Spatial imaging parallel reaction monitoring with parallel accumulation serial fragmentation (iprm-PASEF) investigating metabolically resilient CD38$^{-/-}$ mice

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Overview

Mass spectrometry imaging (MSI) is a rapidly advancing technology that provides mapping of the spatial molecular landscape of tissues for a variety of analytes. One common method employed is matrix-assisted laser desorption/ionization (MALDI)-MSI, however, confident *in situ* identification and accurate quantification of analytes with MALDI-MSI remains challenging without extensive workflows. Here, we present the first study combining trapped ion mobility spectrometry (TIMS)-based parallel accumulation-serial fragmentation (PASEF) with MALDI ionization for targeted **imaging parallel reaction monitoring (iprm-PASEF)**[1,2] to investigate lipids and metabolites in liver tissues from wild-type and CD38 knockout mice (CD38$^{-/-}$). The novel iprm-PASEF workflow enabled confident identification and differentiation of lipid isomers at the MS2 level and revealed greatly increased NAD$^+$ levels and decreased *adenosine diphosphate ribose (ADPR)*, a by-product of NAD$^+$ hydrolysis, in CD38$^{-/-}$ livers. This approach provides highly confident, specific, and robust MS2-based identification and quantification of fragment ions in spatial MSI experiments. Additionally, the innovative iprm-PASEF opens unprecedented opportunities for spatial metabolomics and lipidomics, offering spatially resolved insights into molecular mechanisms.

NAD$^+$, CD38, and Aging

NAD$^+$ (nicotinamide adenine dinucleotide) metabolism plays a critical role in maintaining cellular health and is a central metabolite for physiological and cellular processes. Aging and age-related diseases and cellular conditions, such as cellular senescence, are associated with declines in NAD$^+$. CD38, is a potent enzymatic consumer of NAD$^+$ and is highly expressed in Kupffer cells, the resident macrophages of the liver sinusoids, where its drives cellular senescence and inflammation with aging. Notably, mice lacking CD38 (CD38$^{-/-}$) display higher mitochondrial NAD$^+$ levels in the liver exhibiting a resistance to age-related declines in NAD$^+$, suggesting improved metabolic regulation.[3] Here, we utilized iprm-PASEF to investigate the spatial distribution of metabolites and lipids in the liver of CD38$^{-/-}$ mice and wild type (WT) controls.

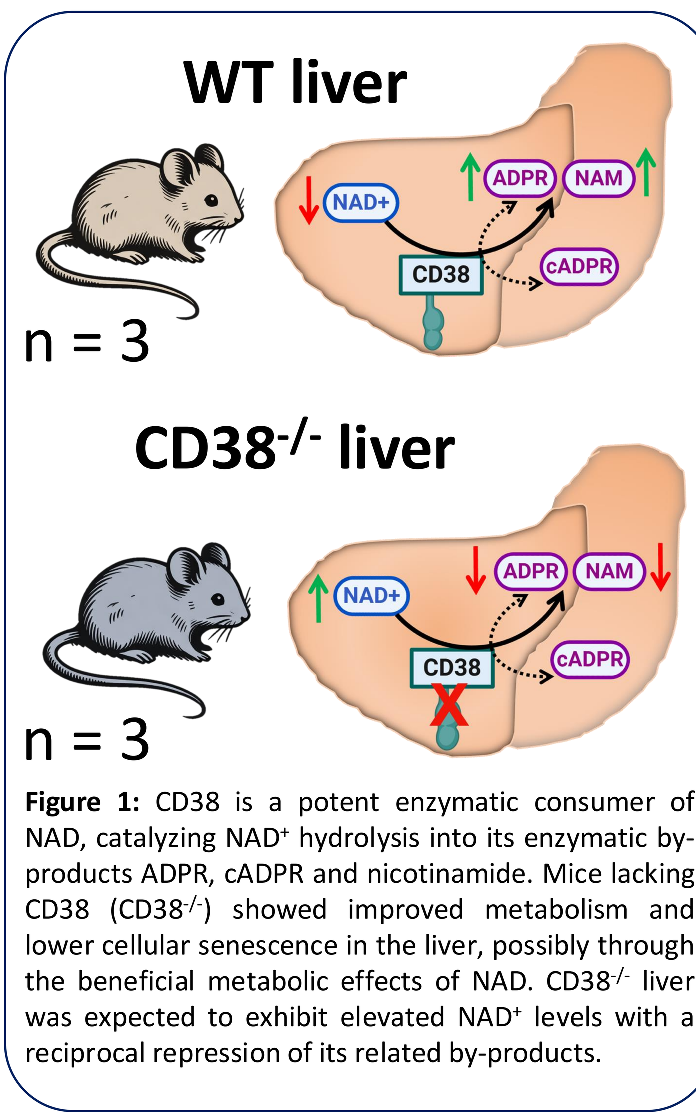

Figure 1: CD38 is a potent enzymatic consumer of NAD, catalyzing NAD$^+$ hydrolysis into its enzymatic by-products ADPR, cADPR and nicotinamide. Mice lacking CD38 (CD38$^{-/-}$) showed improved metabolism and lower cellular senescence in the liver, possibly through the beneficial metabolic effects of NAD. CD38$^{-/-}$ liver was expected to exhibit elevated NAD$^+$ levels with a reciprocal repression of its related by-products.

Workflow

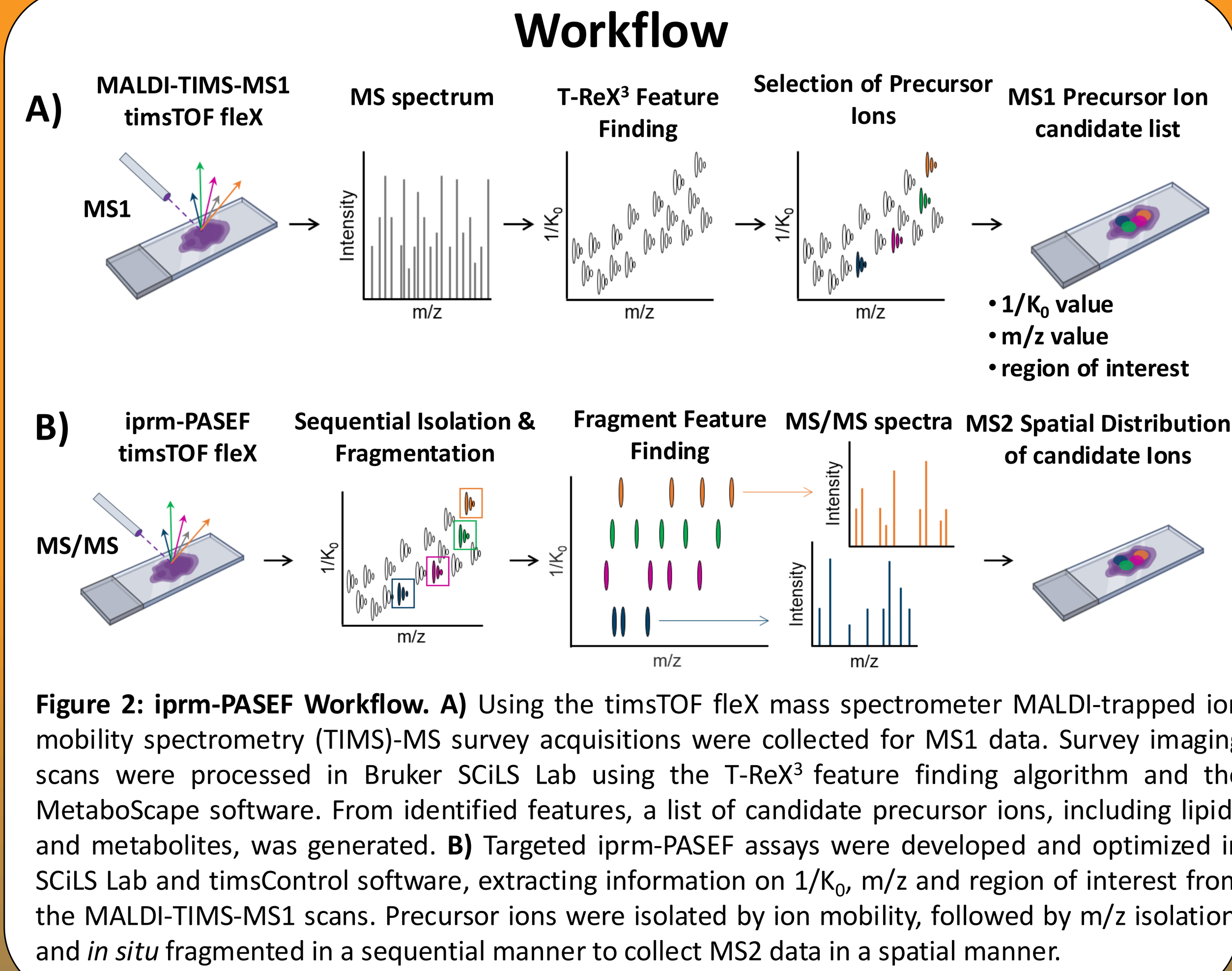

Figure 2: iprm-PASEF Workflow. A) Using the timsTOF fleX mass spectrometer MALDI-trapped ion mobility spectrometry (TIMS)-MS survey acquisitions were collected for MS1 data. Survey imaging scans were processed in Bruker SCiLS Lab using the T-ReX³ feature finding algorithm and the MetaboScape software. From identified features, a list of candidate precursor ions, including lipids and metabolites, was generated. **B)** Targeted iprm-PASEF assays were developed and optimized in SCiLS Lab and timsControl software, extracting information on $1/K_0$, m/z and region of interest from the MALDI-TIMS-MS1 scans. Precursor ions were isolated by ion mobility, followed by m/z isolation, and *in situ* fragmented in a sequential manner to collect MS2 data in a spatial manner.

iprm-PASEF enables spatially resolved fragment ion distribution

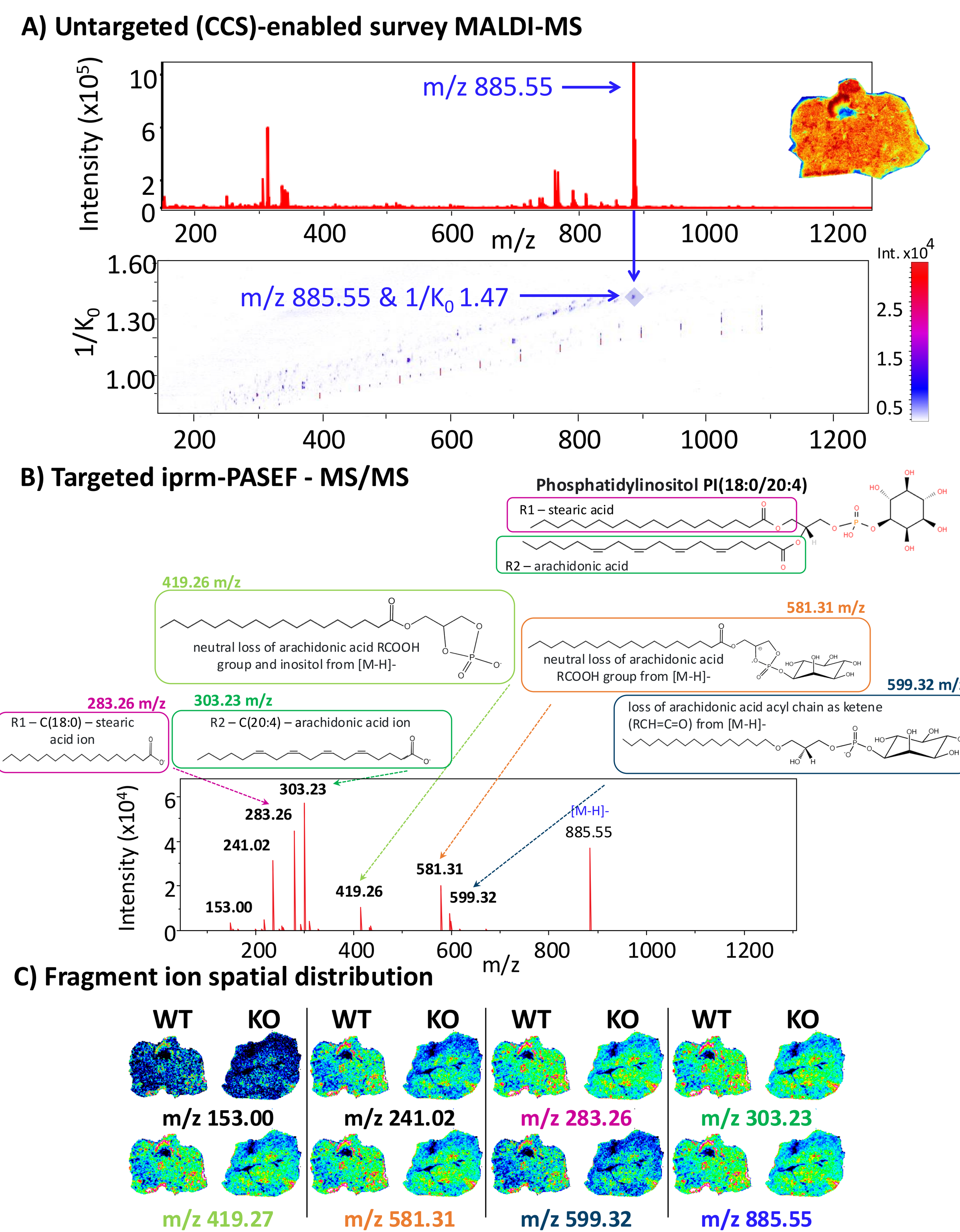

Figure 3: iprm-PASEF enables spatial fragment mapping A) In CD38$^{-/-}$ mouse liver, the composite CCS-enabled survey MALDI-MS scan was acquired in negative ionization mode and detected a feature at at m/z 885.55 and $1/K_0$ 1.47 VS/cm² that was highly abundant across the entire tissue section. **B)** This precursor ion candidate was selected for targeted iprm-PASEF isolation and fragmentation. The generated MS/MS spectrum showed species-specific fragment ions used for both accurate molecular identification and quantification of Phosphatidylinositol PI(18:0/20:4). **C)** The identified fragment ions were spatially mapped for representative images of wild-type (WT) and CD38$^{-/-}$ mouse liver tissue sections based on their m/z. The spatial distribution of the PI(18:0/20:4) fragment ions matched the distribution of the corresponding precursor ion.

irpm-PASEF resolves lipid isomers

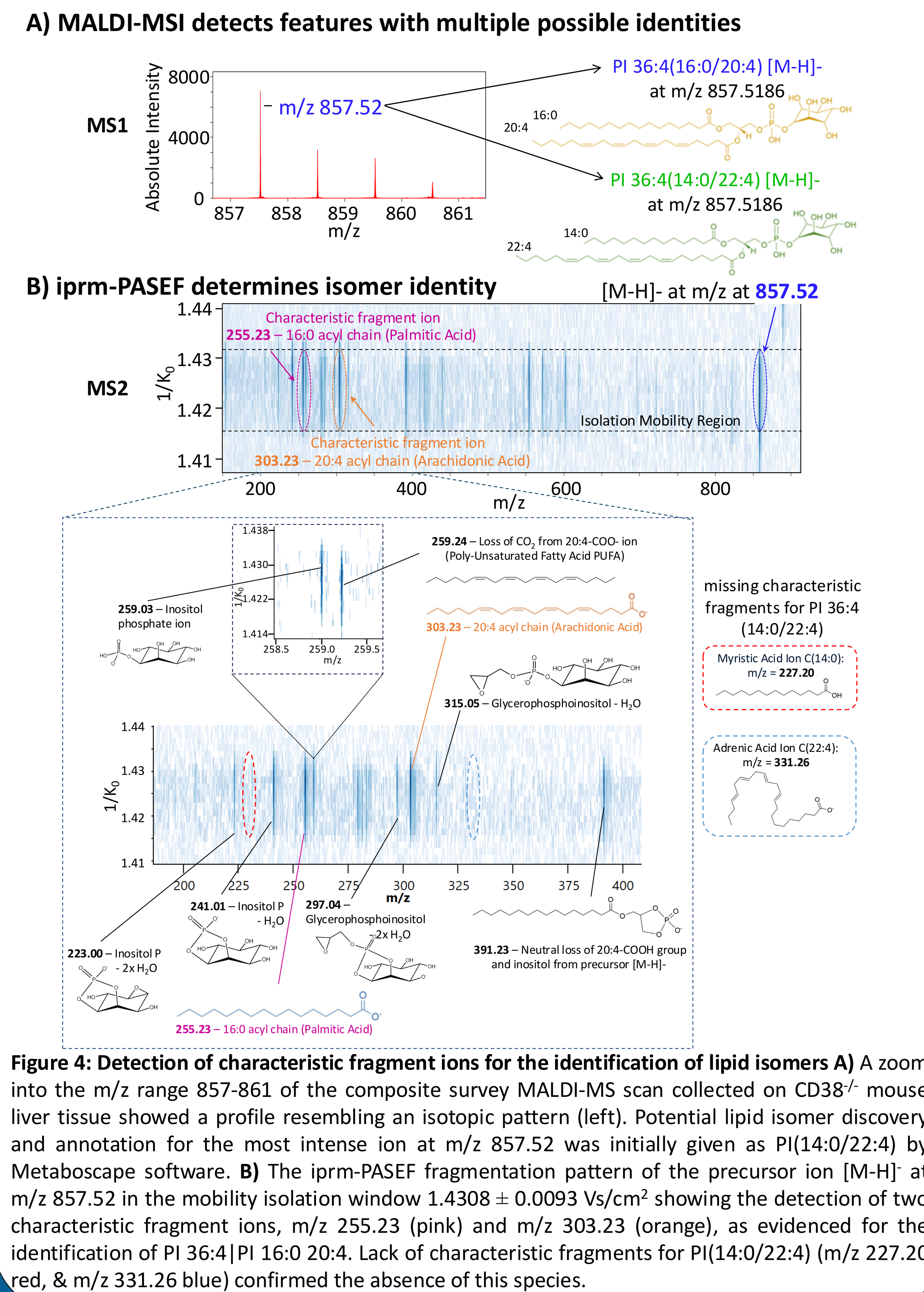

Figure 4: Detection of characteristic fragment ions for the identification of lipid isomers A) A zoom into the m/z range 857-861 of the composite survey MALDI-MS scan collected on CD38$^{-/-}$ mouse liver tissue showed a profile resembling an isotopic pattern (left). Potential lipid isomer discovery and annotation for the most intense ion at m/z 857.52 was initially given as PI(14:0/22:4) by Metaboscape software. **B)** The iprm-PASEF fragmentation pattern of the precursor ion [M-H]$^-$ at m/z 857.52 in the mobility isolation window 1.4308 ± 0.0093 Vs/cm² showing the detection of two characteristic fragment ions, m/z 255.23 (pink) and m/z 303.23 (orange), as evidenced for the identification of PI 36:4|PI 16:0 20:4. Lack of characteristic fragments for PI(14:0/22:4) (m/z 227.20 red, & m/z 331.26 blue) confirmed the absence of this species.

irpm-PASEF identifies NAD and related metabolites

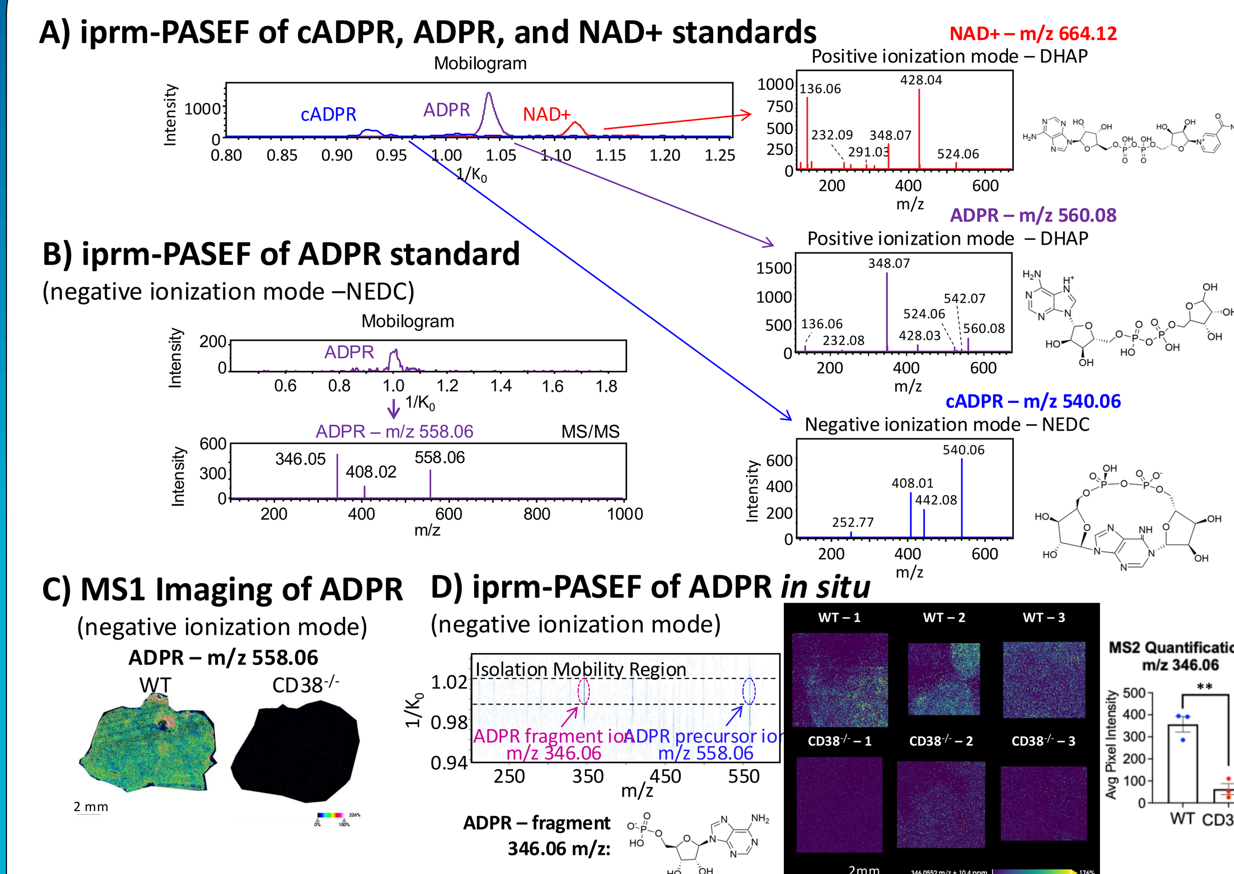

Figure 5: Identification of NAD+ and related metabolites in WT and CD38$^{-/-}$ mouse livers by iprm-PASEF. A) The iprm-PASEF mobilogram and MS/MS spectra of synthetic standards: negative ionization mode with N-(1-naphthyl) ethylenediamine dihydrochloride (NEDC) matrix for cADPR, positive ionization mode with 2,5-dihydroxyacetophenone (DHAP) matrix for ADPR and NAD$^+$. **B)** The iprm-PASEF mobilogram and MS/MS spectrum of the synthetic ADPR standard analyzed in negative ionization mode with NEDC matrix. C) Spatial distribution of ADPR precursor ion [M-H]$^-$ at m/z 558.06 in MS1 survey scans. **D)** iprm-PASEF analysis in WT and CD38$^{-/-}$ mouse liver tissue found a significant decrease of ADPR in CD38$^{-/-}$ mouse liver with MS2-based quantification on the fragment at m/z 348.06. ** Student's t-test p-value < 0.01

irpm-PASEF facilitates *in situ* visualization and quantification of endogenous NAD+

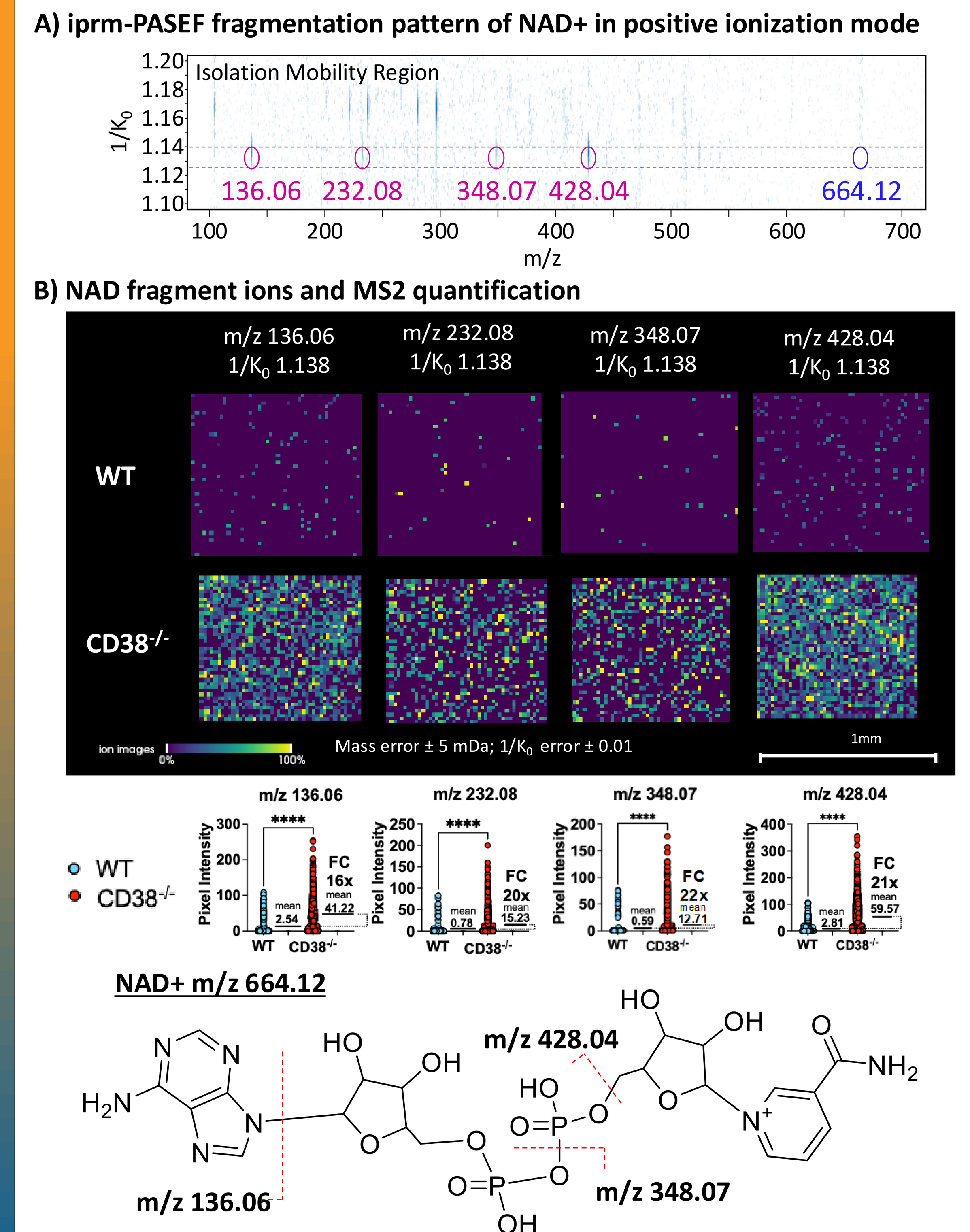

Figure 6. Quantification and *in situ* visualization of endogenous NAD+ fragment ions in WT and CD38$^{-/-}$ mouse livers by iprm-PASEF. A. The iprm-PASEF fragmentation pattern of the endogenous NAD+ precursor ion [M+H]+ at m/z 664.12 in the mobility isolation window 1.1224-1.1400 Vs/cm² showed the detection of fragment ions at m/z 136.06, m/z 232.08, m/z 348.07, and m/z 428.04. B. Spatial distribution of the four NAD$^+$ fragment ions detected by iprm-PASEF in one WT and one CD38$^{-/-}$ mouse liver replicate. Each fragment ion was quantified *in situ* using the pixel intensity within each tissue section, which all demonstrated up-regulated level in CD38$^{-/-}$ vs WT mouse livers. The statistical analysis was performed by comparing the MS2 intensity of each fragment ion in each individual pixel that compose the CD38$^{-/-}$ vs WT image. **** Student's t-test p-value < 0.0001 (n = 1885 pixels for the WT image and n = 2300 pixels for the WT image; only one mouse and one image replicate per condition). Cleavage sites of identified NAD$^+$ fragments are mapped in the NAD+ structure.

Conclusions

- **iprm-PASEF is a powerful application of MALDI-MSI that allows for MS2 level identification and quantification of metabolites and lipids in place during imaging experiments.**
- **Iprm-PASEF can differentiate between multiple related lipid species.**
- **Iprm-PASEF allows for further confidence identification of preliminarily identified analytes through fragment level analysis.**
- **CD38$^{-/-}$ mice were confirmed to have both elevated NAD+ levels and consequently repressed ADPR levels in liver tissue, demonstrating their metabolic resilience.**

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References

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