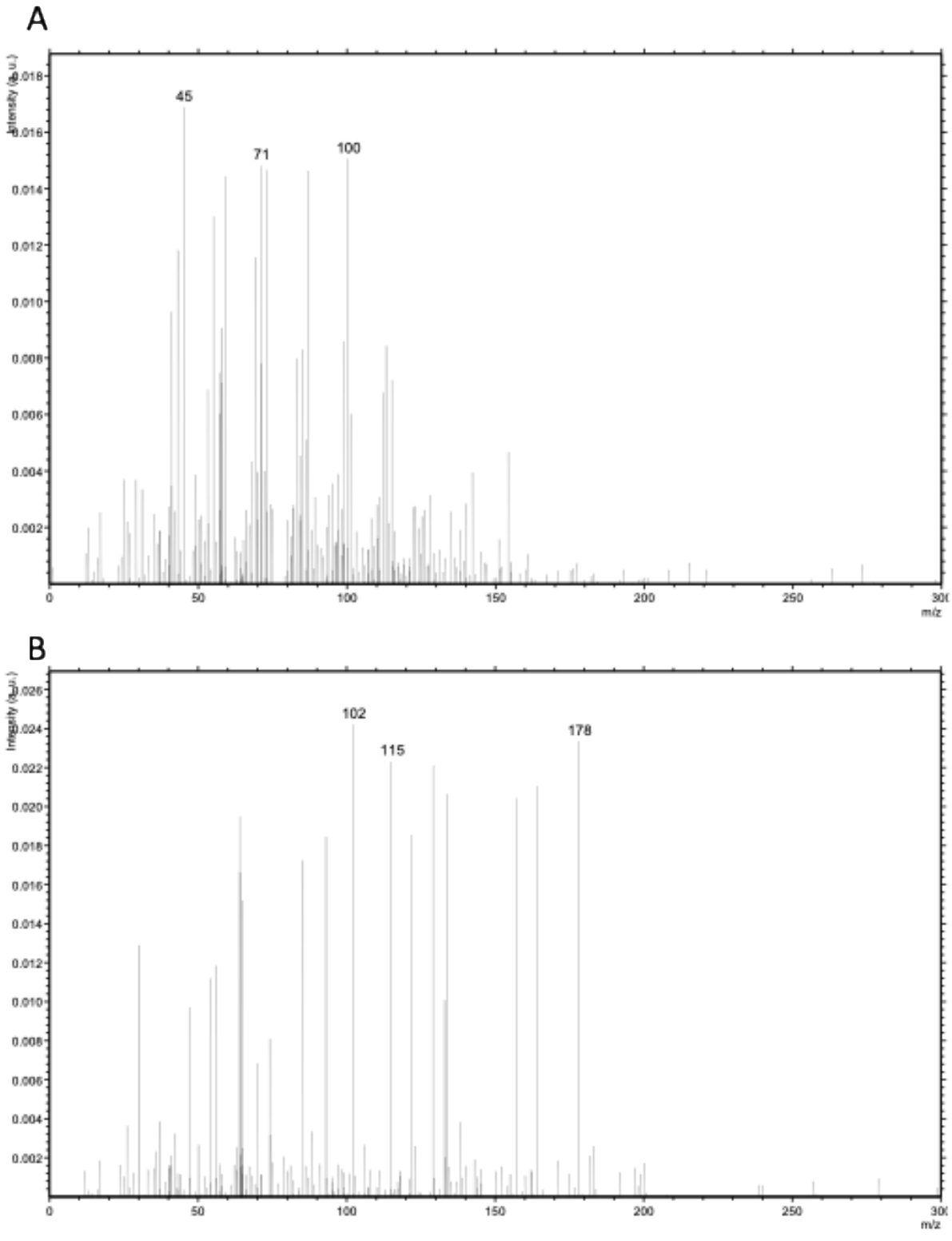


Figure S8. The loading spectra from 6 factors of MCR analysis from negative ion image of the mixed untreated and surviving cancer cells sample from Figure. 7. (**A**) factor 5 and (**B**) factor 6.

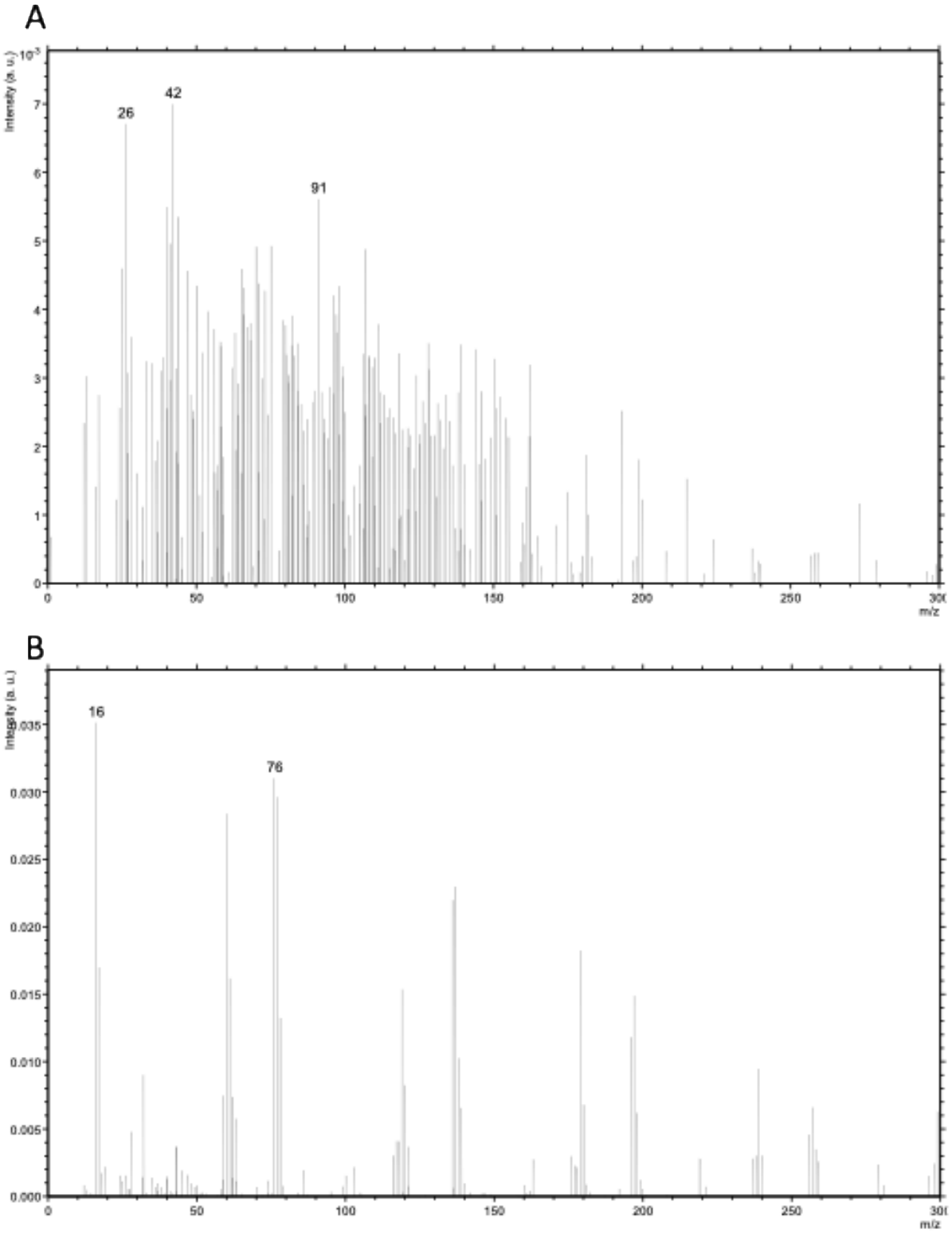


Figure S9. Replicate 2. (**A**) Six factors (F1–F6) derived from PCA analysis of negative ion images for the mixed sample of untreated and surviving cancer cells. (**B**) Six factors (F1–F6) derived from MCR analysis of the same negative ion images. (**C**) PCA analysis, and (**D**) MCR analysis illustrating the overlay of cytoplasm (red), nucleus (blue), and lipid droplets (red).

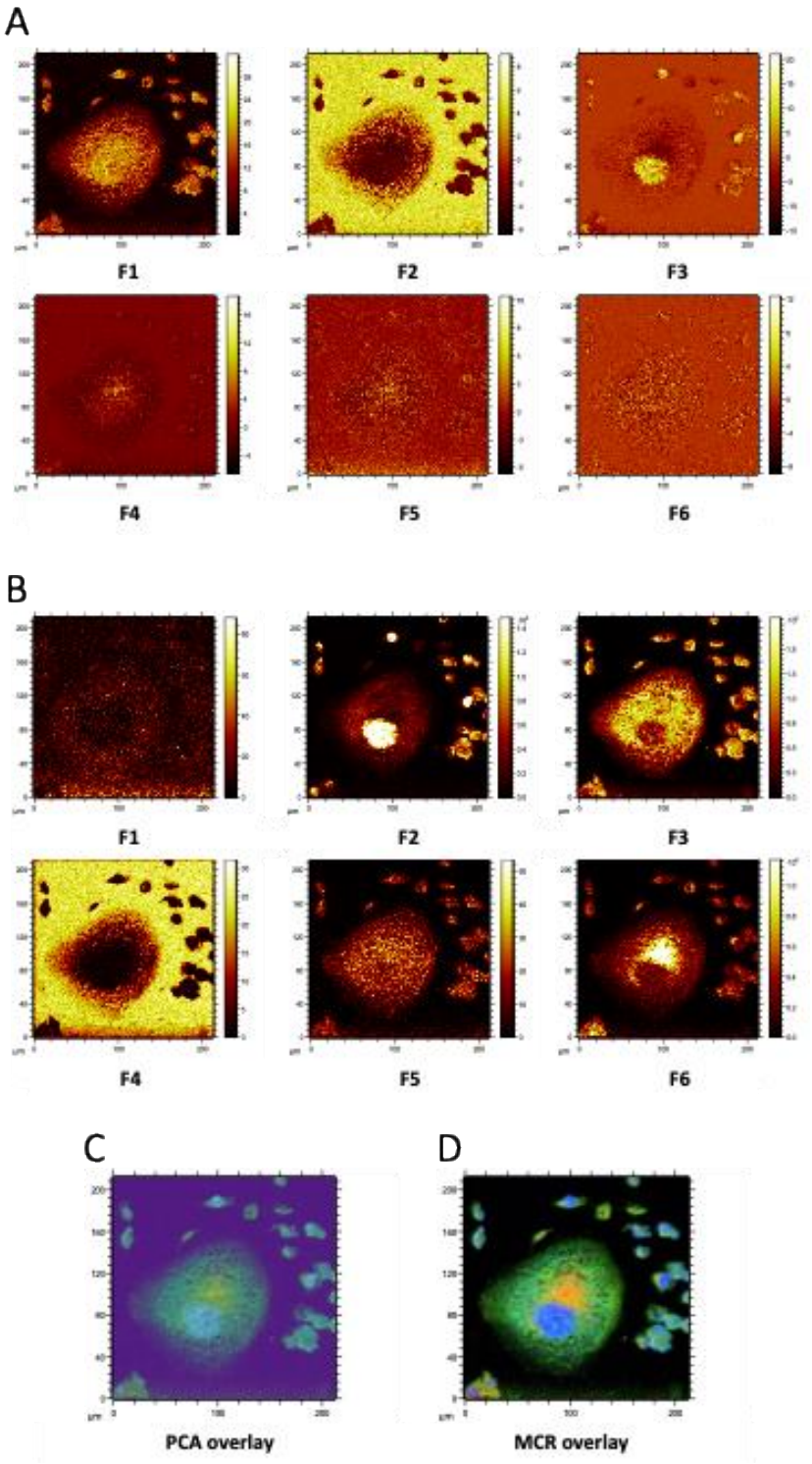


Figure S10. Replicate 2. The loading spectra from 6 factors of PCA analysis from negative ion image of the mixed untreated and surviving cancer cells sample from Figure S9. (**A**) factor 1 and (**B**) factor 2.

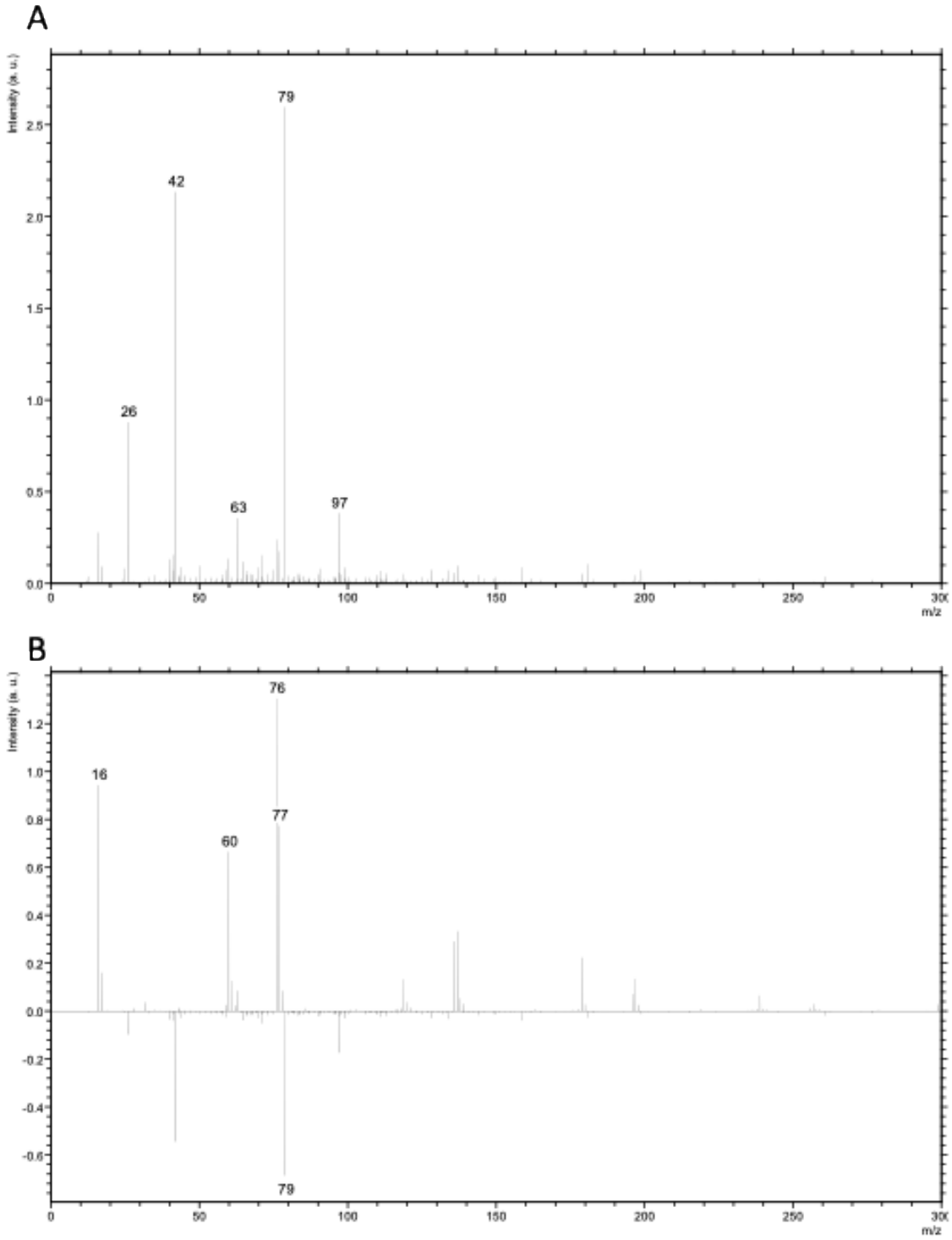


Figure S11. Replicate 2. The loading spectra from 6 factors of PCA analysis from negative ion image of the mixed untreated and surviving cancer cells sample from Figure S9. (**A**) factor 3 and (**B**) factor 4.

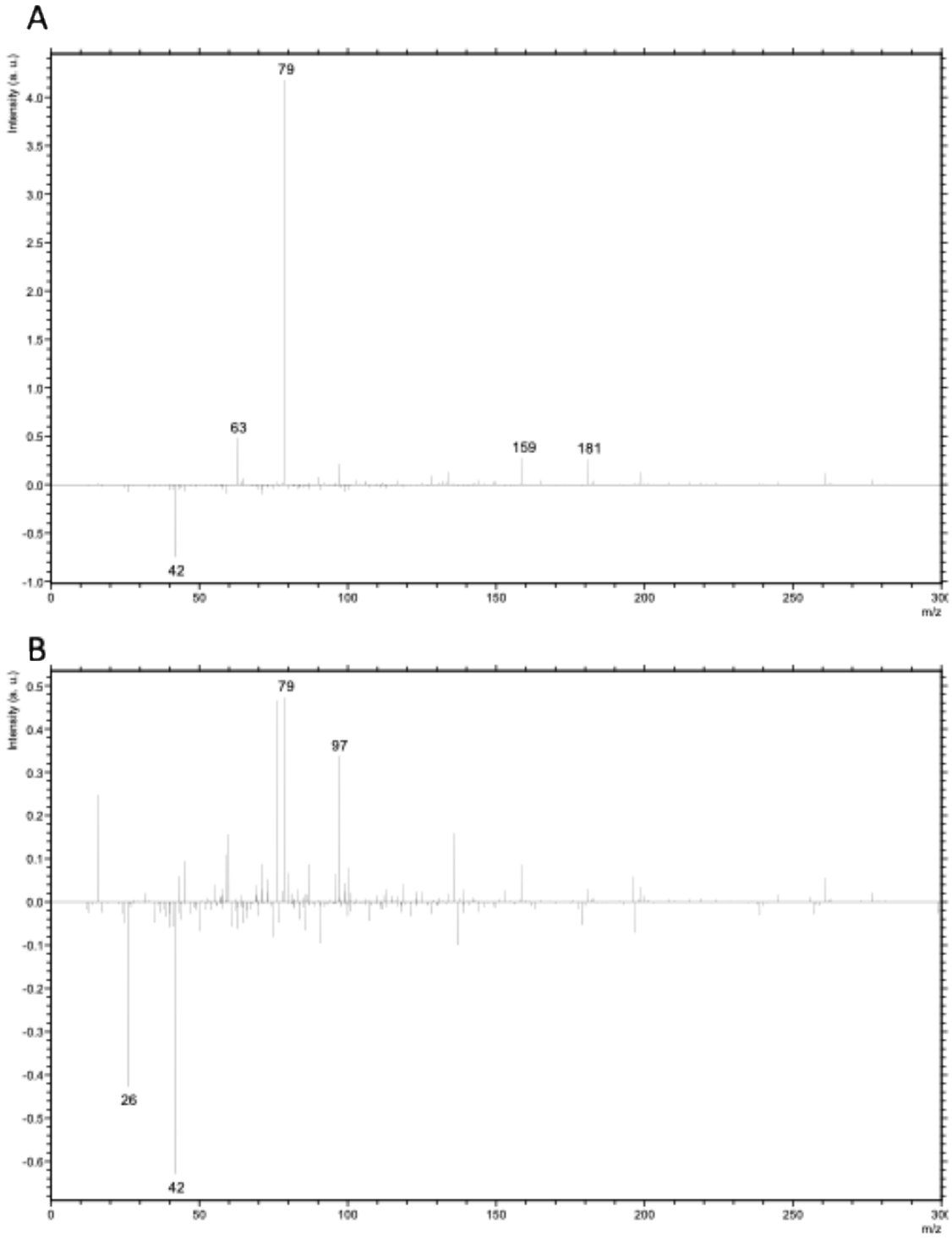


Figure S12. Replicate 2. The loading spectra from 6 factors of PCA analysis from negative ion image of the mixed untreated and surviving cancer cells sample from Figure S9. (**A**) factor 5 and (**B**) factor 6.

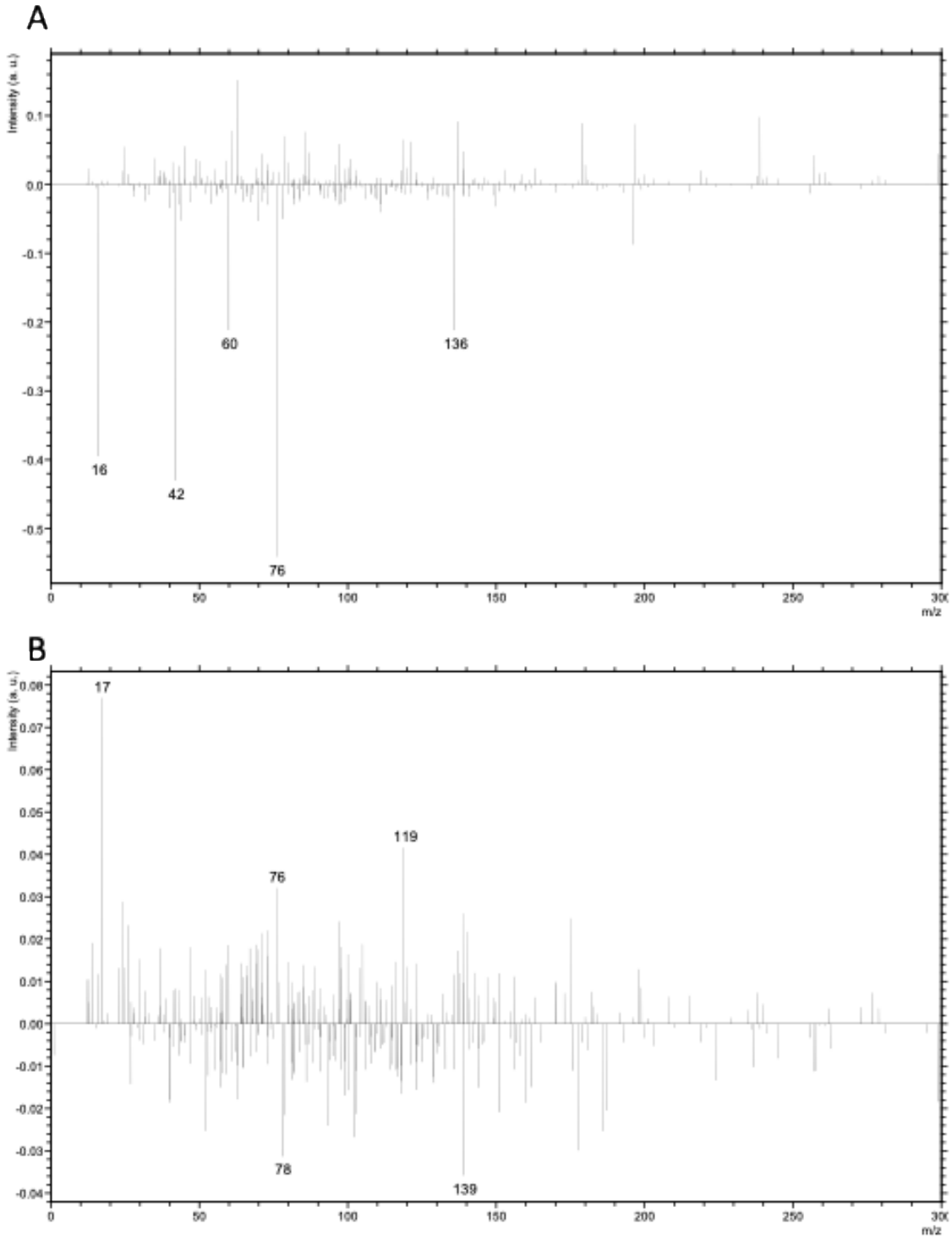


Figure S13. Replicate 2. The loading spectra from 6 factors of MCR analysis from negative ion image of the mixed untreated and surviving cancer cells sample from Figure S9. (**A**) factor 1 and (**B**) factor 2.

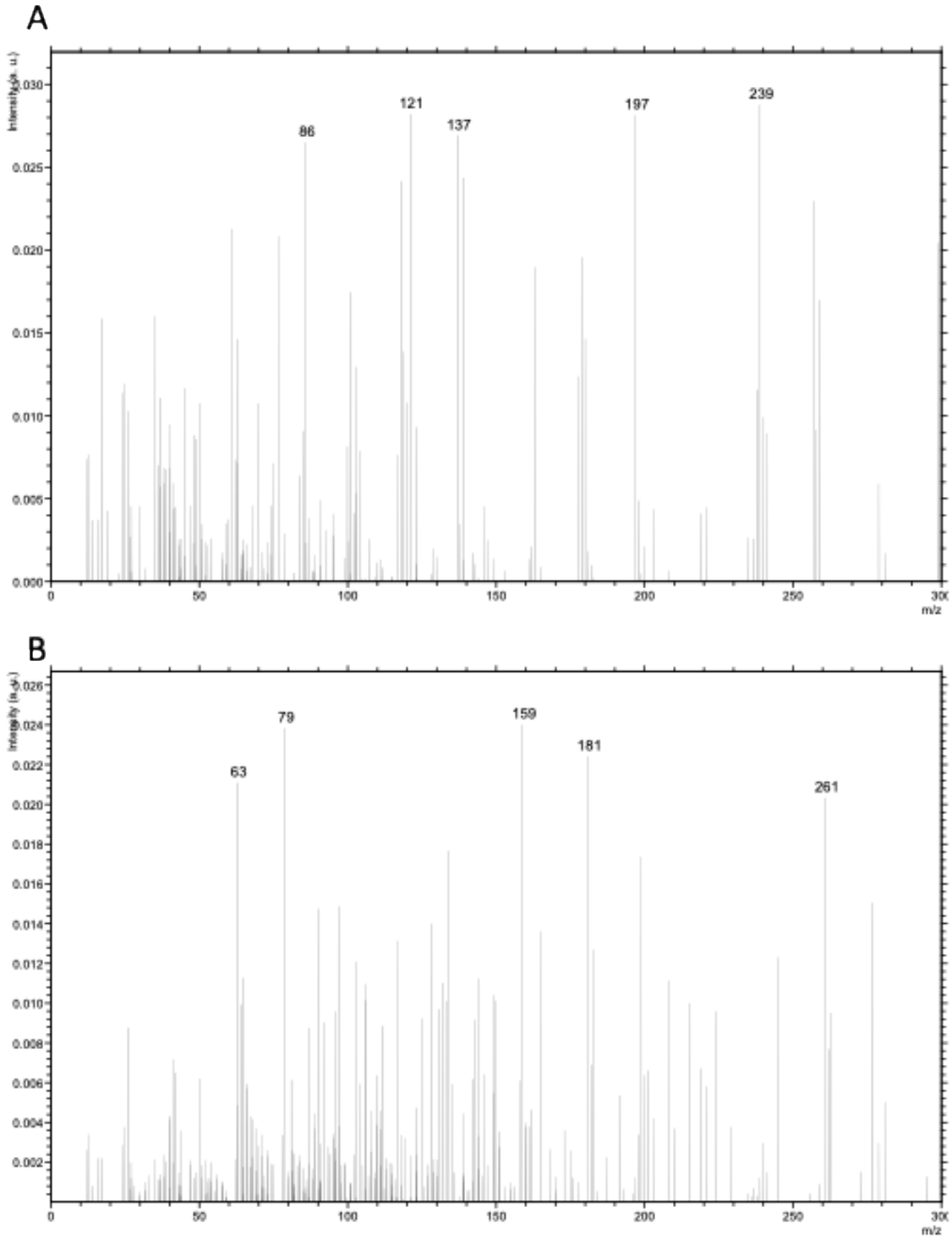


Figure S14. Replicate 2. The loading spectra from 6 factors of MCR analysis from negative ion image of the mixed untreated and surviving cancer cells sample from Figure S9. (**A**) factor 3 and (**B**) factor 4.

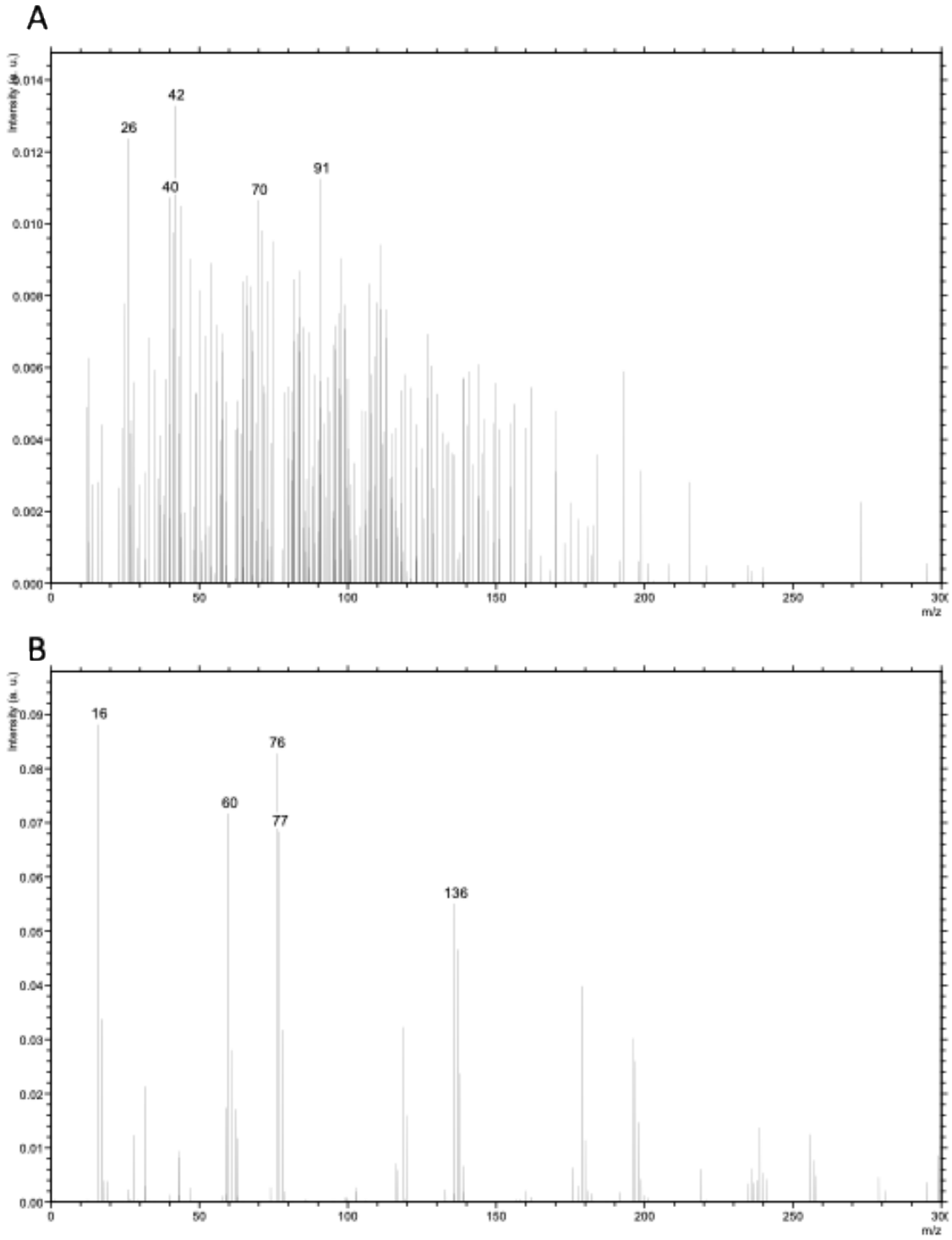


Figure S15. Replicate 2. The loading spectra from 6 factors of MCR analysis from negative ion image of the mixed untreated and surviving cancer cells sample from Figure S9. (**A**) factor 5 and (**B**) factor 6.

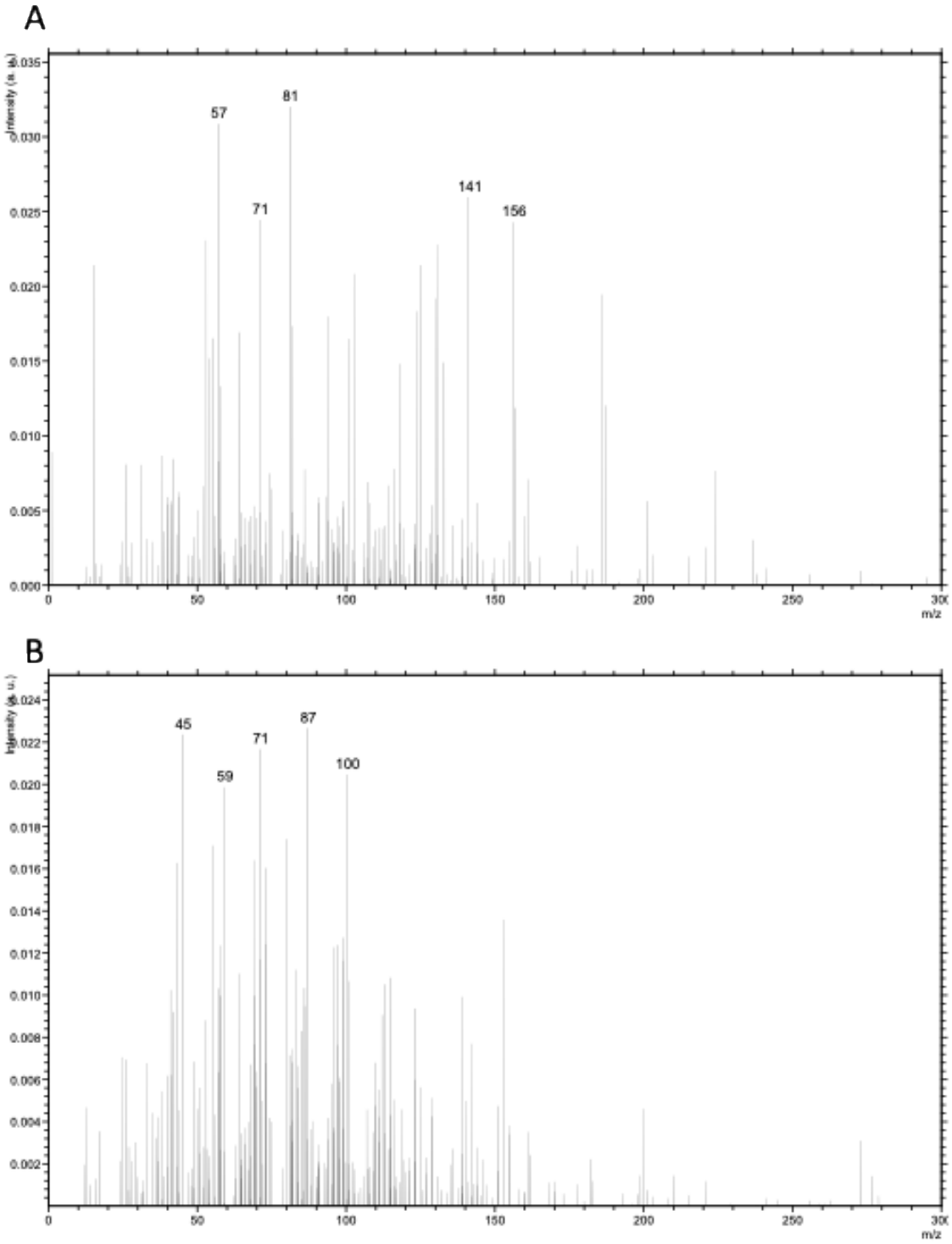


Figure S16. Replicate 3. (**A**) Six factors (F1–F6) derived from PCA analysis of negative ion images for the mixed sample of untreated and surviving cancer cells. (**B**) Six factors (F1–F6) derived from MCR analysis of the same negative ion images. (**C**) PCA analysis, and (**D**) MCR analysis illustrating the overlay of cytoplasm (red), nucleus (blue), and lipid droplets (red).

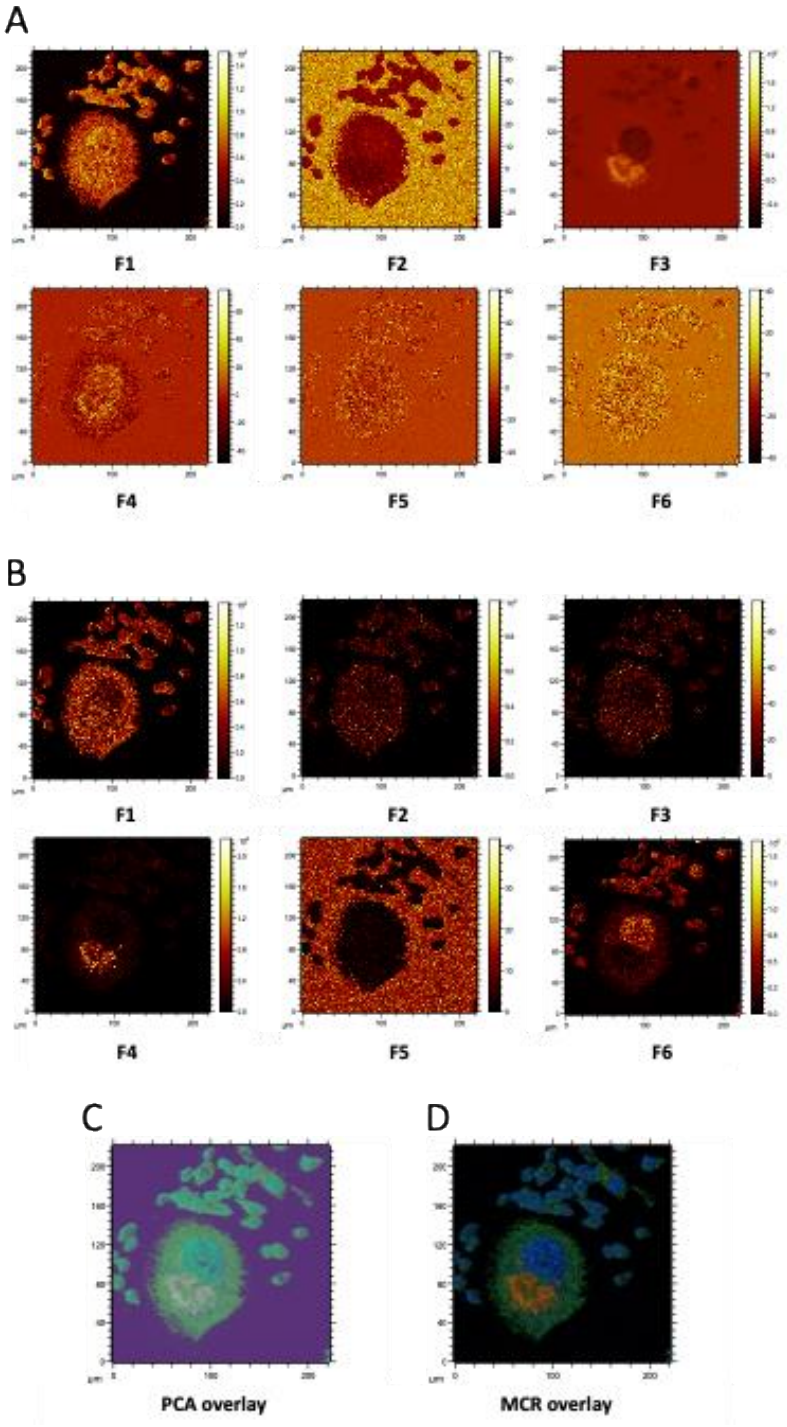


Figure S17. Replicate 3. The loading spectra from 6 factors of PCA analysis from negative ion image of the mixed untreated and surviving cancer cells sample from Figure S16. (**A**) factor 1 and (**B**) factor 2.

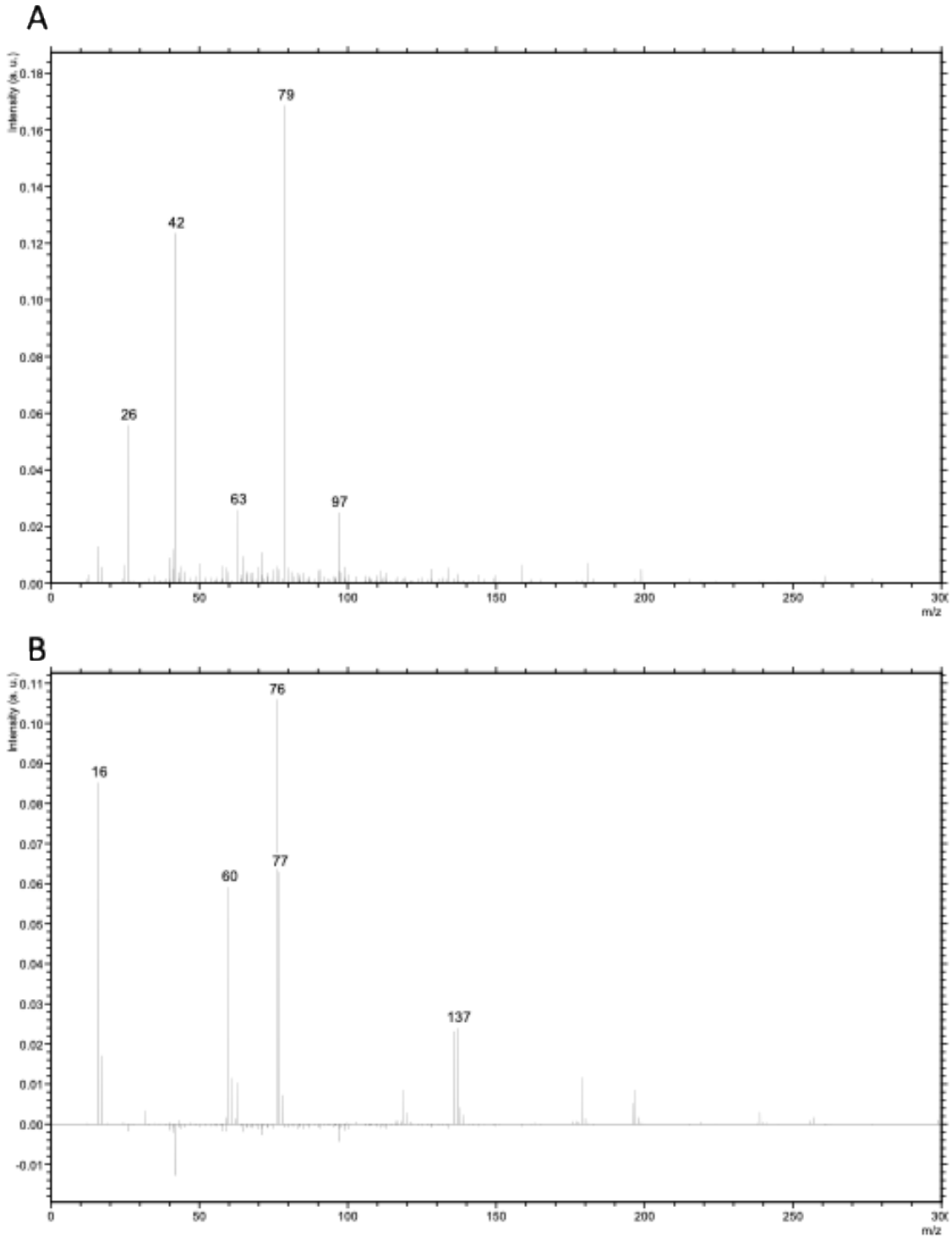


Figure S18. Replicate 3. The loading spectra from 6 factors of PCA analysis from negative ion image of the mixed untreated and surviving cancer cells sample from Figure S16. (**A**) factor 3 and (**B**) factor 4.

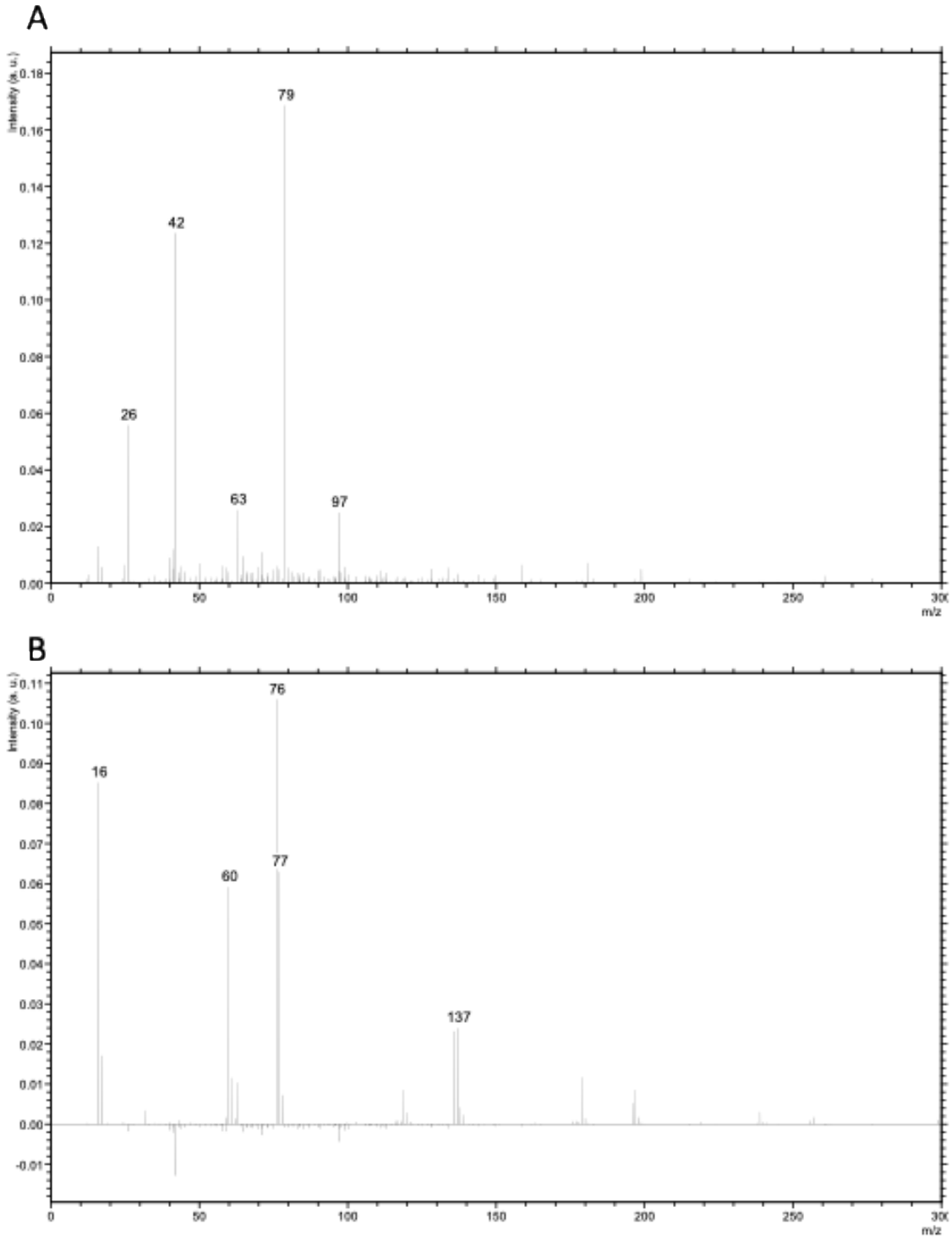


Figure S19. Replicate 3. The loading spectra from 6 factors of PCA analysis from negative ion image of the mixed untreated and surviving cancer cells sample from Figure S16. (**A**) factor 5 and (**B**) factor 6.

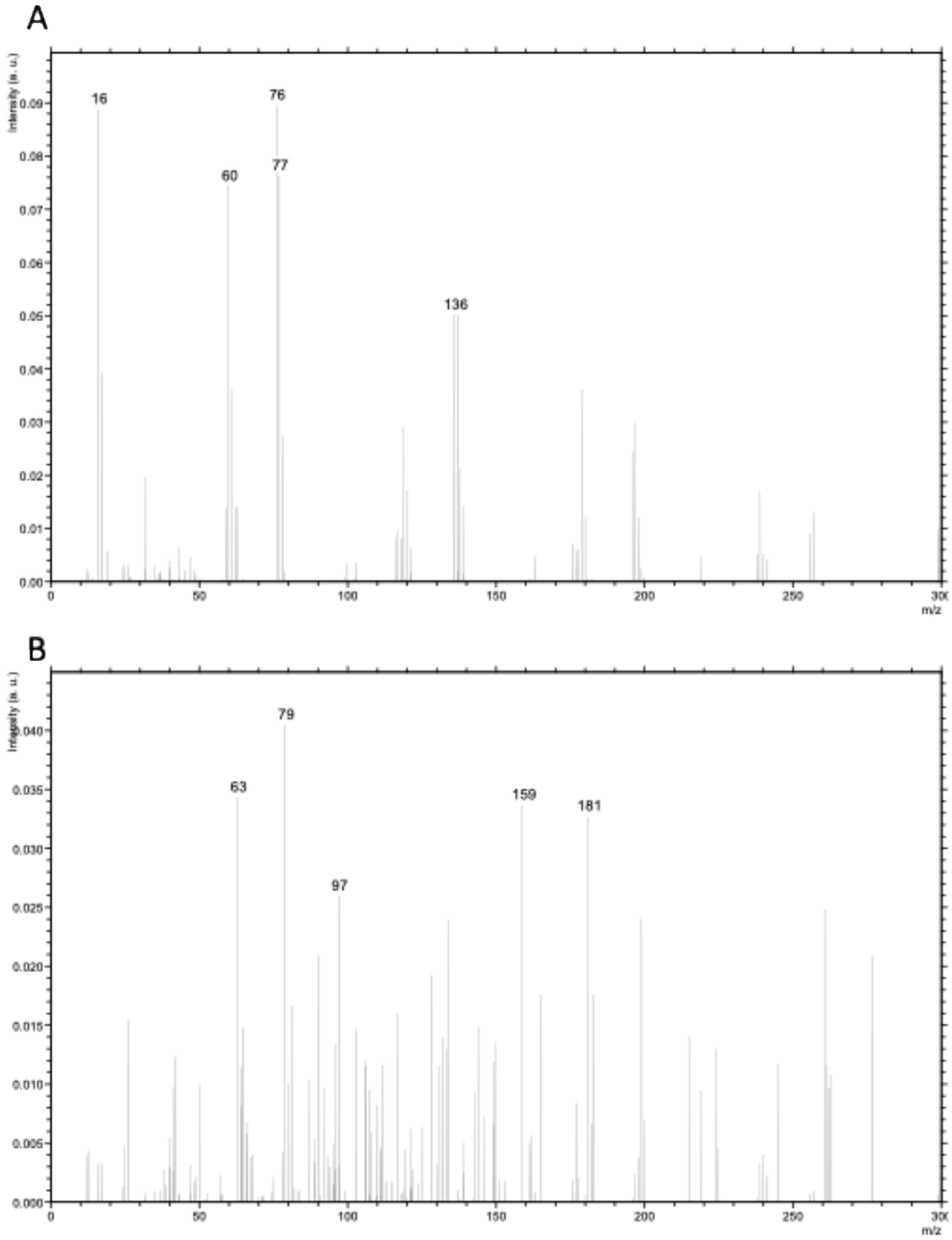


Figure S20. Replicate 3. The loading spectra from 6 factors of MCR analysis from negative ion image of the mixed untreated and surviving cancer cells sample from Figure S16. (**A**) factor 1 and (**B**) factor 2.

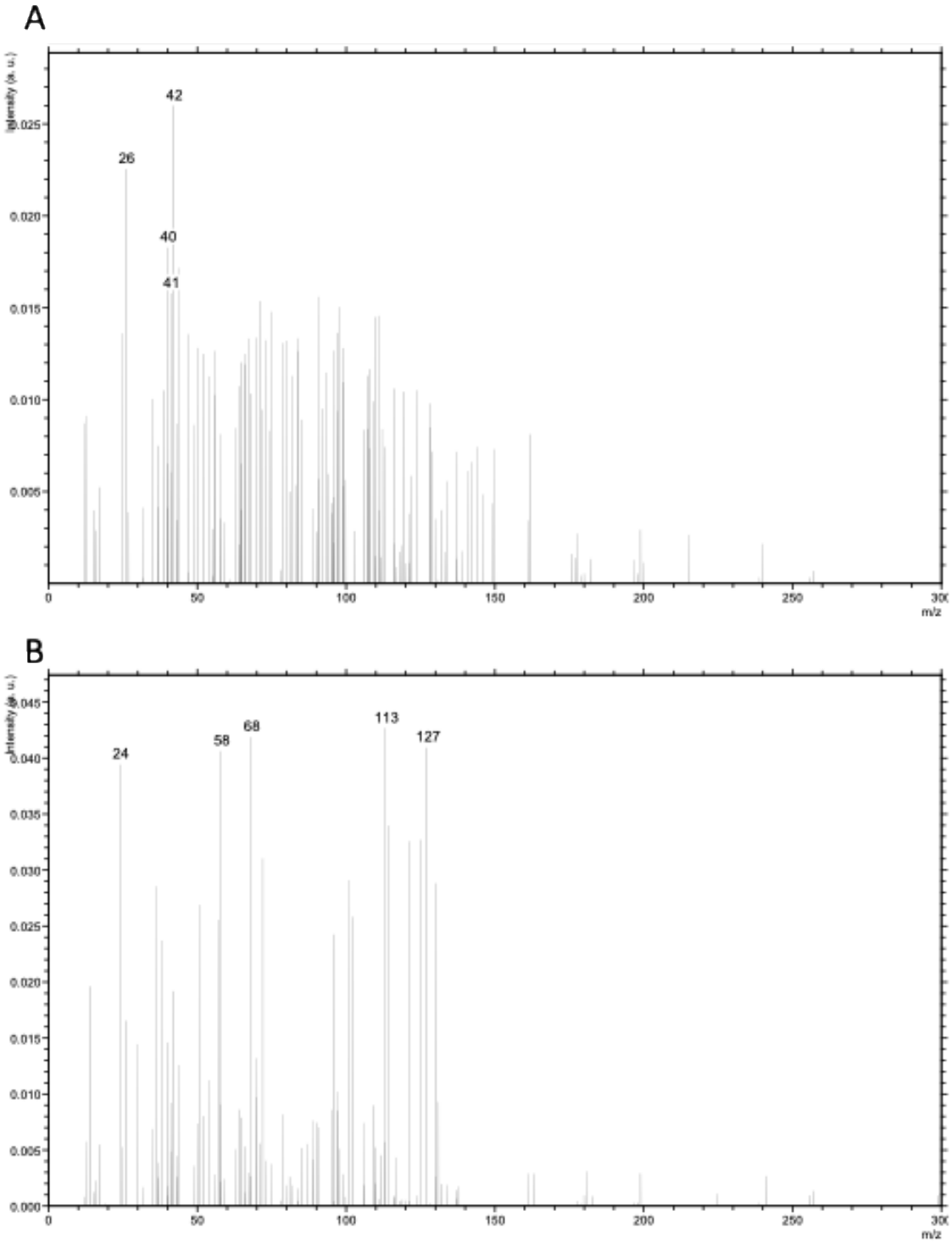
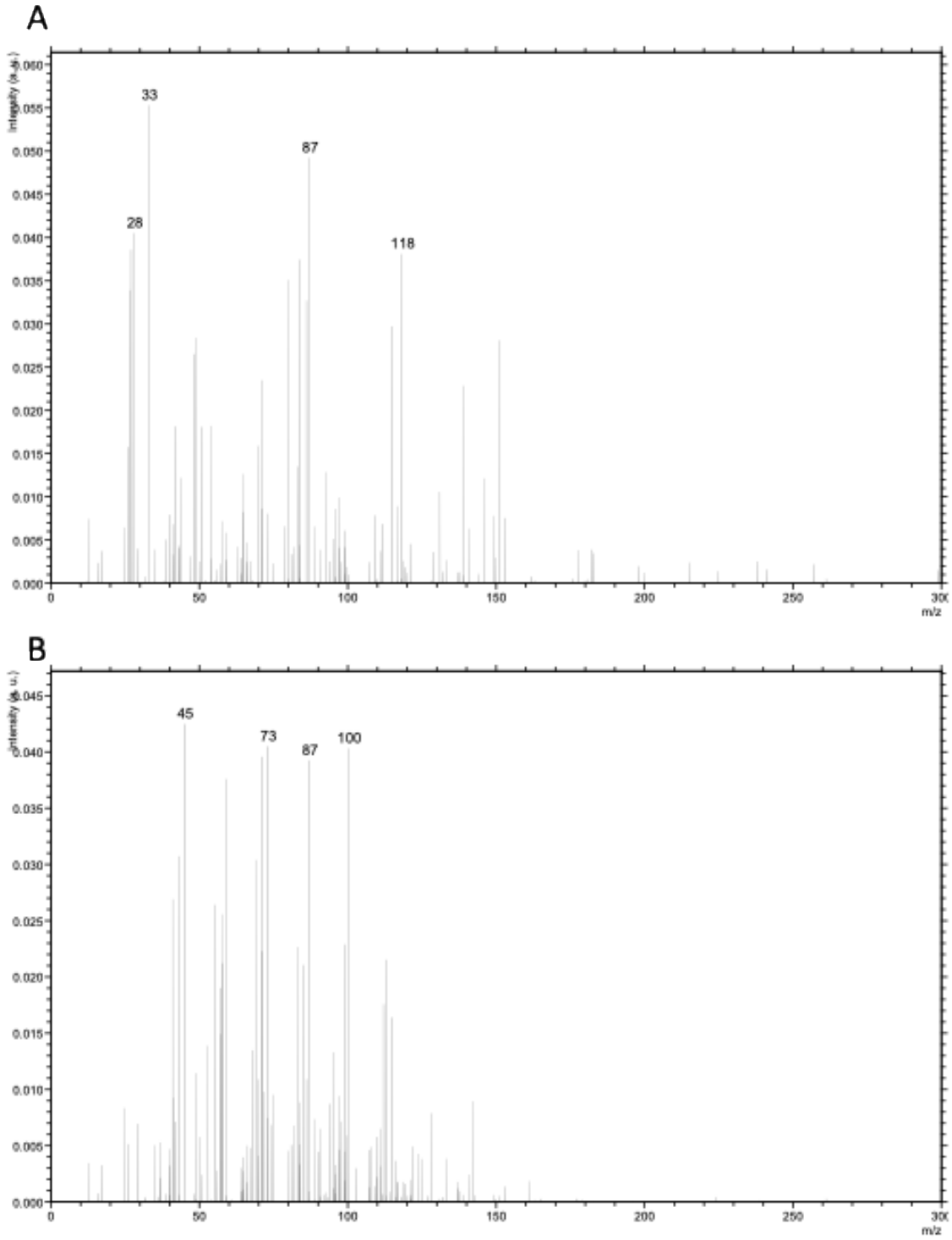


Figure S21. Replicate 3. The loading spectra from 6 factors of MCR analysis from negative ion image of the mixed untreated and surviving cancer cells sample from Figure S16. (**A**) factor 3 and (**B**) factor 4.



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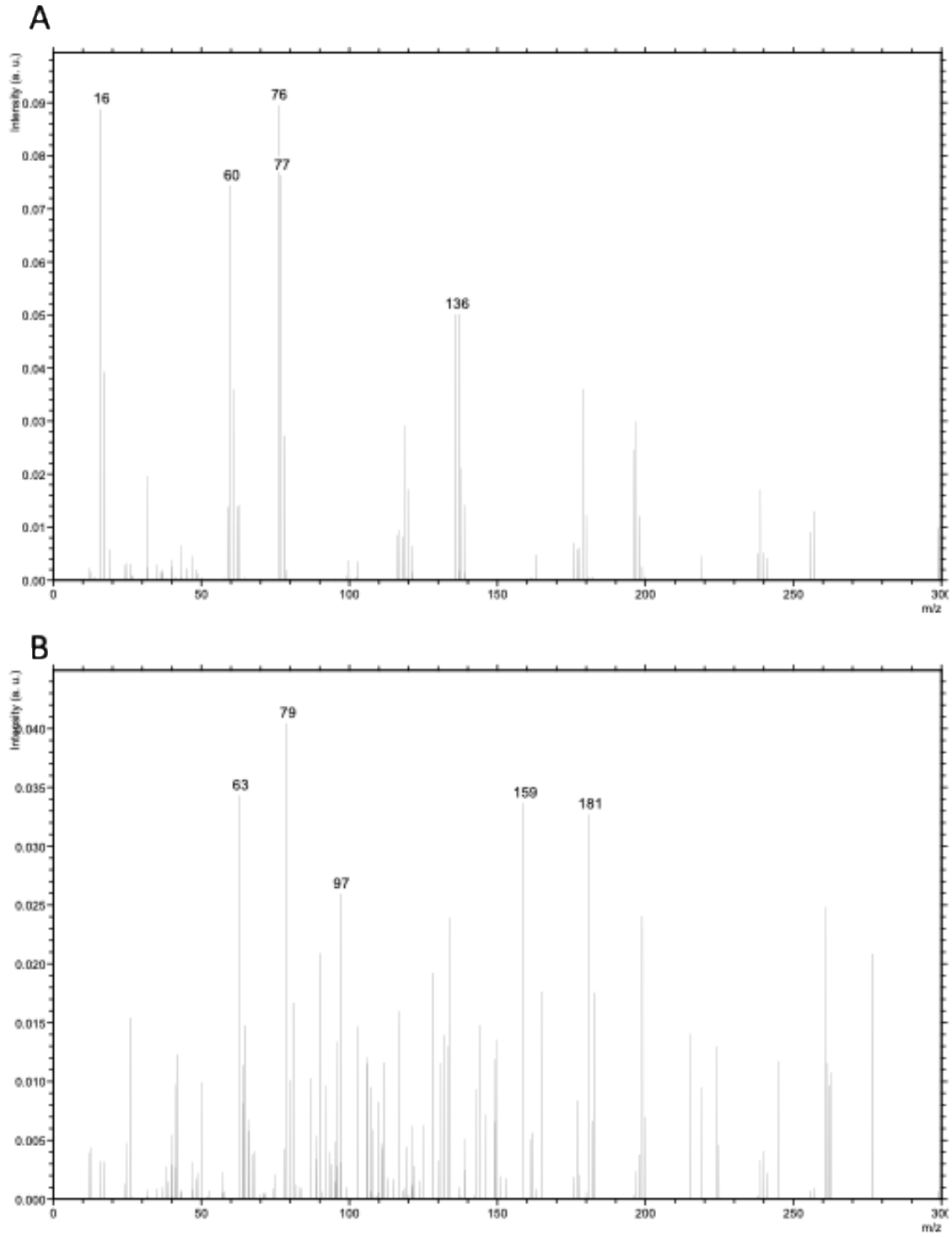

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