Analytical and Bioanalytical Chemistry

Electronic Supplementary Material

Rapid determination of isocitrate dehydrogenase mutation status of human gliomas by extraction nanoelectrospray using a miniature mass spectrometer

Fan Pu, Clint M. Alfaro, Valentina Pirro, Zhuoer Xie, Zheng Ouyang, R. Graham Cooks

Materials

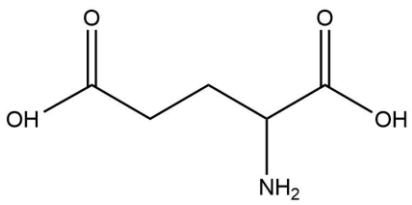
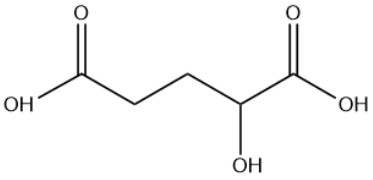
2-Hydroxyglutarate (2-HG), glutamic acid (GLU), acetonitrile and Whatman 1 filter paper were purchased from Sigma-Aldrich (St. Louis, MO). Ultrapure water was obtained from a Milli-Q system (Bedford, MA). Disposable sterile acupuncture needles (0.3 mm x 40 mm) were purchased from Zhongyan Taihe (Beijing, China).

Fabrication of Nanotips

Borosilicate glass capillaries (o.d. 1.5mm, i.d. 0.86 mm, without filament) were purchased from Sutter Instrument (Novato, CA). Borosilicate glass capillaries were pulled into 5 µm tips using a micropipette puller (Model P-97, Sutter Instrument).

GLU and 2-HG Structures and Tandem MS Transitions

Table S1 Chemical structures and MS/MS transitions of GLU and 2-HG

	GLU	**2-HG**
Chemical Structures		
***m/z* of precursor ions (negative mode)**	146	147
***m/z* of product ions (negative mode)**	102, 128	129

MS/MS Product Ion Spectra of GLU and 2-HG

MS/MS product ion spectra of 10 µg/mL GLU and 2-HG in methanol/water (9:1, v/v) solutions were recorded using nanoESI.

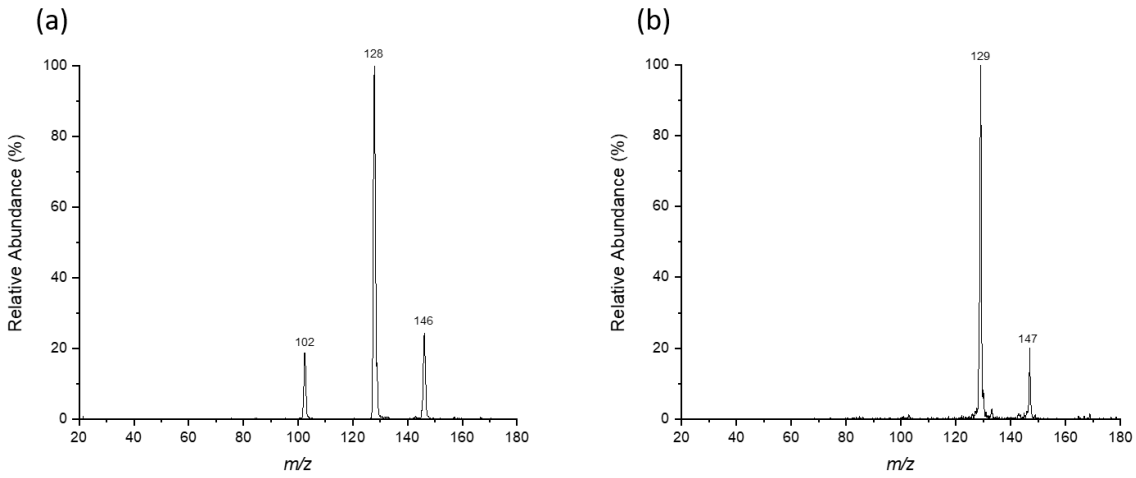


Fig. S1 MS/MS product ion spectra recorded using Mini MS (a) GLU; (b) 2-HG

Calculation of IDH Mutation Score

When calculating IDH mutation scores, the intensities of *m/z* 129 was corrected for isotopic contribution from GLU, the following formula was used to calculate the IDH mutation score:

$$IDH\ Mutation\ Score = \frac{I_{129} - I_{128} \times 6.1\%}{I_{128}}$$

I_{129} and I_{128} are absolute intensities of *m/z* 129 and 128, respectively.

Statistical Analysis and Software

In the box and whisker plots, boxes show median, lower, and upper quartiles, and whiskers are at minimum and maximum values.

Linear regressions were applied to establish correlations of 2HG concentration in IDH mutants and their corresponding IDH mutation scores.

t-Test of two-sample assuming unequal variances was performed on IDH mutant scores between IDH mutants and IDH wildtypes, two tail p-values were reported.

Microsoft Excel was used to perform linear regressions and t-Test. OriginPro 2018b was used to plot all spectra and box and whisker plots. ChemDraw Professional 16.0 was used to draw chemical structures.

IDH Mutation Scores of Tissue Sections

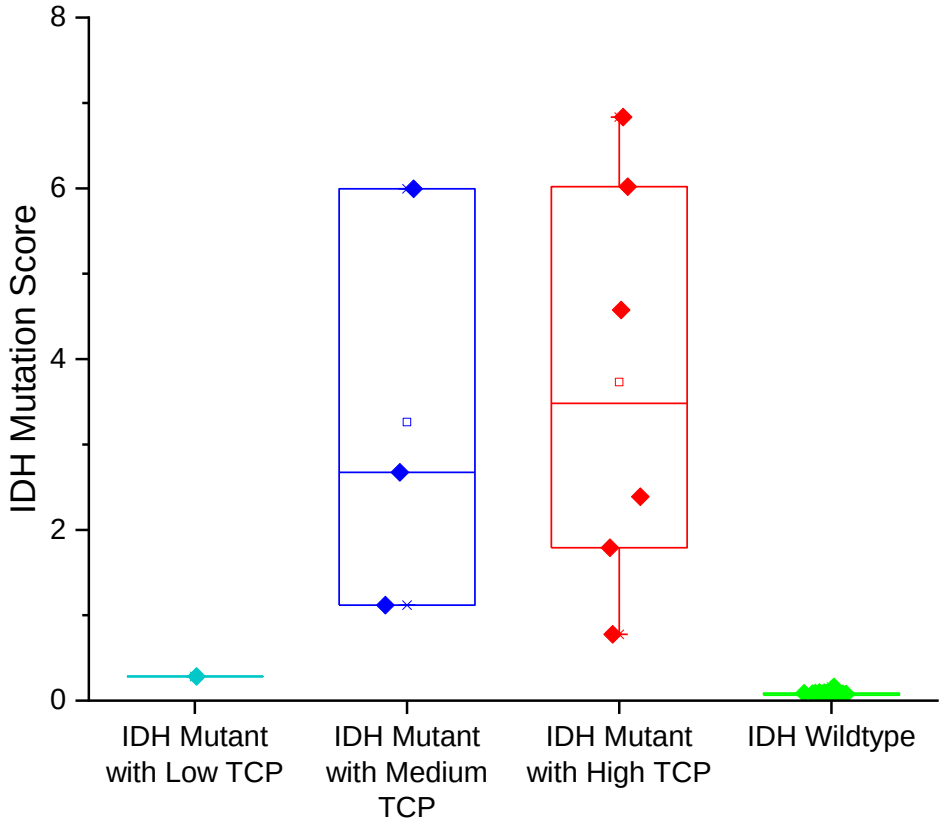


Fig. S2 IDH mutation scores of tissue sections. 29 IDH wildtype, 10 IDH mutant (1 low TCP, 3 medium TCP and 6 high TCP)

Table S2 IDH mutation scores of tissue sections

Subject No.	Sample No.	IDH Mutation Score	IDH Mutation Status	TCP	Diagnosis
1	1-S1	0.0634	Wildtype	Low	Normal
	1-S2	0.0846	Wildtype	Low	Normal
2	2-S1	0.0909	Wildtype	Low	Normal

	2-S2	0.0794	Wildtype	Low	Normal
3	3-S1	0.0703	Wildtype	Low	Normal
4	4-S1	0.0908	Wildtype	Low	Normal
5	5-S1	0.0934	Wildtype	Low	Infiltrated margin
6	6-S1	0.0888	Wildtype	Medium	Glioma
	6-S2	0.0668	Wildtype	Medium	Glioma
7	7-S1	0.0852	Wildtype	Low	Normal
	7-S2	0.0610	Wildtype	Low	Normal
8	8-S1	0.0760	Wildtype	Low	Infiltrated margin
9	9-S1	0.0795	Wildtype	Medium	Glioma
10	10-S1	0.0618	Wildtype	Low	Infiltrated margin
	10-S2	0.0810	Wildtype	Low	Infiltrated margin
11	11-S1	0.1370	Wildtype	High	Glioma
12	12-S1	0.0512	Wildtype	Low	Infiltrated margin
	12-S2	0.0525	Wildtype	Low	Infiltrated margin
	12-S3	0.0868	Wildtype	Low	Infiltrated margin
13	13-S1	0.0953	Wildtype	High	Glioma
14	14-S1	0.0810	Wildtype	Medium	Glioma
15	15-S1	0.1609	Wildtype	High	Glioma
16	16-S1	0.0506	Wildtype	Low	Normal
17	17-S1	0.0387	Wildtype	Low	Infiltrated margin
18	18-S1	0.0625	Wildtype	Low	Infiltrated margin
19	19-S1	0.0521	Wildtype	Medium	Normal
20	20-S1	0.0888	Wildtype	Medium	Normal
21	21-S1	0.0686	Wildtype	Low	Normal
	21-S2	0.0765	Wildtype	Low	Normal
22	22-S1	0.2822	Mutant	Low	Glioma
23	23-S1	0.7763	Mutant	High	Glioma
24	24-S1	1.1191	Mutant	Medium	Glioma
25	25-S1	1.7910	Mutant	High	Glioma
26	26-S1	2.3883	Mutant	High	Glioma
27	27-S1	2.6741	Mutant	Medium	Glioma
28	28-S1	4.5745	Mutant	High	Glioma
	28-S2	6.0185	Mutant	High	Glioma
	28-S3	6.8349	Mutant	High	Glioma
29	29-S1	5.9937	Mutant	Medium	Glioma

IDH Mutation Scores of Bulk Tissue Biopsies

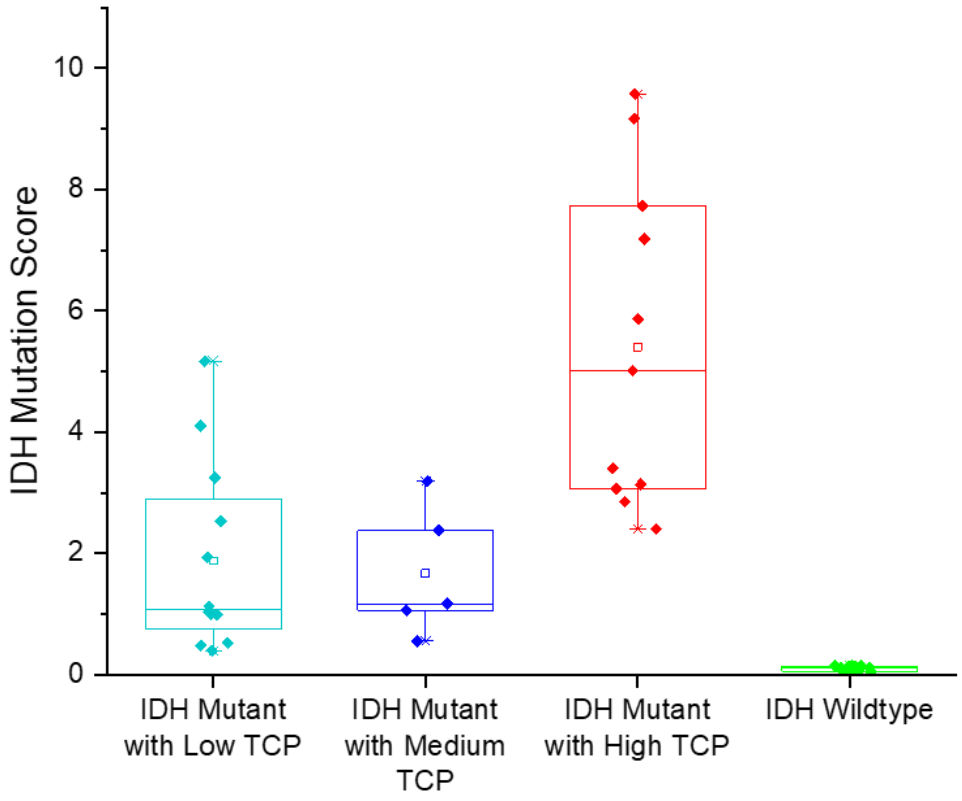


Fig. S3 IDH mutation scores of tissue biopsies. 16 were IDH Wildtype, 28 were IDH mutant (12 low TCP, 5 medium TCP and 11 high TCP)

Table S3 Reproducibility of extraction nESI using Mini MS, n=3

Sample No.	IDH Mutation Score	IDH Mutation Status	RSD
M1	11.5377	Mutant	26.72%
M2	5.1516	Mutant	27.05%
M3	2.6115	Mutant	33.08%
W1	0.0699	Wildtype	25.72%
W2	0.0656	Wildtype	34.74%
W3	0.0689	Wildtype	6.32%
W4	0.0741	Wildtype	33.70%
W5	0.0842	Wildtype	23.53%

Table S4 IDH mutation scores of tissue biopsies

Subject No.	Sample No.	IDH Mutation Score	IDH Mutation Status	TCP	Glioma Diagnosis
30	30-B1	0.0811	Wildtype	Low	Infiltrated white matter
	30-B2	0.1190	Wildtype	High	Glioma
	30-B3	0.1100	Wildtype	Low	Infiltrated white matter
	30-B4	0.1539	Wildtype	Low	Infiltrated white matter
	30-B5	0.1460	Wildtype	Low	Infiltrated white matter
31	31-B1	0.1394	Wildtype	High	Glioma
	31-B2	0.0900	Wildtype	High	Glioma
	31-B3	0.1012	Wildtype	High	Glioma
32	32-B1	0.0376	Wildtype	Low	Grey and white matter (80%)
	32-B2	0.0518	Wildtype	Low	White matter
	32-B3	0.1463	Wildtype	Low	White matter
33	33-B1	0.1166	Wildtype	High	Glioma
	33-B2	0.0466	Wildtype	High	Glioma
	33-B3	0.0659	Wildtype	Low	Infiltrated white matter
34	34-B1	0.1295	Wildtype	High	Tumor
35	35-B1	0.0425	Wildtype	Low	Infiltrated grey matter
36	36-B1	3.1891	Mutant	Medium	Glioma
	36-B2	2.3792	Mutant	Medium	Glioma
	36-B3	3.2451	Mutant	Low	Infiltrative margin
	36-B4	0.9918	Mutant	Low	Infiltrative margin
37	37-B1	9.5765	Mutant	High	Glioma
	37-B2	1.9356	Mutant	Low	Infiltrated white matter
	37-B3	1.1297	Mutant	Low	Infiltrated white matter
	37-B4	5.1713	Mutant	Low	Infiltrated white matter
38	38-B1	7.7310	Mutant	High	Glioma
	38-B2	9.1739	Mutant	High	Glioma
39	39-B1	0.9982	Mutant	Low	White matter
	39-B2	4.1080	Mutant	Low	Glioma
	39-B3	0.5529	Mutant	Medium	Infiltrated margin
	39-B4	1.0613	Mutant	Medium	Infiltrated white matter
40	40-B1	1.0244	Mutant	Low	Infiltrated white matter
	40-B2	0.4832	Mutant	Low	Infiltrated white matter
	40-B3	0.5211	Mutant	Low	Grey matter
41	41-B1	5.0162	Mutant	High	Tumor
	41-B2	5.8643	Mutant	High	Tumor
	41-B3	3.1335	Mutant	High	Tumor
	41-B4	7.1849	Mutant	High	Tumor
	41-B5	3.4018	Mutant	High	Tumor

42	42-B1	0.3971	Mutant	Low	Grey matter
	42-B2	1.1677	Mutant	Medium	Glioma
43	43-B1	2.8553	Mutant	High	Glioma
	43-B2	2.4004	Mutant	High	Glioma
44	44-B1	3.0641	Mutant	High	Glioma
	44-B2	2.5315	Mutant	Low	Infiltrative margin

Typical MS/MS Spectra from Extraction Nanoelectrospray of Bulk Tissue Biopsies

Typical MS/MS spectra recorded from extraction nanoelectrospray of another four different bulk tissue biopsies are presented here, insets are enlarged views of the peaks of interests.

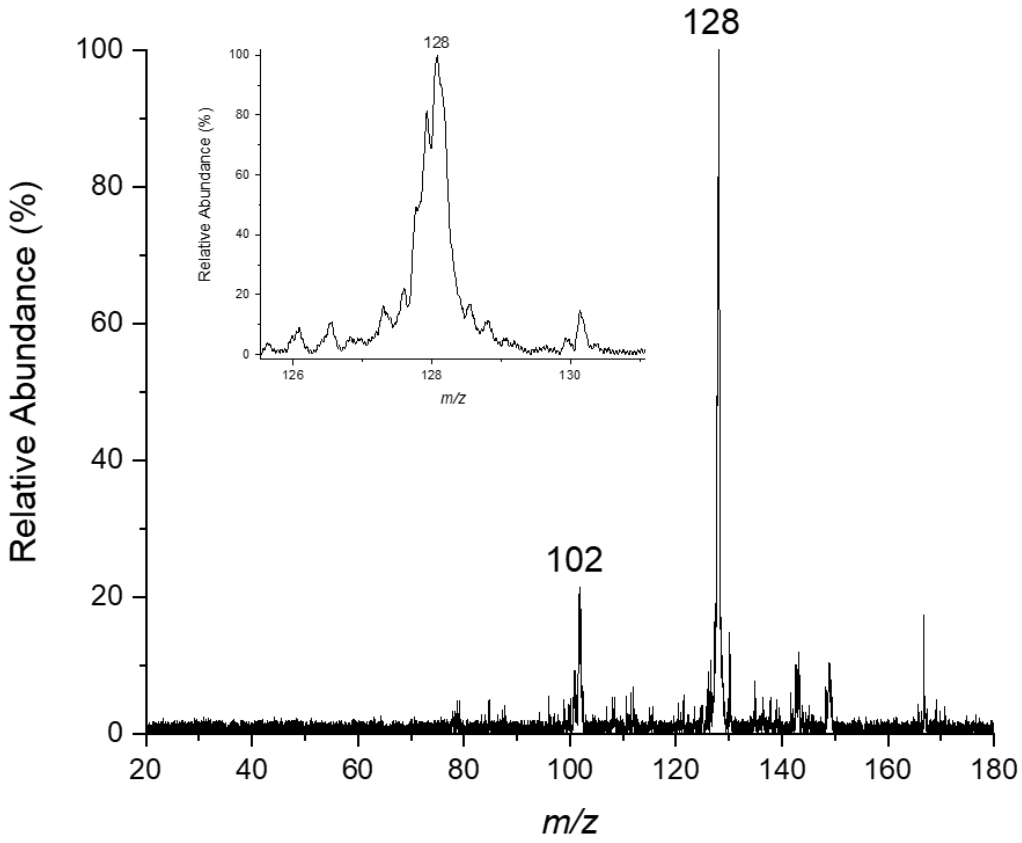


Fig. S4 MS/MS spectra, IDH wildtype, low TCP

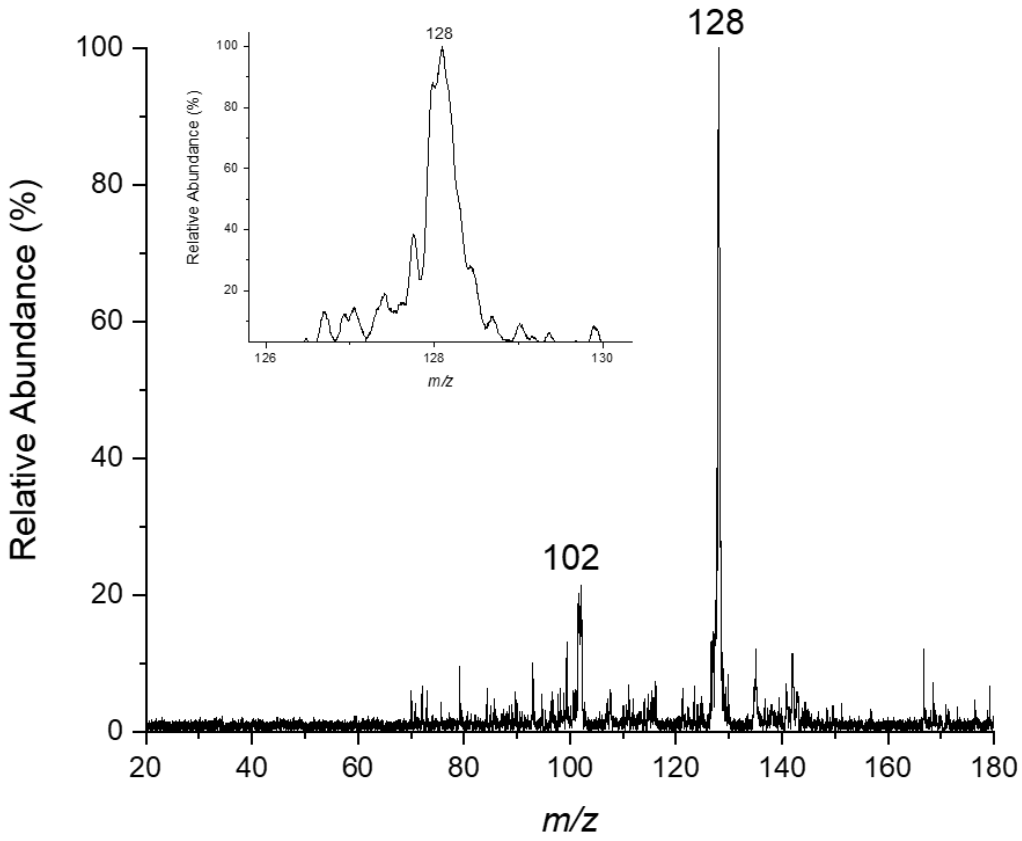


Fig. S5 MS/MS spectra, IDH wildtype, high TCP

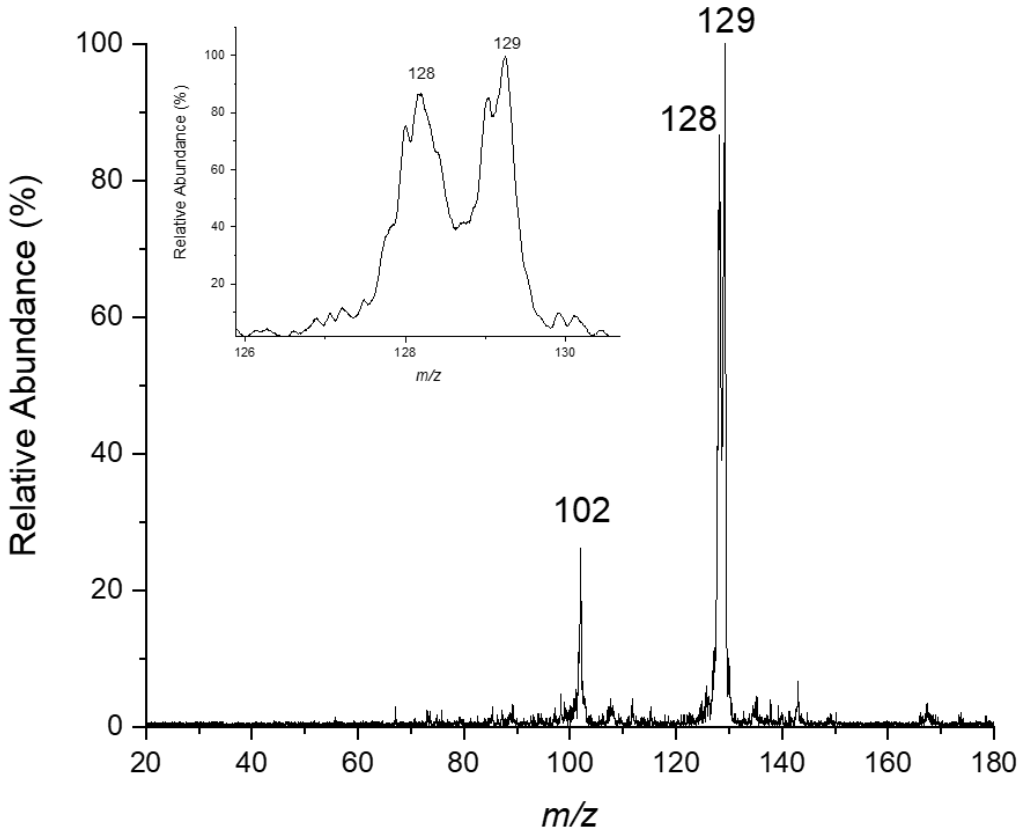


Fig. S6 MS/MS spectra, IDH mutant, low TCP

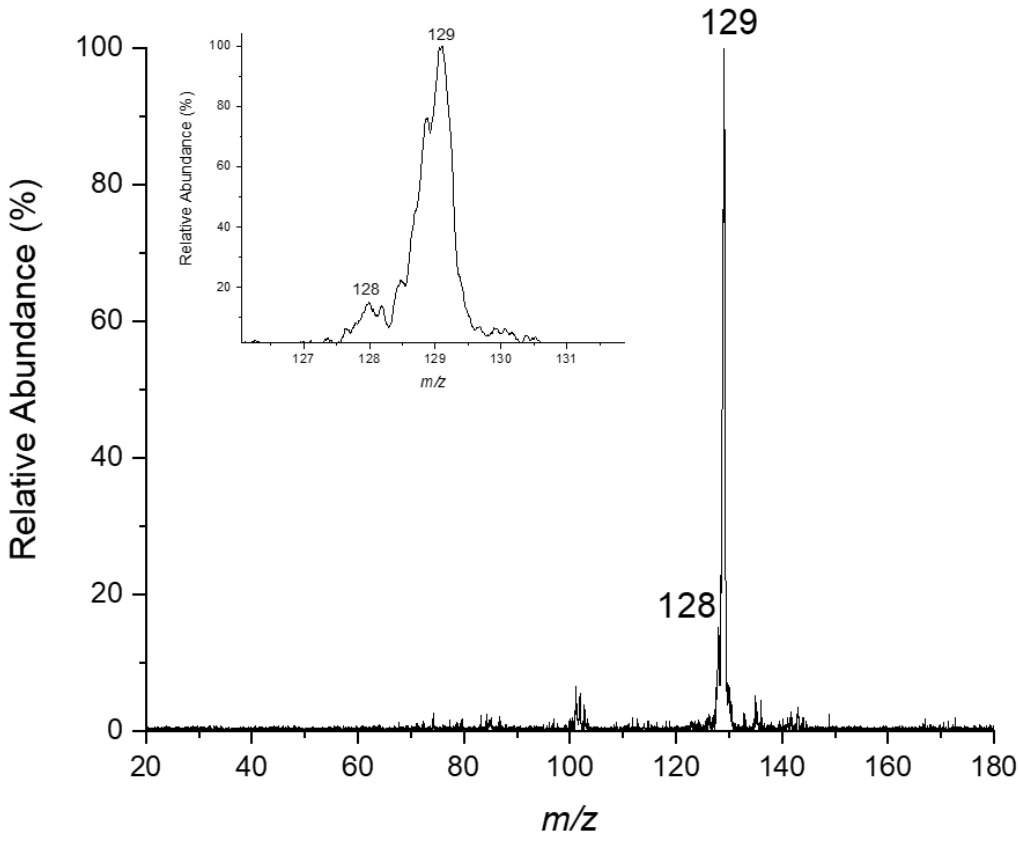


Fig. S7 MS/MS spectra, IDH mutant, high TCP