Cell Reports, Volume *23*

Supplemental Information

Comprehensive Quantification of the Modified Proteome Reveals Oxidative Heart Damage in Mitochondrial Heteroplasmy

Navratan Bagwan, Elena Bonzon-Kulichenko, Enrique Calvo, Ana Victoria Lechuga-Vieco, Spiros Michalakopoulos, Marco Trevisan-Herraz, Iakes Ezkurdia, José Manuel Rodríguez, Ricardo Magni, Ana Latorre-Pellicer, José Antonio Enríquez, and Jesús Vázquez

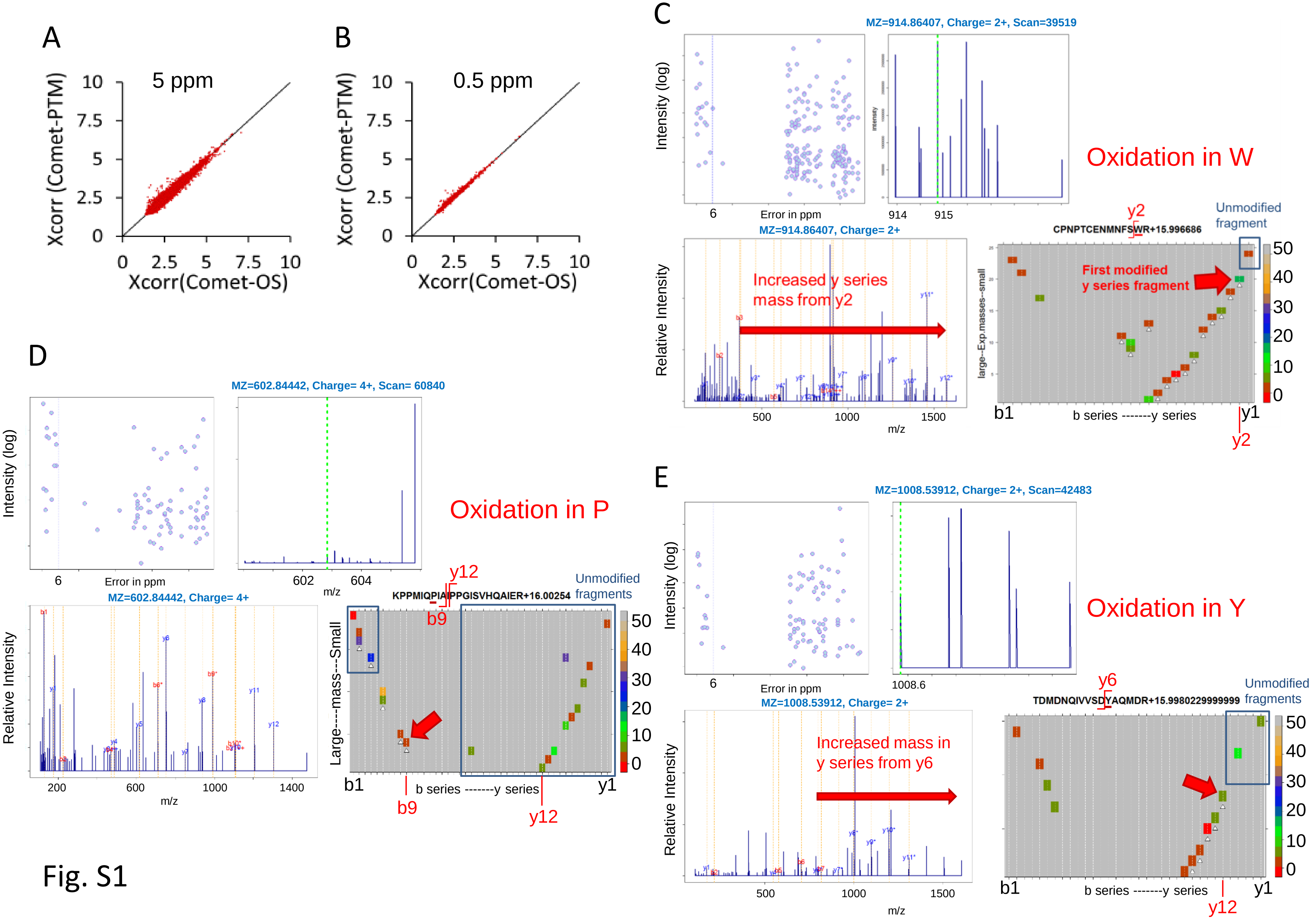

A
5 ppm
Xcorr (Comet-PTM)
Xcorr(Comet-OS)
0
2.5
5
7.5
10
B
0.5 ppm
C
MZ=914.86407, Charge= 2+, Scan=39519
Intensity (log)
Error in ppm
6
914
915
Oxidation in W
MZ=914.86407, Charge= 2+
Relative Intensity
Increased y series mass from y2
m/z
500
1000
1500
y2
CPNPTCENMNFSWR+15.996686
Unmodified fragment
First modified y series fragment
large--Exp.masses--small
b1
b series -------y series
y1
y2
50
40
30
20
10
0
D
MZ=602.84442, Charge= 4+, Scan= 60840
Intensity (log)
Error in ppm
6
602
604
m/z
Oxidation in P
MZ=602.84442, Charge= 4+
Relative Intensity
200
600
1000
1400
m/z
y12
b9
KPPMIQPIAIPPGISVHQAIER+16.00254
Unmodified fragments
Large---mass---Small
b1
b series -------y series
y1
b9
y12
50
40
30
20
10
0
E
MZ=1008.53912, Charge= 2+, Scan=42483
Intensity (log)
Error in ppm
6
1008.6
Oxidation in Y
MZ=1008.53912, Charge= 2+
Relative Intensity
Increased mass in y series from y6
500
1000
1500
m/z
y6
TDMDNQIVVSDYAQMDR+15.9980229999999
Unmodified fragments
b1
b series -------y series
y1
y12
50
40
30
20
10
0

Fig. S1

Figure S1. Related to Figure 1. Peptide identification performance of Comet-PTM. (A-B) Comparison of scores obtained from Comet-PTM and CS in the population of PSM that produced the same match with the two engines. A match was considered identical when the peptide sequence was the same and the difference between ΔMass obtained by Comet-PTM and the theoretical mass of the modification selected in CS was within 5 ppm **(A)** or 0.5 ppm **(B).** Note that the scores were practically identical, and the dispersion around the identity line was diminished when the tolerance decreased; this demonstrates that the small differences in the score are a consequence of the error in the estimation of ΔMass and not in the design of the score in Comet-PTM. **(C-E)** Vseq analyses of 3 peptides containing oxidations incorrectly assigned to Met by CS. Comet-PTM correctly assigns the oxidation to Trp **(C)** Pro **(D)** or Tyr **(E).**

ΔMass(Kynurenin): 3.994915 Da
ΔMass(Pro > Thr): 3.994915 Da

D**P**CNSSIASIR	----------------------	D**T**CNSSIASIR
Pituitary homeobox 3 isoform X1		Pituitary homeobox 2 isoform c
EAMEH**P**YFYPVVK	--------------------	EAMEH**T**YFYPVVK
Casein kinase II subunit alpha		Casein kinase II subunit alpha isoform X1
EGFHFEETI**P**GFK	--------------------	EGFHFEETI**T**GFK
Glucose 1,6-bisphosphate synthase		Phosphoglucomutase-1 isoform X1
II**P**GGAAAEDGR	---------------------	II**T**GGAAAEDGR
Disks large homolog 2 isoform X1		Disks large homolog 2 isoform X1
VDNSSITGESEPQ**P**R	------------------	VDNSSITGESEPQ**T**R
ATPase, H+/K+ transporting alpha polypeptide		Na+/K+ transporting ATPase subunit alpha 3
YAAEIHIVHWN**P**K	--------------------	YAAEIHIVHWN**T**K
Carbonic anhydrase 3 isoform CRAb		Carbonic anhydrase 2 isoform X1

Figure S2. Related to Figure 2. List of peptides containing a Pro > Thr substitution assigned as a kynurenin modification in Pro. Peptide sequences together with their corresponding proteins are listed, highlighting (in red) the position of Pro and Thr residues.

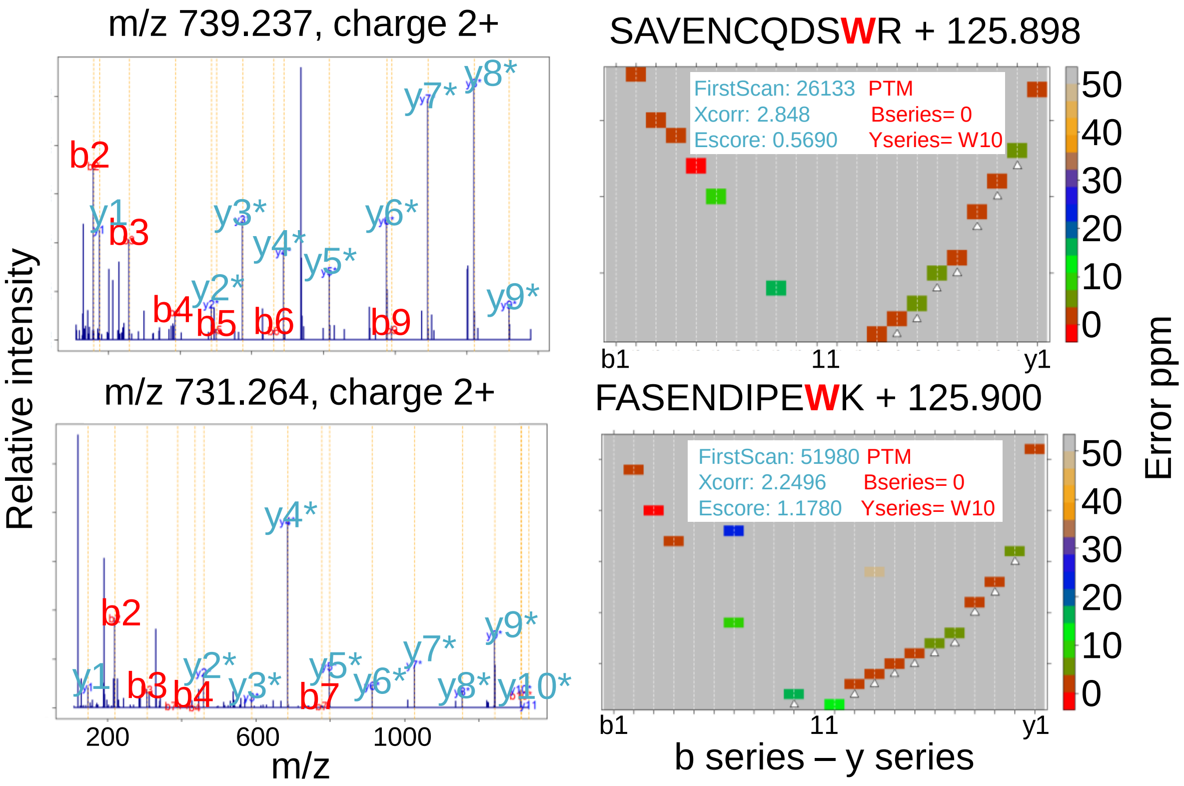
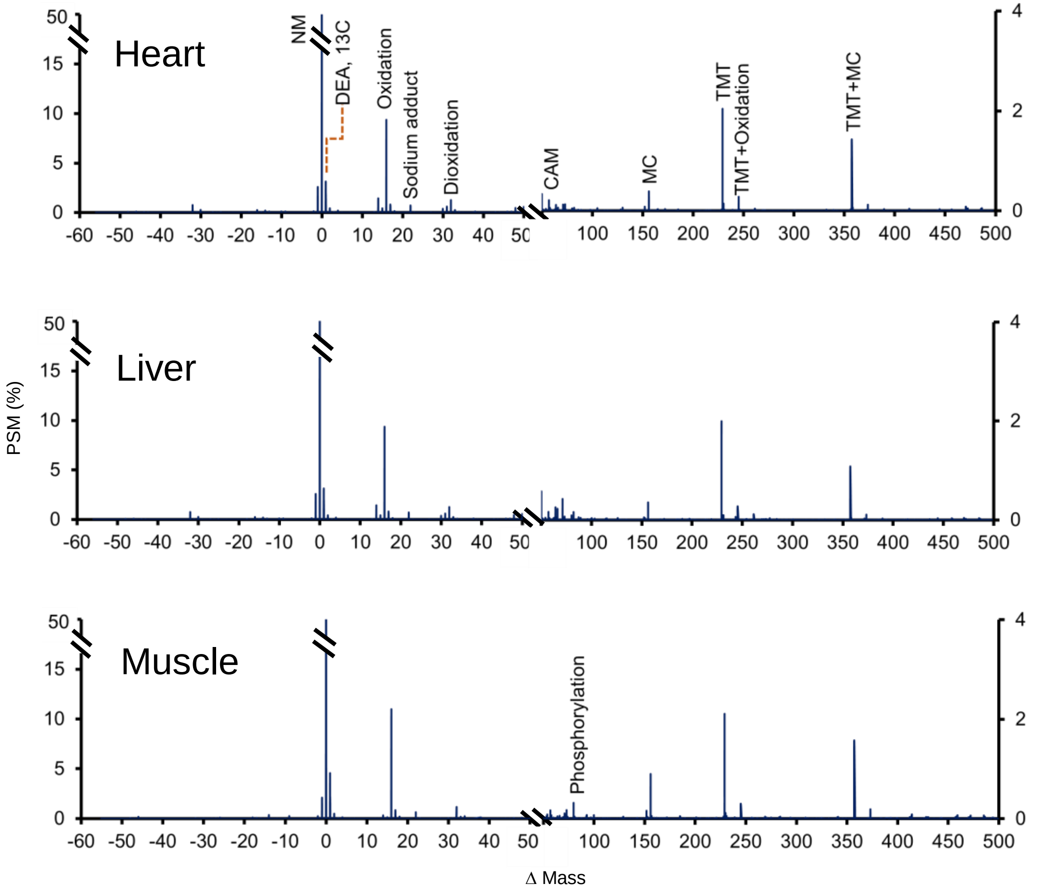


Figure S3. Related to Figure 2. Detection of a Trp iodination. Vseq results for 2 representative peptides containing iodinated Trp. Both the MS2 spectra (left panels) and the V-shaped heatmap distributions for the main fragmentation series (right panels), demonstrate the location of the modification in Trp.



NM: non-modified
CAM: carbamidomethyl

DEA: deamidation
MC: missed cleavage

Figure S4. Related to Figure 4. ΔMass distribution of modified peptides identified in the 3 mouse tissues. Assignations of the most frequent ΔMass peaks are indicated.

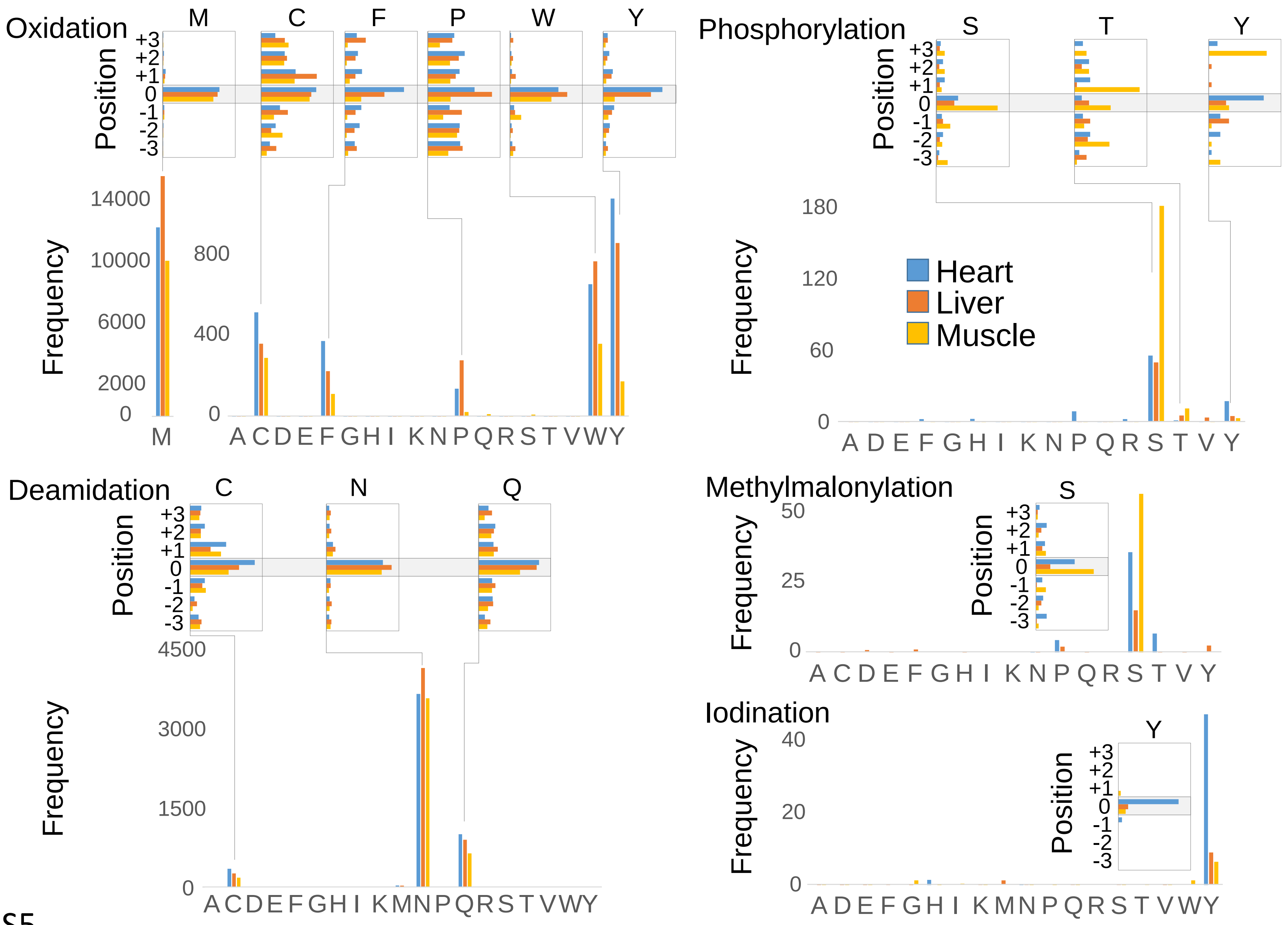

Oxidation
M C F P W Y
Position
+3 +2 +1 0 -1 -2 -3
Frequency
14000 10000 6000 2000 0
800 400 0
M
A C D E F G H I K N P Q R S T V W Y
Phosphorylation
S T Y
Position
+3 +2 +1 0 -1 -2 -3
Frequency
180 120 60 0
A D E F G H I K N P Q R S T V Y
Heart
Liver
Muscle
Deamidation
C N Q
Position
+3 +2 +1 0 -1 -2 -3
Frequency
4500 3000 1500 0
A C D E F G H I K M N P Q R S T V W Y
Methylmalonylation
S
Frequency
50 25 0
Position
+3 +2 +1 0 -1 -2 -3
A C D E F G H I K N P Q R S T V Y
Iodination
Y
Frequency
40 20 0
Position
+3 +2 +1 0 -1 -2 -3
A D E F G H I K M N P Q R S T V W Y

Fig. S5

Figure S5. Related to Figure 4. Distribution of amino acids containing the most frequent modifications. The horizontal and vertical bar graphs have the same meaning as in Figure 2E.

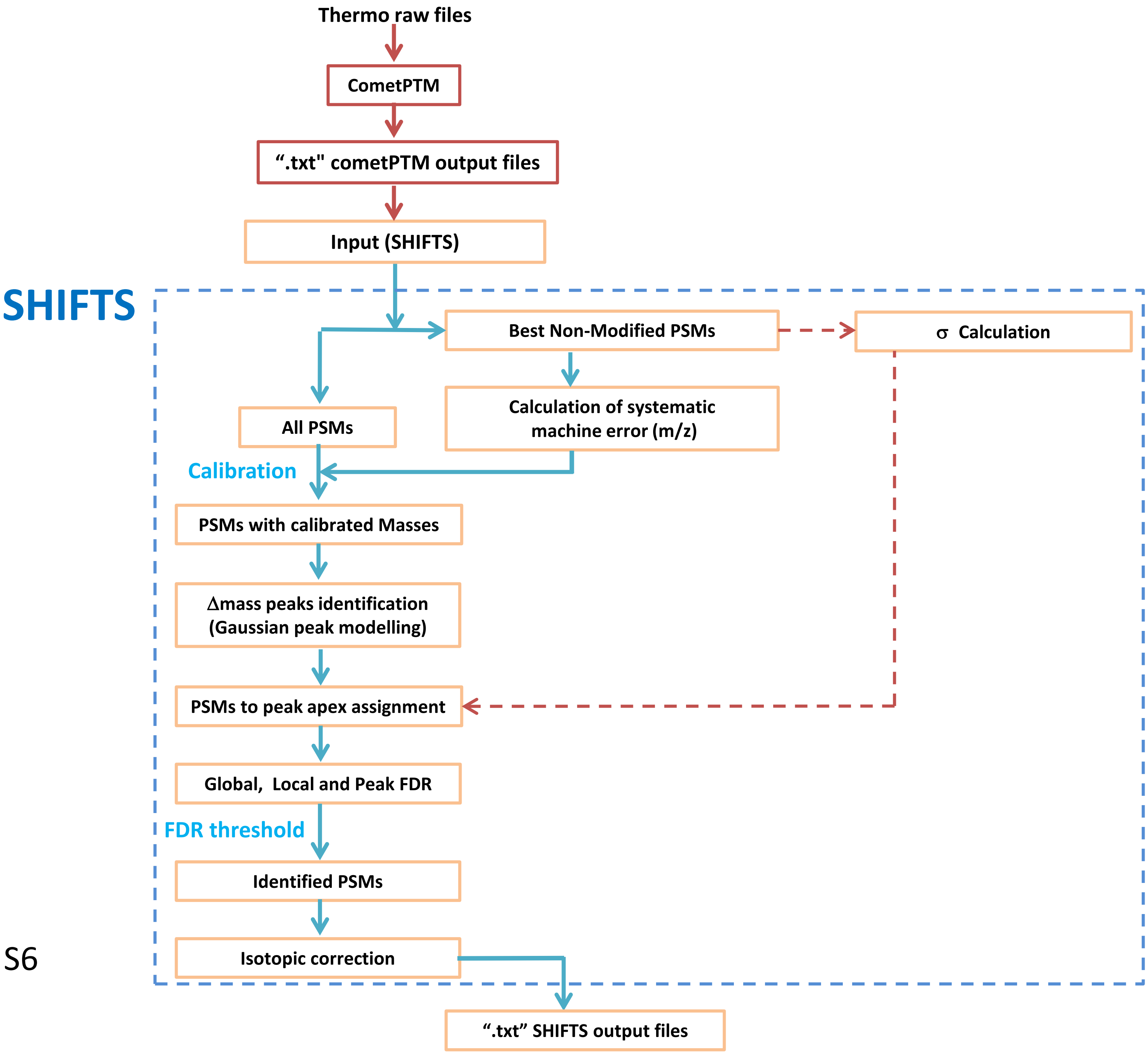

Thermo raw files
CometPTM
".txt" cometPTM output files
Input (SHIFTS)
SHIFTS
Best Non-Modified PSMs
σ Calculation
All PSMs
Calculation of systematic machine error (m/z)
Calibration
PSMs with calibrated Masses
Δmass peaks identification (Gaussian peak modelling)
PSMs to peak apex assignment
Global, Local and Peak FDR
FDR threshold
Identified PSMs
Isotopic correction
".txt" SHIFTS output files

Fig. S6

Figure S6. Related to Experimental Procedures. Scheme of SHIFTS algorithm. Schematic representation of SHIFTS (Systematic, Hypothesis-free Identification of modifications with controlled FDR based on ultra-Tolerant database Search), depicting how the output from Comet-PTM is processed, including mass recalibration, peak detection and FDR calculation.