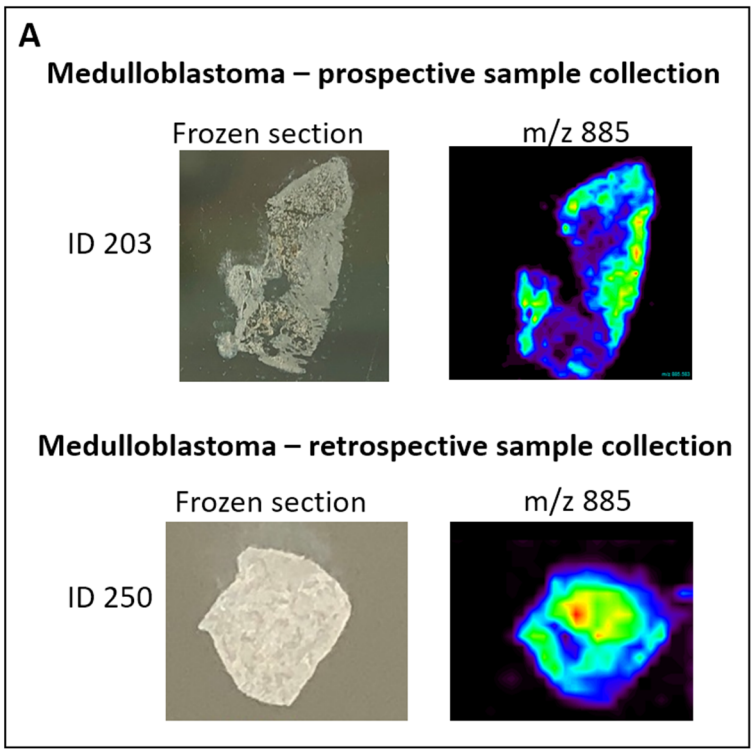
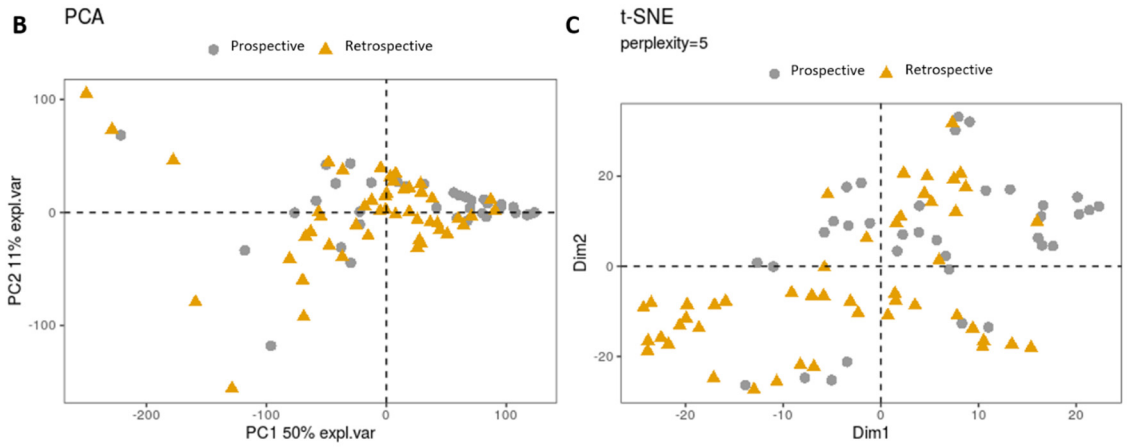
Supplementary Data

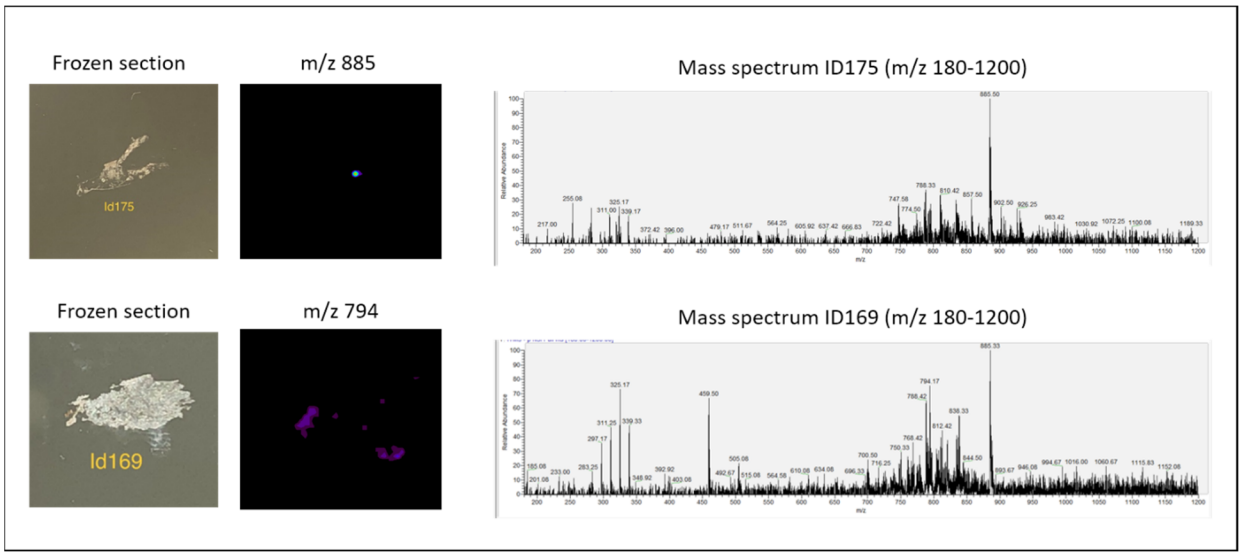
Supplementary Figure 1A DESI-MSI analysis of two tumor samples diagnosed as medulloblastoma. The left panels show the frozen section before the DESI-MSI analysis. The right panels show the 2D chemical image generated from the DESI-MSI analysis, evidencing the distribution of ion 885. The comparison between the prospective and retrospective sample shows that there is no significant difference in the ionization and resolution of the image generated when the two forms of collection are compared. **Supplementary Figure 1B** Unsupervised analysis (Principal Component Analysis, PCA) of the data obtained from the prospective and retrospective samples. **Supplementary Figure 1C** The nonlinear dimensionality reduction analysis using the t-SNE algorithm shows that it is not possible to distinguish the two groups of samples in relation to the type of recruitment

Supplementary Figure 1

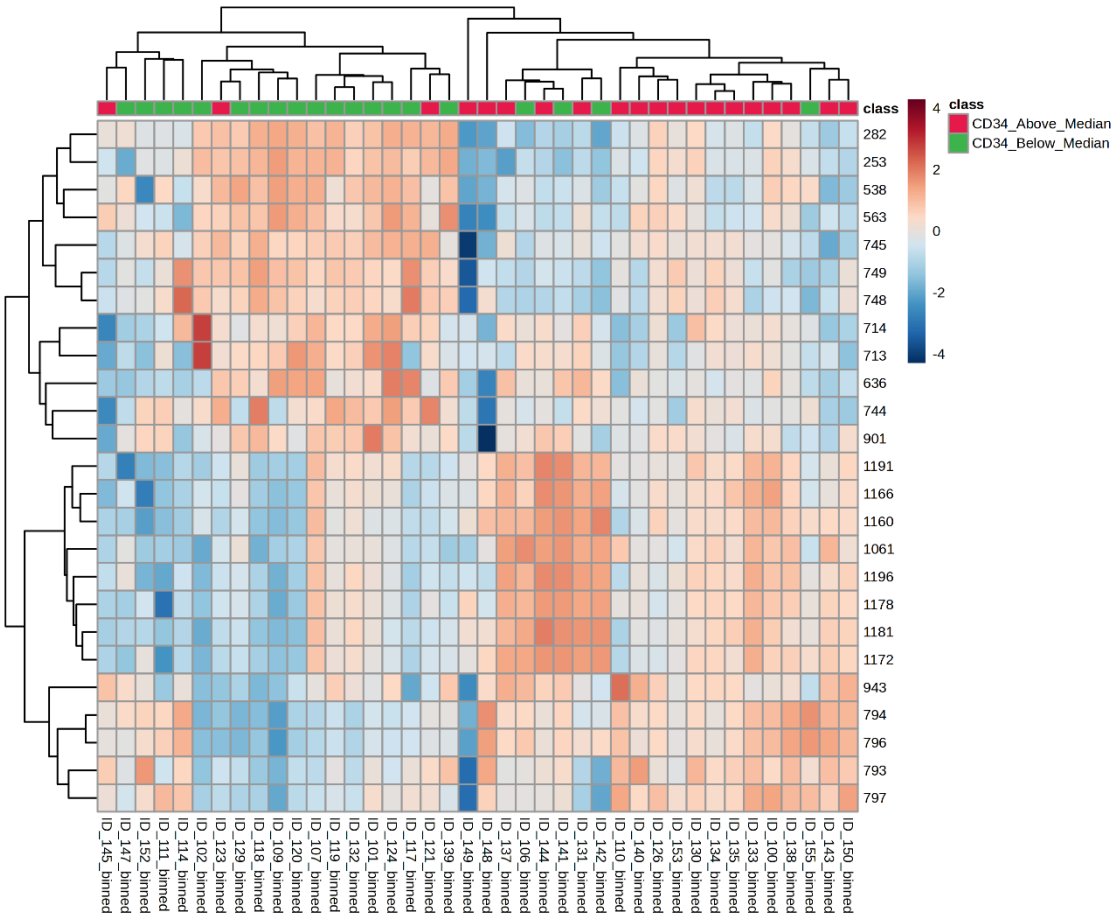




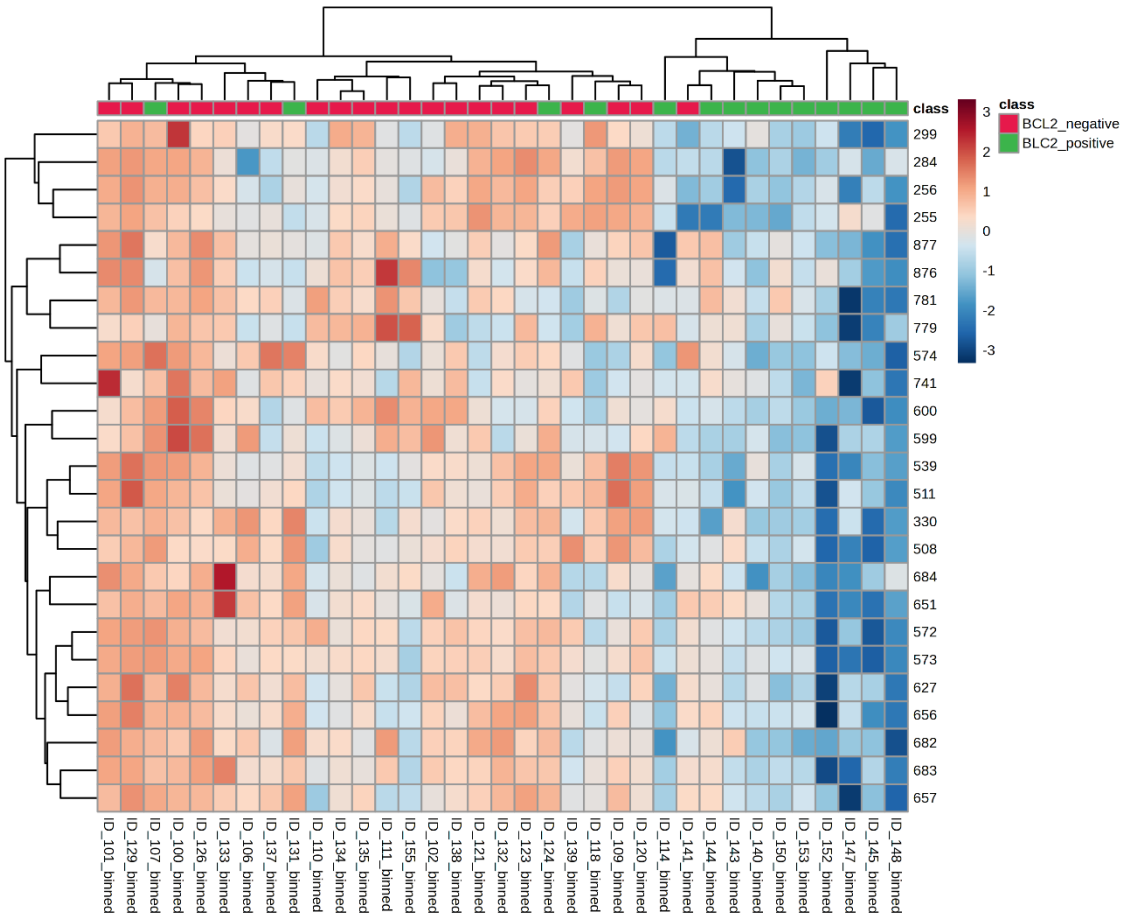
Supplementary Figure 2 Representative results of ionization failures. In the two left panels, it is observed a lack of correlation between the chemical image generated and the frozen section analyzed. On the right panels, it is possible to observe the chemical spectra obtained demonstrating the relative abundance of ions between m/z 180 and m/z 1200. As a result of inadequate ionization, nonspecific ions can significantly interfere with the interpretation of results, evidenced by the background noise observed in the spectra.



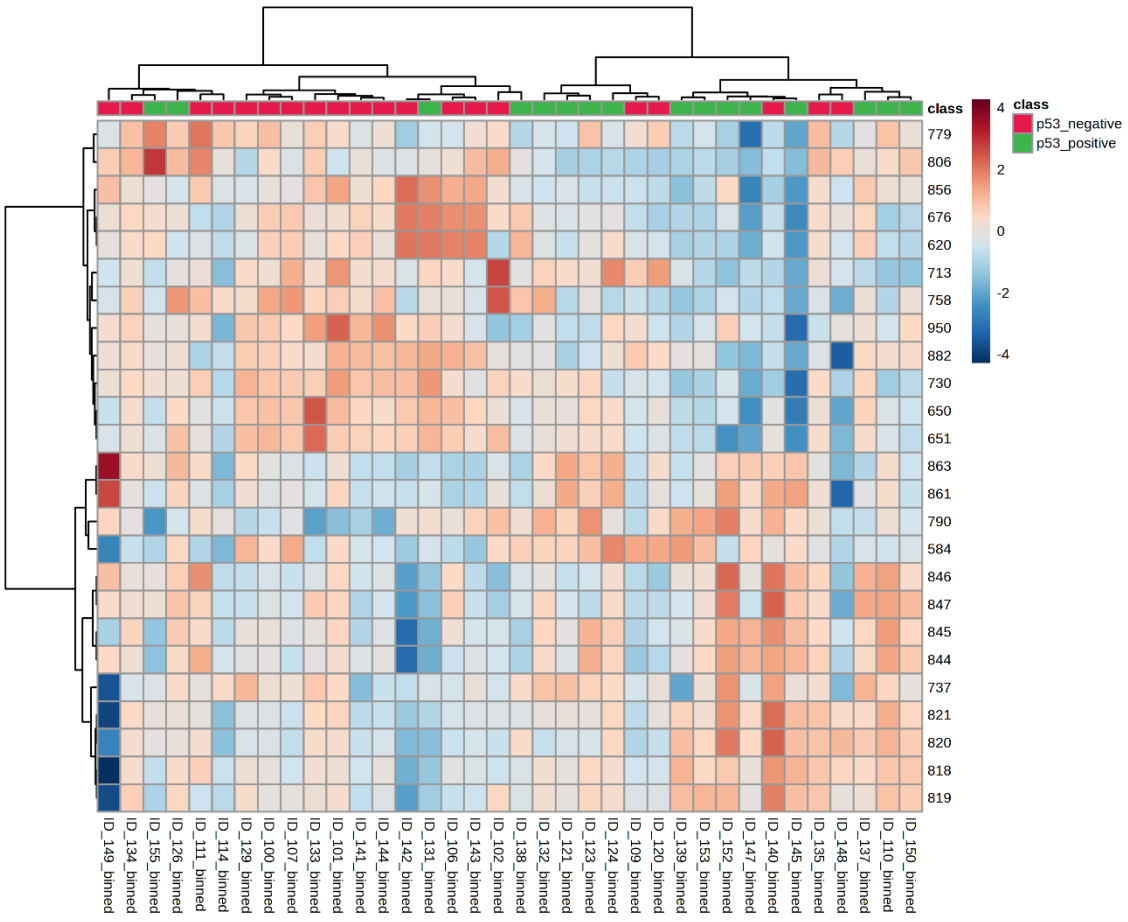
Supplementary Figure 3 Hierarchical clustering analysis based on complete clustering method and Euclidean distance representing the 25 main variables responsible for grouping the samples into the respective groups: tumors with CD34-positive vessels below and above the median. The color bar represents the data values across the samples, where blue means lower ion abundance and red means higher ion abundance



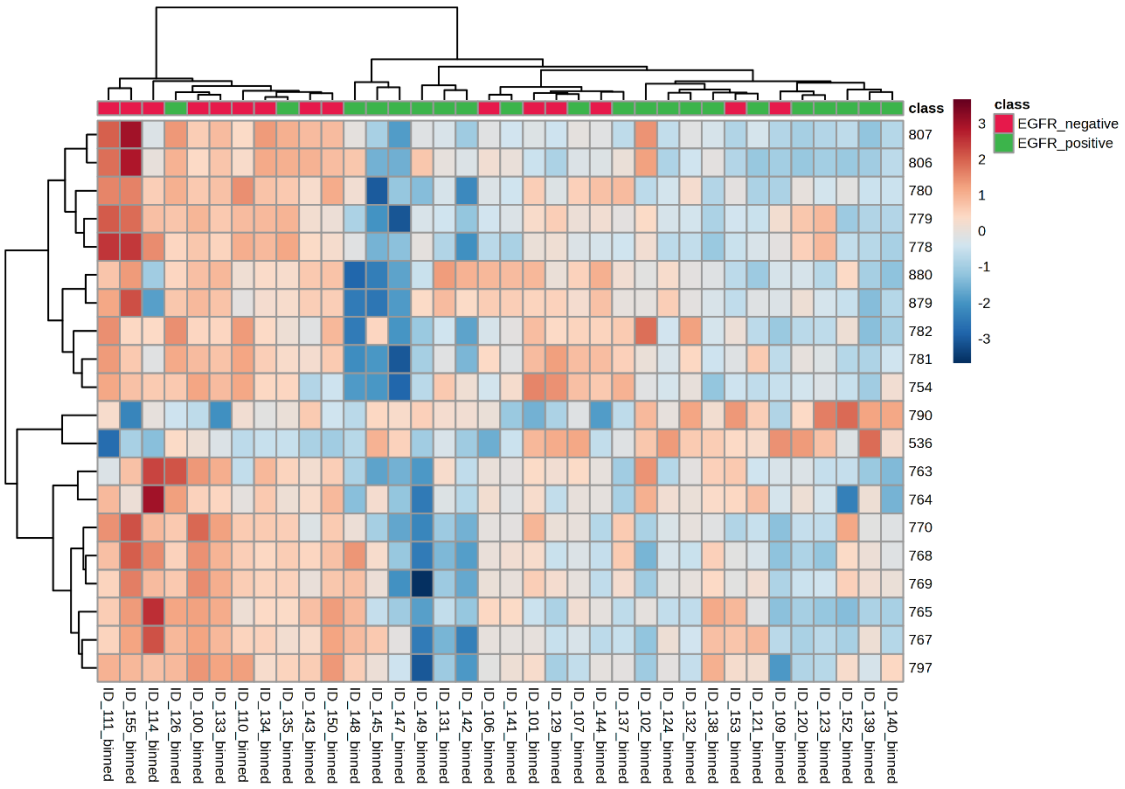
Supplementary Figure 4 Hierarchical clustering analysis based on complete-linkage clustering and Euclidean distance representing the 25 main variables responsible for grouping the samples into the respective groups: positive and negative tumors for BCL2 immunoexpression. The color bar represents the data values across the samples, where blue means lower ion abundance and red means higher ion abundance



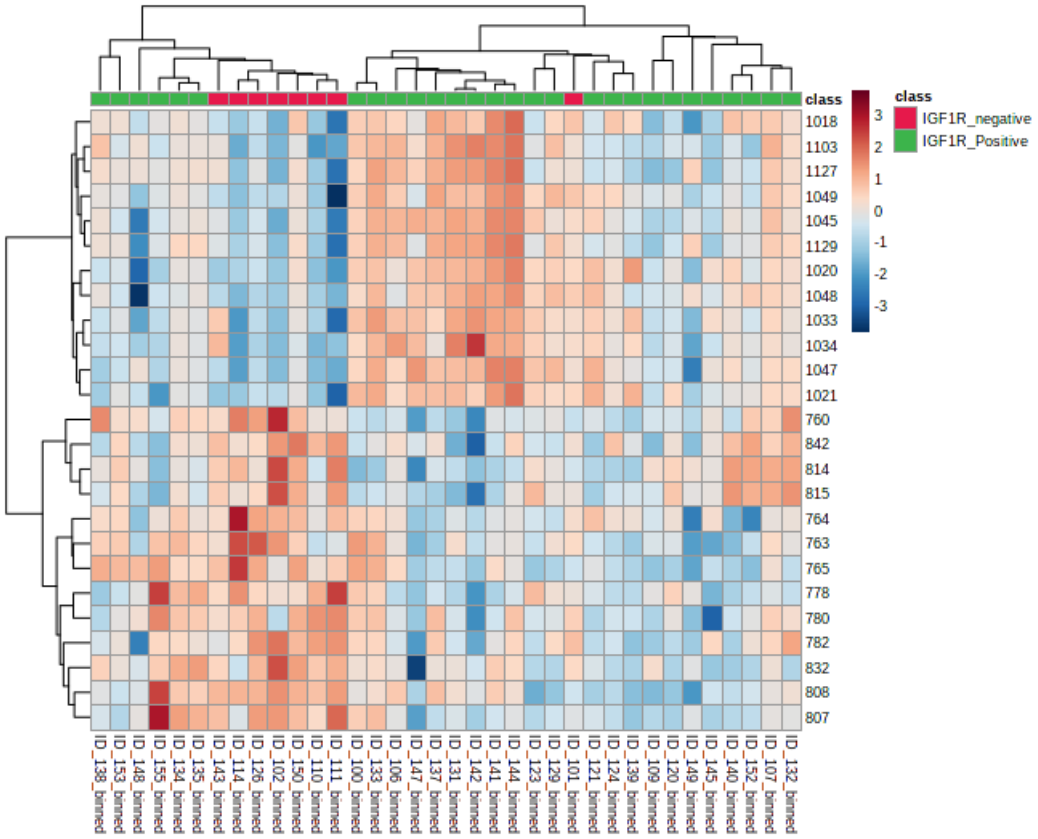
Supplementary Figure 5 Hierarchical clustering analysis based on Ward clustering method and Pearson distance representing 25 variables responsible for grouping the samples into the respective groups: positive and negative tumors for p53 immunoexpression. The color bar represents the data values across the samples, where blue means lower ion abundance and red means higher ion abundance



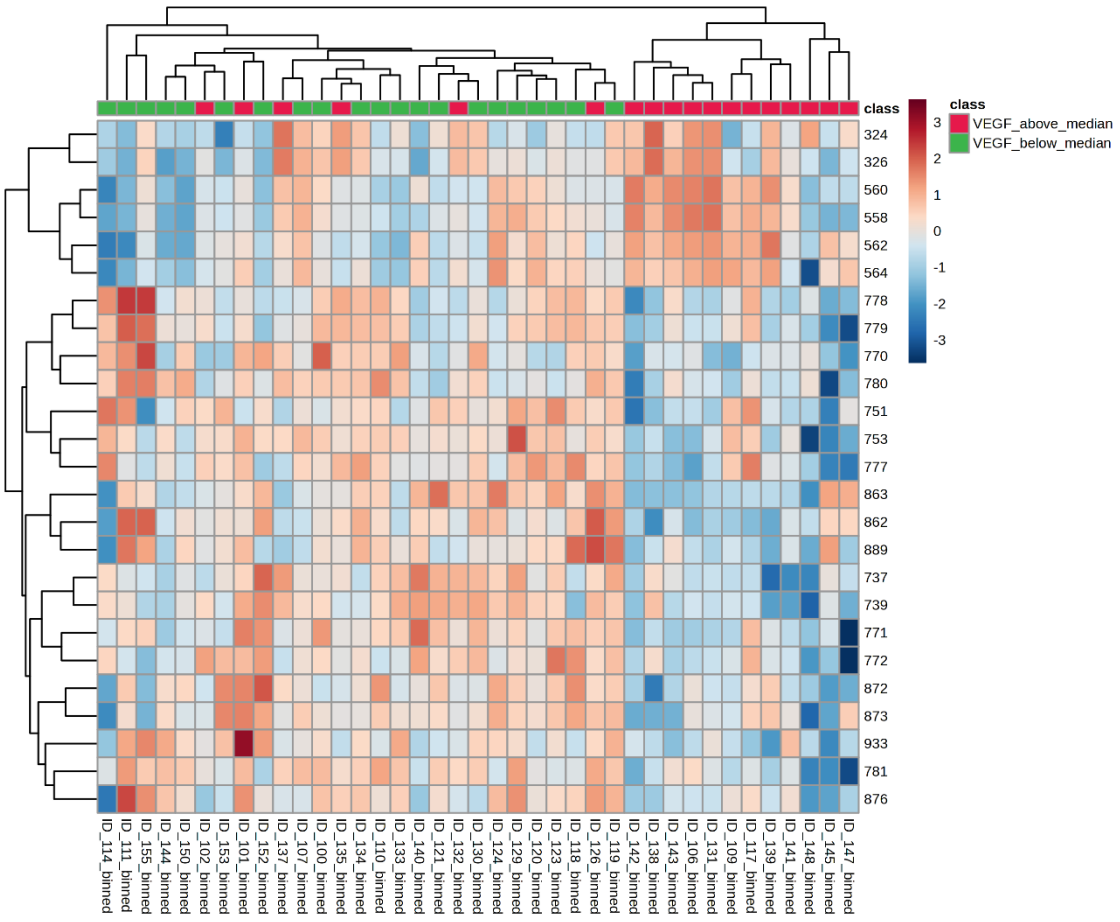
Supplementary Figure 6 Hierarchical clustering analysis based on Ward clustering method and Euclidean distance representing the main 20 variables responsible for grouping the samples into the respective groups: positive and negative tumors for EGFR immunoexpression. The color bar represents the data values across the samples, where blue means lower ion abundance and red means higher ion abundance



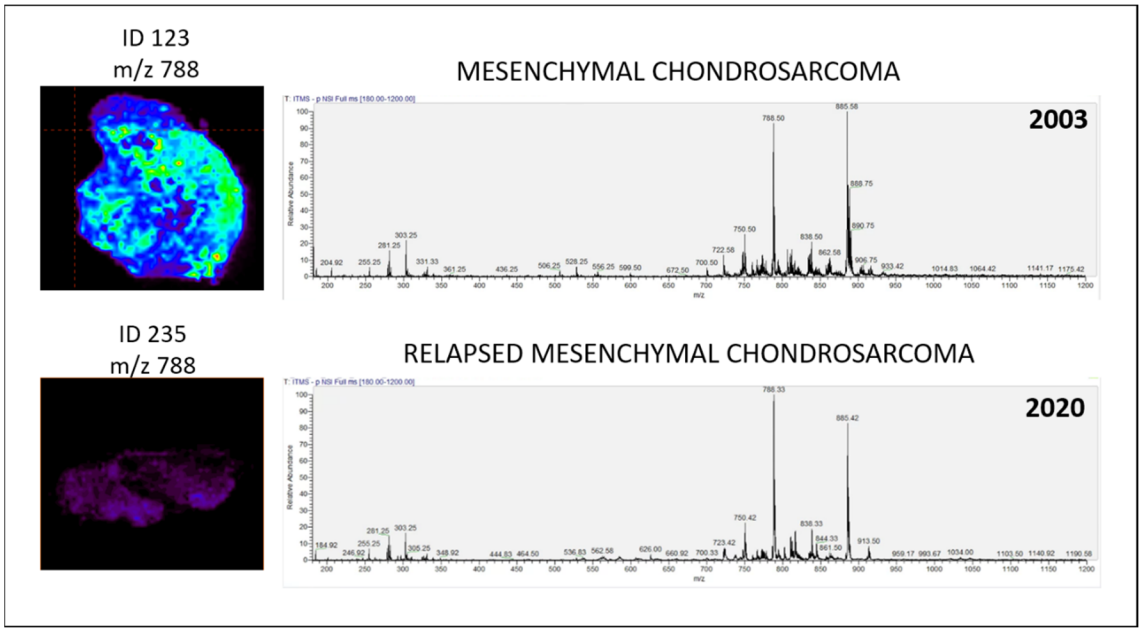
Supplementary Figure 7 Hierarchical clustering analysis representing the main 25 variables responsible for grouping the samples into the respective groups: positive and negative tumors for IGF1R immunoexpression. The color bar represents the data values across the samples, where blue means lower ion abundance and red means higher ion abundance



Supplementary Figure 8 Hierarchical clustering analysis based on complete-linkage clustering and Euclidean distance representing the 25 main variables responsible for grouping the samples into the respective groups: positive and negative tumors for VEGF immunoexpression. The color bar represents the data values across the samples, where blue means lower ion abundance and red means higher ion abundance



Supplementary Figure 9 DESI-MSI analysis of tumor samples from the same patient, at the time of initial diagnosis (year 2003) and at tumor recurrence (year 2020). The left panels depict the chemical images generated from DESI-MSI analysis of frozen sections, evidencing the distribution of the *m/z* 788 ion in the tissue. The right panels show the chemical spectra of representative areas of the analyzed tissues, evidencing a high similarity between the chemical profile of the samples, with slight alterations in the relative abundances of the ions. X axis: *m/z* range 180-1200. Y axis: relative abundance of ions. The pathology diagnosis confirmed tumor recurrence



Supplementary Table 1: Histopathology diagnosis of patients included in the study.

	N. Patients
Primary CNS tumor	129
Metastatic	3
Total	132

Histology	**N. patients**
ASTROCYTOMA*	37
MEDULLOBLASTOMA	21
EPENDYMOMA	11
CRANIOPHARYONGIOMA	7
GANGLIOGLIOMA	7
CHOROID PLEXUS CARCINOMA	6
DNET	6
SCHWANNOMA	3
Other**	34
Total	132

* This group includes both low-grade and high-grade astrocytomas.

** Tumors that were diagnosed in two or less patients were not detailed separately, it were included in the "other" category.

Supplementary Table 2: List of primary antibodies used in the immunohistochemical studies.

STAINING PURPOSE	ANTIBODY ANTI-	DETAILS	SUPPLIER
Angiogenesis	CD34	clone QBEnd/10	Agilent DAKO
	VEGF *	clone VG1	Biogen / Agilent DAKO
Cell proliferation	KI-67	clone SP6	Agilent DAKO
Apoptosis	Bcl-2	clone E17	Agilent DAKO
	Bax	clone 2D2	Biogen
Oncogene	ALK	SP8	Agilent DAKO
Tumor supressor	P53	DO7	Agilent DAKO
Growth factor	EGFR *	Clone EP22	Biogen
	PDGFR-alpha*	Policlonal	Biogen / Analítica (ABCAM)
	IGF2*	Policlonal	Biogen / Analítica (ABCAM)
	IGF1R*	Policlonal	Biogen / Sigma
Cell adhesion	Beta-catenin	Beta-catenin-1	Agilent DAKO
	E-cadherin	NCH-38	Agilent DAKO

*VEGF: *Vascular Endothelial Growth Factor*, EGFR: *Epidermal Growth Factor Receptor*, PDGFR-alpha: *Platelet-derived growth factor receptor*, *alpha polypeptide*, IGF2: *Insulin-like growth factor* 2, IGF1R: *Insulin-like growth factor 1 receptor*.

Supplementary Table 3: List of 119 differentially abundant ions found between normal and tumor tissues.

	FC	log2(FC)	p.ajusted	-LOG10(p)
917	4.7238	2.2399	3.3248e-07	6.4782
918	4.4968	2.1689	3.3248e-07	6.4782
907	5.6393	2.4955	8.1611e-07	6.0883
834	4.7777	2.2563	8.1611e-07	6.0883
891	4.3275	2.1135	8.1611e-07	6.0883
835	4.117	2.0416	8.1611e-07	6.0883
919	3.8221	1.9344	8.1611e-07	6.0883
892	3.5793	1.8397	8.1611e-07	6.0883
909	2.6647	1.414	1.1828e-06	5.9271
906	5.419	2.438	1.2743e-06	5.8947
916	4.4783	2.163	1.2743e-06	5.8947
902	4.0079	2.0028	1.2743e-06	5.8947
893	3.1033	1.6338	1.4943e-06	5.8256
890	3.8488	1.9444	8.2145e-06	5.0854
920	3.5264	1.8182	1.0061e-05	4.9974
932	2.6524	1.4073	1.0061e-05	4.9974
823	0.404	-1.3076	1.2727e-05	4.8953
908	3.2613	1.7054	1.4438e-05	4.8405
800	0.37898	-1.3998	1.8294e-05	4.7377
905	5.0311	2.3309	2.3176e-05	4.635
904	4.6448	2.2156	2.4088e-05	4.6182
903	4.1732	2.0612	2.4088e-05	4.6182
842	0.44306	-1.1744	2.4088e-05	4.6182
771	0.39107	-1.3545	2.6602e-05	4.5751
741	0.38348	-1.3828	3.8906e-05	4.41
740	0.39589	-1.3368	4.7675e-05	4.3217
934	2.4429	1.2886	6.4425e-05	4.1909
833	2.4369	1.285	6.4425e-05	4.1909
877	2.2784	1.188	6.4425e-05	4.1909
921	2.9584	1.5648	8.4545e-05	4.0729
801	0.41949	-1.2533	8.4765e-05	4.0718
824	0.46317	-1.1104	9.3978e-05	4.027
889	3.7451	1.905	0.00012402	3.9065
943	0.31812	-1.6524	0.00014874	3.8276
802	0.46846	-1.094	0.00014874	3.8276
933	2.1339	1.0935	0.00014987	3.8243
796	0.36597	-1.4502	0.00015603	3.8068
822	0.44799	-1.1585	0.00020491	3.6884
769	0.48178	-1.0536	0.0002088	3.6803
793	0.48145	-1.0545	0.00031651	3.4996
770	0.47805	-1.0648	0.00032966	3.4819
768	0.4142	-1.2716	0.0004169	3.38

795	0.42282	-1.2419	0.00042134	3.3754
794	0.37339	-1.4212	0.00045764	3.3395
742	0.49786	-1.0062	0.00046292	3.3345
1001	0.39646	-1.3348	0.00067758	3.169
830	0.4747	-1.0749	0.00075771	3.1205
722	0.24726	-2.0159	0.0009791	3.0092
684	0.31917	-1.6476	0.0011437	2.9417
828	0.45325	-1.1416	0.0019575	2.7083
829	0.48496	-1.0441	0.0025639	2.5911
184	0.095344	-3.3907	0.0030785	2.5117
944	0.44611	-1.1645	0.0036553	2.4371
1174	0.38278	-1.3854	0.0039031	2.4086
999	0.48119	-1.0553	0.0040602	2.3915
1000	0.47221	-1.0825	0.0042234	2.3743
324	0.14119	-2.8243	0.0042399	2.3726
888	2.512	1.3288	0.0044119	2.3554
630	3.4324	1.7792	0.0044748	2.3492
1176	0.36692	-1.4465	0.004539	2.343
945	0.47128	-1.0853	0.0059659	2.2243
758	0.48153	-1.0543	0.006052	2.2181
1002	0.49154	-1.0246	0.0072131	2.1419
1047	0.49294	-1.0205	0.008632	2.0639
1184	0.41694	-1.2621	0.008678	2.0616
970	0.39313	-1.3469	0.0088574	2.0527
698	0.42239	-1.2434	0.0088574	2.0527
984	0.48291	-1.0502	0.0093451	2.0294
1060	0.48801	-1.035	0.0098588	2.0062
968	0.40112	-1.3179	0.010877	1.9635
572	0.3451	-1.5349	0.011109	1.9543
1059	0.46411	-1.1075	0.011109	1.9543
1179	0.4211	-1.2478	0.01128	1.9477
732	0.40404	-1.3074	0.011541	1.9378
1178	0.39411	-1.3433	0.01172	1.9311
631	3.1387	1.6502	0.012274	1.911
339	0.33395	-1.5823	0.012779	1.8935
607	2.6691	1.4163	0.012779	1.8935
1061	0.46897	-1.0924	0.01298	1.8867
1196	0.40767	-1.2945	0.0133	1.8762
311	0.28632	-1.8043	0.014066	1.8518
1085	0.38756	-1.3675	0.014066	1.8518
598	2.5126	1.3292	0.014195	1.8479
1197	0.39947	-1.3238	0.01646	1.7836
1175	0.40248	-1.313	0.01646	1.7836
325	0.28484	-1.8118	0.018193	1.7401
567	2.329	1.2197	0.018193	1.7401
600	3.0792	1.6226	0.019942	1.7002

1198	0.42684	-1.2282	0.021228	1.6731
972	0.49207	-1.0231	0.0219	1.6596
1067	0.4856	-1.0422	0.022243	1.6528
734	0.41479	-1.2696	0.022822	1.6417
608	2.1461	1.1017	0.022822	1.6417
278	0.35445	-1.4964	0.023658	1.626
606	2.6139	1.3862	0.023658	1.626
1182	0.44854	-1.1567	0.024659	1.608
1181	0.44132	-1.1801	0.025974	1.5855
1084	0.44156	-1.1793	0.025974	1.5855
1187	0.46195	-1.1142	0.028637	1.5431
1124	0.49112	-1.0259	0.028637	1.5431
1113	0.468	-1.0954	0.029388	1.5318
1119	0.49779	-1.0064	0.029388	1.5318
1151	0.45316	-1.1419	0.029842	1.5252
1150	0.44478	-1.1688	0.030481	1.516
1083	0.4729	-1.0804	0.030665	1.5134
1173	0.46065	-1.1183	0.031139	1.5067
1123	0.44898	-1.1553	0.033309	1.4774
185	0.27213	-1.8776	0.033821	1.4708
1172	0.47065	-1.0873	0.034188	1.4661
340	0.40963	-1.2876	0.034787	1.4586
1185	0.46836	-1.0943	0.03595	1.4443
1177	0.44413	-1.1709	0.039798	1.4001
682	0.45809	-1.1263	0.040076	1.3971
1180	0.47548	-1.0726	0.042852	1.368
309	0.28127	-1.83	0.044148	1.3551
1195	0.4818	-1.0535	0.044148	1.3551
765	0.48329	-1.049	0.045805	1.3391
1147	0.4436	-1.1727	0.046487	1.3327
460	0.27442	-1.8655	0.047179	1.3263

Supplementary Table 4: List of 104 differentially abundant ions found between low- and high-grade tumors.

	FC	log2(FC)	p.ajusted	-LOG10(p)
806	0.3352	-1.5769	0.00087815	3.0564
282	2.8242	1.4978	0.00087815	3.0564
1170	0.39509	-1.3398	0.00087815	3.0564
281	2.2044	1.1404	0.00087815	3.0564
305	2.9518	1.5616	0.00091175	3.0401
1169	0.36689	-1.4466	0.00091175	3.0401
1175	0.38821	-1.3651	0.00091175	3.0401
1157	0.3932	-1.3467	0.00091175	3.0401
1159	0.40505	-1.3038	0.00091175	3.0401
1174	0.41485	-1.2693	0.00091175	3.0401
1155	0.42681	-1.2283	0.00091175	3.0401
329	3.1241	1.6434	0.0009889	3.0048
1144	0.35454	-1.496	0.0009889	3.0048
837	2.4017	1.2641	0.0009889	3.0048
1176	0.43067	-1.2154	0.0009889	3.0048
1166	0.45037	-1.1508	0.0009889	3.0048
253	3.5582	1.8311	0.0010929	2.9614
509	2.043	1.0307	0.001165	2.9337
1143	0.34869	-1.52	0.0013145	2.8812
1199	0.36731	-1.4449	0.0013145	2.8812
1085	0.37684	-1.408	0.0013145	2.8812
1198	0.41467	-1.27	0.0013157	2.8808
1115	0.42385	-1.2384	0.0013157	2.8808
1114	0.46892	-1.0926	0.0013157	2.8808
307	4.4184	2.1435	0.0013178	2.8802
309	3.6188	1.8555	0.0013178	2.8802
1147	0.38572	-1.3744	0.0013178	2.8802
970	0.39221	-1.3503	0.0013178	2.8802
1150	0.41142	-1.2813	0.0013178	2.8802
1173	0.41897	-1.2551	0.0013178	2.8802
1160	0.43123	-1.2135	0.0013178	2.8802
1178	0.43307	-1.2073	0.0013178	2.8802
1179	0.43892	-1.188	0.0013178	2.8802
1183	0.44002	-1.1844	0.0013178	2.8802
1099	0.44135	-1.18	0.0013178	2.8802
1152	0.44347	-1.1731	0.0013178	2.8802
1100	0.44735	-1.1605	0.0013178	2.8802
1167	0.45168	-1.1466	0.0013178	2.8802
1172	0.45375	-1.14	0.0013178	2.8802
1128	0.48891	-1.0324	0.0013178	2.8802
1096	0.45581	-1.1335	0.0013859	2.8583
807	0.41089	-1.2832	0.0014072	2.8516

1139	0.47464	-1.0751	0.0014293	2.8449
1142	0.38732	-1.3684	0.0014521	2.838
1101	0.46651	-1.1	0.0014758	2.831
1177	0.41424	-1.2715	0.0015529	2.8088
1156	0.43088	-1.2147	0.0015529	2.8088
1102	0.44593	-1.1651	0.0015529	2.8088
1182	0.43117	-1.2137	0.001611	2.7929
1153	0.44262	-1.1759	0.0016413	2.7848
1148	0.36006	-1.4737	0.0016468	2.7834
1123	0.44566	-1.166	0.0016468	2.7834
1185	0.45597	-1.133	0.0016788	2.775
331	2.7763	1.4732	0.0017305	2.7618
1171	0.39839	-1.3277	0.0017305	2.7618
1158	0.43112	-1.2138	0.0017305	2.7618
1089	0.4318	-1.2116	0.0017305	2.7618
1084	0.43695	-1.1944	0.0017305	2.7618
1188	0.44119	-1.1805	0.0017305	2.7618
1194	0.45578	-1.1336	0.0017305	2.7618
1184	0.4662	-1.101	0.0017305	2.7618
1191	0.47395	-1.0772	0.0017305	2.7618
280	2.0835	1.059	0.0017305	2.7618
1146	0.35202	-1.5063	0.0017598	2.7545
1193	0.4722	-1.0825	0.0017981	2.7452
1186	0.44241	-1.1765	0.0018152	2.7411
1116	0.49272	-1.0212	0.0018152	2.7411
1145	0.36457	-1.4557	0.0018965	2.7221
1086	0.38547	-1.3753	0.0018965	2.7221
1151	0.44734	-1.1605	0.0018965	2.7221
1125	0.46279	-1.1116	0.0018965	2.7221
1121	0.46798	-1.0955	0.0018965	2.7221
1097	0.47238	-1.082	0.0018965	2.7221
1168	0.47329	-1.0792	0.0018965	2.7221
1083	0.4823	-1.052	0.0018965	2.7221
1126	0.4951	-1.0142	0.0018965	2.7221
1180	0.43369	-1.2053	0.0019852	2.7022
765	0.46611	-1.1012	0.0019852	2.7022
804	0.49864	-1.0039	0.0021269	2.6723
1122	0.45186	-1.146	0.0021423	2.6691
1113	0.47305	-1.0799	0.0021423	2.6691
1118	0.48564	-1.042	0.0021423	2.6691
883	2.0521	1.0371	0.0021423	2.6691
1154	0.4599	-1.1206	0.0021727	2.663
1181	0.46891	-1.0926	0.0021727	2.663
1149	0.40469	-1.3051	0.0022366	2.6504
1090	0.48402	-1.0469	0.0022366	2.6504
1192	0.49865	-1.0039	0.0022366	2.6504

1087	0.38707	-1.3693	0.0022904	2.6401
1095	0.45227	-1.1447	0.0025032	2.6015
1141	0.43262	-1.2088	0.0025408	2.595
1073	0.49939	-1.0018	0.0025408	2.595
1140	0.47137	-1.0851	0.0026417	2.5781
1117	0.49373	-1.0182	0.0029295	2.5332
1195	0.48694	-1.0382	0.0030227	2.5196
836	2.0286	1.0205	0.0034051	2.4679
1088	0.38435	-1.3795	0.003571	2.4472
1187	0.46654	-1.0999	0.0038348	2.4163
1190	0.47398	-1.0771	0.0038348	2.4163
1197	0.47917	-1.0614	0.0047447	2.3238
1132	0.4948	-1.0151	0.0063024	2.2005
1189	0.47862	-1.063	0.0065151	2.1861
184	0.12862	-2.9588	0.0073119	2.136
968	0.42442	-1.2364	0.0096652	2.0148