

## Supplementary info

### Ultra-High Mass Resolution MALDI Imaging Mass Spectrometry of Proteins and Metabolites in a Mouse Model of Glioblastoma

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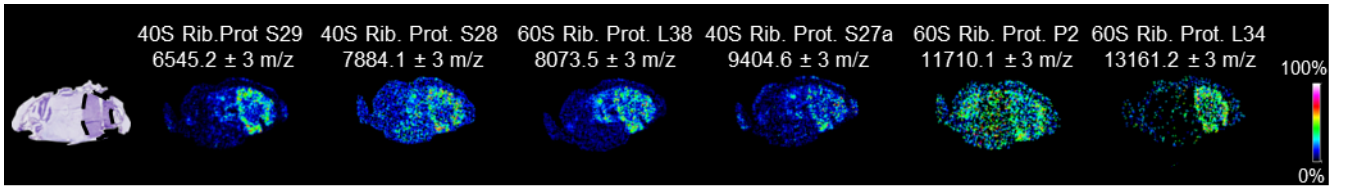
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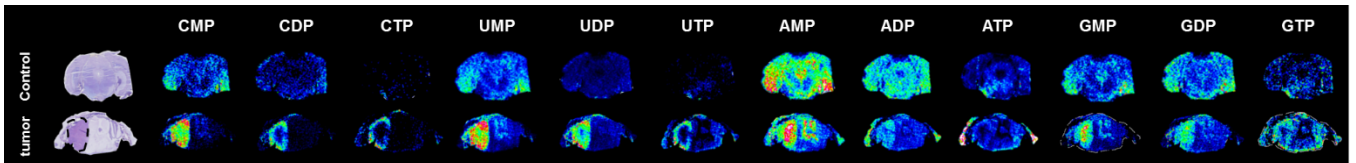
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Supplementary Figure SF1 MALDI FTICR images of ribosomal proteins highlighted in the text



Supplementary Figure SF2 MALDI FTICR images of mono-, di-, and triphosphate ribonucleotides

**Supplementary Table ST1:** Protein identified in the MALDI - FTICR average spectra with alignment with the data obtained with the LCM-LC-MS/MS results and available mass lists <sup>1,2</sup>. Me: methylation; Ac: acetylation; S-S: disulphide bond; deam: deamidation; heme: heme group; Ox: oxidation; SA: Sinapinic acid adduct;

| Prot. Entry | Protein name                           | Sequence | PTMs and Chemical mod. | Average Mass detected |
|-------------|--|----------|------------------------|-----------------------|
| F8WIX8      | Histone H2A                            | 87-121   |                        | 3805.18               |
| Q7M088      | Beta-amyloid protein                   | 1-37     |                        | 4075.30               |
| Q7M088      | Beta-amyloid protein                   | 1-37     | Me                     | 4089.24               |
| Q7M088      | Beta-amyloid protein                   | 1-38     | 2Me                    | 4160.27               |
| Q7M088      | Beta-amyloid protein                   | 1-39     |                        | 4231.21               |
| Q7M088      | Beta-amyloid protein                   | 1-39     | Ac                     | 4274.12               |
| Q6ZWY8      | Thymosin $\beta$ 10                    | 2-44     | Ac                     | 4937.30               |
| P20065      | Thymosin $\beta$ 4                     | 8-50     | Ac                     | 4964.25               |
| P20065      | Thymosin $\beta$ 4                     | 8-50     | Ac / Ox                | 4980.24               |
| P17665      | Cytochrome C oxidase polypeptide VIIC  | 17-63    |                        | 5444.60               |
| P62274      | 40s ribosomal protein 29               | 2-56     |                        | 6546.52               |
| P48771      | Cytocrome C oxidase polypeptide VIIa   | 24-83    |                        | 6573.66               |
| P48771      | Cytocrome C oxidase polypeptide VIIa   | 24-83    | 2Ox                    | 6605.62               |
| P48771      | Cytocrome C oxidase polypeptide VIIa   | 24-83    | Ac / 2Ox               | 6648.02               |
| P56394      | Cytochrome C oxidase copper chaperone  | 2-63     | Ox                     | 6669.99               |
| P48771      | Cytocrome C oxidase polypeptide VIIa   | 24-83    | Ac / 2Ox / SA          | 6872.06               |
| P62858      | 40S ribosomal protein S28              | 1-69     | 2deam                  | 7844.21               |
| P62858      | 40s ribosomal protein S28              | Full     | Ac                     | 7883.14               |
| Q9JJI8      | 60s ribosomal protein L38              | 2-70     |                        | 8073.60               |
| Q06185      | ATP synthase subunit e, mitochondrial  | 2-71     |                        | 8106.11               |
| P62983      | Ubiquitin - 40S ribosomal protein S27a | 1- 76    | Ubiquitin              | 8565.41               |
| D6RH49      | 40S ribosomal protein S27              | Full     | Ac S-S                 | 8660.17               |
| P62983      | Ubiquitin - 40S ribosomal protein S27a | 1-76     | Ubiquitin / SA         | 8790.32               |
| P97450      | ATP synthase Coupling Factor 6         | 33-108   |                        | 8946.23               |
| Q9D8X0      | Protein MANBAL                         | Full     | 2Me                    | 9369.45               |
| P62983      | Ubiquitin - 40S ribosomal protein S27a | 77-156   | 40S rib.p.S27a         | 9405.61               |
| P14069      | Calcyclin                              | 2-89     | Ac                     | 9962.35               |
| P14069      | Calcyclin                              | 2-89     | Ac / Ox                | 9978.44               |
| P56391      | Cytochrome C oxydase subunit 6B        | 2-86     | 2Me / Ac               | 10011.35              |
| P27005      | S100-A8 (Calgranulin A)                | 2-93     |                        | 10164.58              |
| P14069      | Calcyclin                              | 2-89     | Ac / Ox / SA           | 10202.55              |
| P14069      | Calcyclin                              | 2-89     | Ac / Ox / 2SA          | 10426.56              |
| Q62426      | Cystatin B                             | Full     | Ac                     | 11088.86              |
| P62806      | Histone H4                             | 2-103    | Ac / 2Me               | 11306.52              |
| P62806      | Histone H4                             | 2-103    | Ac / 3Me               | 11321.52              |
| P62806      | Histone H4                             | 2-103    | Ac / 4Me               | 11334.51              |
| P62806      | Histone H4                             | 2-103    | Ac / 5Me               | 11348.50              |
| P62806      | Histone H4                             | 2-103    | Ac / 6Me               | 11363.50              |
| P62806      | Histone H4                             | 2-103    | Ac / 7Me               | 11376.49              |

|        |  |       |                 |             |
|--------|--|-------|-----------------|-------------|
| P62806 | Histone H4   | 2-103 | 3Ac / 2Me       | 11390.98    |
| P62806 | Histone H4   | 2-103 | 2Ac / 5Me       | 11390.98    |
| P62806 | Histone H4   | 2-103 | Ac / 2Me / SA   | 11530.42    |
| P62806 | Histone H4   | 2-103 | Ac / 3Me / SA   | 11545.87    |
| P62806 | Histone H4   | 2-103 | Ac / 5Me / SA   | 11573.96    |
| P17095 | High mobility group protein HMG-I/HMG-Y                                      | 1-107 | 2Me             | 11643.32    |
| P99027 | 60S acidic ribosomal protein P2  | 1-115 | Ac / OX         | 11710.11    |
| P62806 | Histone H4   | 2-103 | Ac / 2Me / 2SA  | 11756.00    |
| D3YWS3 | Profilin   | 1-110 |                 | 11778.46    |
| P01837 | Ig kappa chain C region  | 1-106 |                 | 11778.37    |
| P62897 | Cytochrome c, somatic  | 2-104 | Heme / Ac       | 12132.63    |
| P62897 | Cytochrome c, somatic  | 2-104 | Heme / Ac / ox  | 12148.61    |
| P62897 | Cytochrome c, somatic  | 2-104 | Heme / Ac / SA  | 12357.47    |
| P19788 | Matrix Gla protein   | Full  | K+              | 12398.45    |
| P61804 | Dolichyl-diphosphooligosaccharide--protein glycosyltransferase subunit DAD-1 | Full  | Ox              | 12513.44    |
| P62897 | Cytochrome c, somatic  | 2-104 | Heme / Ac / 2SA | 12581.39    |
| Q9D1R9 | 60S ribosomal protein L34  | 2-117 |                 | 13163.13    |
| P0C0S6 | Histone Ha.z   | 2-128 |                 | 13421.33    |
| Q9CQB4 | Cytochrome b-c1 complex subunit 7  | Full  |                 | 13562.32    |
| P62852 | 40S ribosomal protein S25  | Full  | Ox              | 13758.38    |
| Q6ZWY9 | Histone H2B type 1-C/E/g   | 2-126 |                 | 13775.39    |
| Q64478 | Histone H2B type 1-H   | 2-126 |                 | 13789.65    |
| Q8CGP1 | Histone H2B type 1-K   | 2-126 |                 | 13789.65    |
| Q6ZWY9 | Histone H2B type 1-C/E/g   | 2-126 | Me              | 13789.65    |
| P10853 | Histone H2B type 1-F/J/L   | 2-126 |                 | 13805.63    |
| P10853 | Histone H2B type 1-F/J/L   | 2-126 | Me              | 13819.72    |
| Q64478 | Histone H2B type 1-H   | 2-126 | K+              | 13826.59    |
| P10853 | Histone H2B type 1-F/J/L   | 2-126 | 2Me             | 13833.59    |
| P10853 | Histone H2B type 1-F/J/L   | 2-126 | 3Me             | 13847.61    |
| Q64524 | Histone H2B type 2-E   | 2-126 |                 | 13863.59    |
| Q6GSS7 | Histone H2A type 2-A   | 2-131 | Ac              | 14007.98    |
| Q6GSS7 | Histone H2A type 2-A   | 2-131 | Me / Ac         | 14021.36    |
| A2AB79 | Histone H2A  | 2-129 | Ac              | 14033.21    |
| P22752 | Histone H2A type 1   | 2-130 | Ac              | 14048.03    |
| P22752 | Histone H2A type 1   | 2-130 | Ac / Me         | 14062.37    |
| Q6GSS7 | Histone H2A type 2-A   | 2-131 | 2Ac / 2Me       | 14078.38    |
| Q6GSS7 | Histone H2A type 2-A   | 2-131 | 2Ac / 3Me       | 14093.39    |
| A2AB79 | Histone H2A  | 2-129 | Ac / 5Me        | 14106.39    |
| Q6GSS7 | Histone H2A type 2-A   | Full  | Ac              | 14137.40455 |
| Q6GSS7 | Histone H2A type 2-A   | Full  | Ac / Me         | 14152.41    |
| Q6GSS7 | Histone H2A type 2-A   | Full  | Ac / SA         | 14362.46    |
| Q6GSS7 | Histone H2A type 2-A   | Full  | Ac / Me / SA    | 14376.43    |
| P01942 | Hemoglobin subunit beta  | 2-142 | 2Me             | 14982.66    |
| P84244 | Histone H3.3   | 2-136 | Ac              | 15240.21    |
| P84244 | Histone H3.3   | 2-136 | Ac / Me         | 15253.24    |
| P84244 | Histone H3.3   | 2-136 | Ac / 2Me        | 15267.42    |
| P84244 | Histone H3.3   | 2-136 | Ac / 3Me        | 15281.45    |

|        |              |       |          |          |
|--------|--------------|-------|----------|----------|
| P84228 | Histone H3.2 | 2-136 | Ac       | 15297.45 |
| P68433 | Histone H3.1 | 2-135 | Ac / Me  | 15327.25 |
| P84228 | Histone H3.2 | 2-136 | Ac / 3Me | 15341.45 |
| P84228 | Histone H3.2 | 2-136 | Ac / 4Me | 15355.42 |
| P84228 | Histone H3.2 | 2-136 | Ac / 5Me | 15369.40 |
| P84228 | Histone H3.2 | 2-136 | 3AC      | 15838.42 |
| P84228 | Histone H3.2 | 2-136 | Ac / 6Me | 15383.42 |
| P84228 | Histone H3.2 | 2-136 | Ac / 7Me | 15397.38 |

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1. McDonnell, L. a., Walch, A., Stoeckli, M. & Corthals, G. L. MSiMass list: A public database of identifications for protein MALDI MS imaging. *J. Proteome Res.* **13**, 1138–1142 (2014).
2. Maier, S. K. *et al.* Comprehensive identification of proteins from MALDI imaging. *Mol. Cell. Proteomics* **12**, 2901–10 (2013).

**Supplementary Table ST2:** List of metabolites detected with MALDI-FTICR-MSI with two experimental approaches. Second column: list of masses detected in negative ionization with 9-AA as matrix. Third column: list of detected m/z values identified as derivatized metabolites, derivatization performed with DPP-TFB and detection in positive mode .

| Metabolite                       | Mass detected with 9-AA | Mass detected with DPP-TFB derivatization [M+ 233.0961 ]+ |
|----------------------------------|-------------------------|---|
| Hydroxyproline                   | 130.05                  |   |
| Glutamine                        | 145.061                 |   |
| Glutamate                        | 146.045                 |   |
| Uric Acid                        | 167.02                  |   |
| Glycerol monophosphate           | 171.005                 |   |
| N - acetylaspartate              | 174.04                  |   |
| Ascorbate                        | 175.024                 |   |
| Citric Acid                      | 191.019                 |   |
| Phosphocreatinine                | 210.027                 |   |
| Ribose - phosphate               | 229.011                 |   |
| Inositol cyclic phosphate        | 241.011                 |   |
| Palmitic acid                    | 255.232                 |   |
| Glucose/Fructose - monophosphate | 259.022                 |   |
| 6 – phosphogluconic acid         | 275.017                 |   |
| Linoleic acid                    | 279.232                 |   |
| Oleic acid                       | 281.248                 |   |
| NAAG                             | 303.083                 |   |
| Arachidonic Acid                 | 303.232                 |   |
| Glutathione                      | 306.076                 |   |
| CMP                              | 322.044                 |   |
| UMP                              | 323.028                 |   |
| Docosaehaenoic Acid              | 327.232                 |   |
| AMP                              | 346.055                 |   |
| IMP                              | 347.039                 |   |
| GMP                              | 362.05                  |   |
| CDP                              | 402.01                  |   |
| UDP                              | 402.994                 |   |
| ADP                              | 426.022                 |   |
| GDP                              | 442.016                 |   |
| CTP                              | 481.977                 |   |
| UTP                              | 482.961                 |   |

|                             |         |
|-----------------------------|---------|
| ATP                         | 505.988 |
| GTP                         | 521.983 |
| Udp - glucose               | 565.047 |
| UDP-N acetylglucosamine     | 606.074 |
| Phosphadidylinositol (18:1) | 883.534 |
| Phosphadidylinositol (18:0) | 885.549 |
| Glycine                     | 290.118 |
| Alanine                     | 304.133 |
| GABA                        | 318.149 |
| Proline                     | 330.149 |
| Valine                      | 332.165 |
| Threonine                   | 334.144 |
| Taurine                     | 340.1   |
| Leucine                     | 346.18  |
| Aspartate                   | 348.123 |
| Tyramine                    | 352.169 |
| Glutamine                   | 361.155 |
| Lysine                      | 361.191 |
| Glutamate                   | 362.139 |
| Dopamine                    | 368.164 |
| Tryptamine                  | 375.186 |
| 3-MT                        | 382.18  |
| Tyrosine                    | 396.159 |
| L-DOPA                      | 412.154 |

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