Supplementary Material

Proteomic Profiling of S-acylated Macrophage Proteins Identifies a Role for Palmitoylation in Mitochondrial Targeting of Phospholipid Scramblase 3

B. Alex Merrick,[1*] Suraj Dhungana,[1*] Jason G. Williams,[2] Jim J. Aloor,[1] Shyamal Peddada,[3] Kenneth B. Tomer,[2] Michael B. Fessler[1]

[1]Laboratory of Respiratory Biology, [2]Laboratory of Structural Biology, and [3]Biostatistics Branch; National Institute of Environmental Health Sciences, NIH, Research Triangle Park, NC 27709

* Equal contribution

Supplemental Figure Legends

Figure S1. MS/MS of ion m/z 885.5 from stomatin. The cysteic acid form of tryptic peptide 12 from stomatin is identified by precursor mass and extensive b- and y-series ions.

Figure S2. MS/MS of ion m/z 899.7 from stomatin. A BME-modified version of tryptic peptide 12 from stomatin is identified by precursor mass and extensive b- and y-series ions.

Figure S3. Extracted ion chromatograms of ion m/z 885.5 from stomatin. Extracted ion chromatograms were drawn for ion m/z 885.5 (corresponding to the doubly charged T12 peptide from stomatin with the cysteine residue oxidized to cysteic acid), for HA^+ -treated (dotted lines) and HA^- -treated (solid lines) conditions from all 4 experiments. For clarity, only one EIC (the most abundant) of the 24 gel lane slices is shown for each HA^+ sample, for a total of 4 EICs. The EICs for the corresponding HA^- gel slice for each experiment, as well as the 6 slices both above and below the corresponding slice (13 total) are shown, for a total of 52 EICs.

Figure S4. Subcellular fraction markers. Mitochondrial and nuclear fractions of RAW 264.7 macrophages that had been transfected with empty vector (EV), wild type (WT) Plscr3, or mutant Plscr3 were confirmed by immunoblotting for mitochondrial (Grp75) and nuclear (lamin A/C) markers.

Supplemental Tables

Tables S1-S8. Protein identification data for acyl biotinyl exchange experiments. Spectral count and associated data are shown for proteins identified under hydroxylamine cleavage (HA^+) and mock cleavage (HA^-) conditions in four independent experiments.

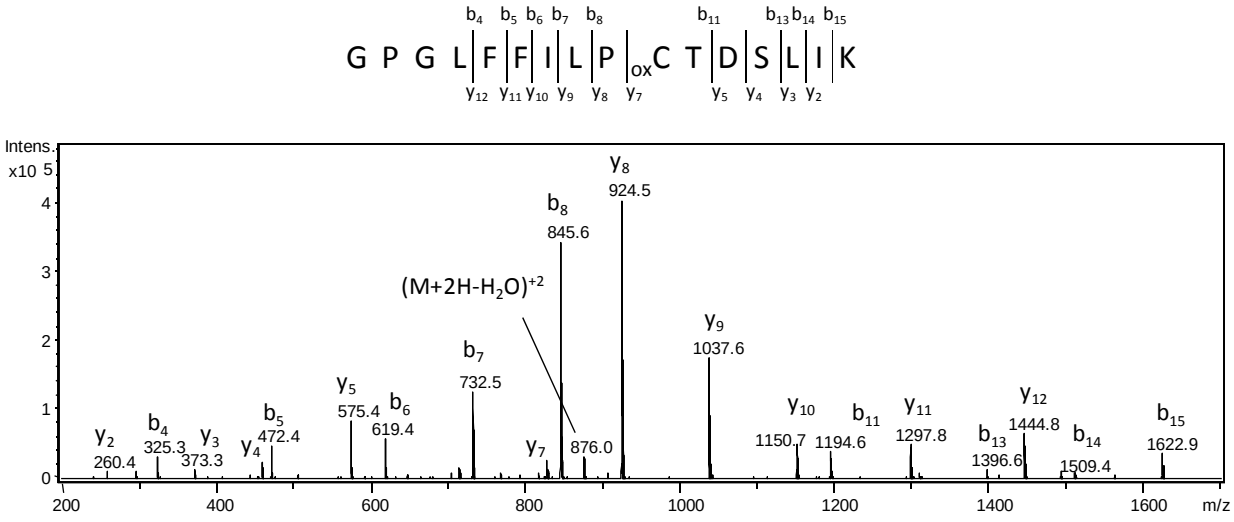
Table S9. Cross-experiment spectral count data. Spectral counts and mean HA^+/HA^- spectral count ratio are shown across 4 independent experiments for 1,183 proteins identified. Proteins in yellow were omitted from the statistical analysis of S-acylation due to their detection in <2 HA^+ experiments. Proteins in green were additionally omitted due to mean abundance under HA^- conditions that was equal to or higher than that under HA^+ conditions (mean HA^+/HA^- ratio ≤1). The remaining proteins in white were entered into the statistical analysis that yielded the S-acylprotein candidates identified in Tables I and S10. In calculating HA^+/HA^- ratio, a value of 0.2 was arbitrarily assigned to spectral count values of zero in order to avoid division by zero, as previously reported (6).

Table S10. Data for candidate S-acylproteins significant at FDR=0.10. *P*-values for S-acylation and mean HA^+/HA^- ratios are shown. Also shown are Pubmed identification numbers (PMID) for prior reports confirming biochemical palmitoylation.

Table S11. S-acylprotein candidates detected in macrophage detergent-resistant membranes. Listed are S-acylprotein candidates that are putative raft-localized proteins as they were identified by the authors in macrophage detergent-resistant membranes (Dhungana S et al., *Mol Cell Proteomics* 2009).

Table S12. S-acylprotein candidates not detected in macrophage detergent-resistant membranes. Listed are the S-acylprotein candidates identified in the present report that were not detected by the authors in macrophage detergent-resistant membranes (Dhungana S et al., *Mol Cell Proteomics* 2009).

Figure S1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G P G L F F I L P oxC T D S L I K
y12 y11 y10 y9 y8 y7 y5 y4 y3 y2
Intens.
x10 5
4
3
2
1
0
y8
924.5
b8
845.6
(M+2H-H2O)+2
y9
1037.6
b7
732.5
y5
575.4
b6
619.4
b5
472.4
y2
260.4
b4
325.3
y3
373.3
y4
y7
876.0
y10
1150.7
1194.6
b11
y11
1297.8
y12
1444.8
b13
1396.6
b14
1509.4
b15
1622.9
200
400
600
800
1000
1200
1400
1600
m/z

Figure S2

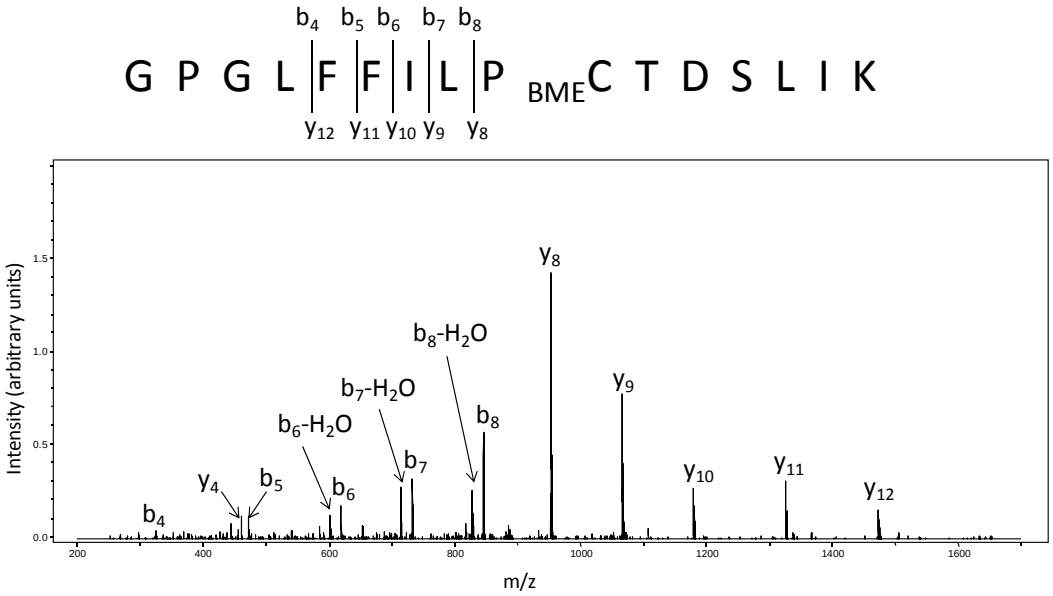

b4 b5 b6 b7 b8
G P G L F F I L P BME C T D S L I K
y12 y11 y10 y9 y8
Intensity (arbitrary units)
m/z
y8
b8-H2O
y9
b7-H2O
b8
b6-H2O
b7
y4
b5
b6
y10
y11
b4
y12

Figure S3

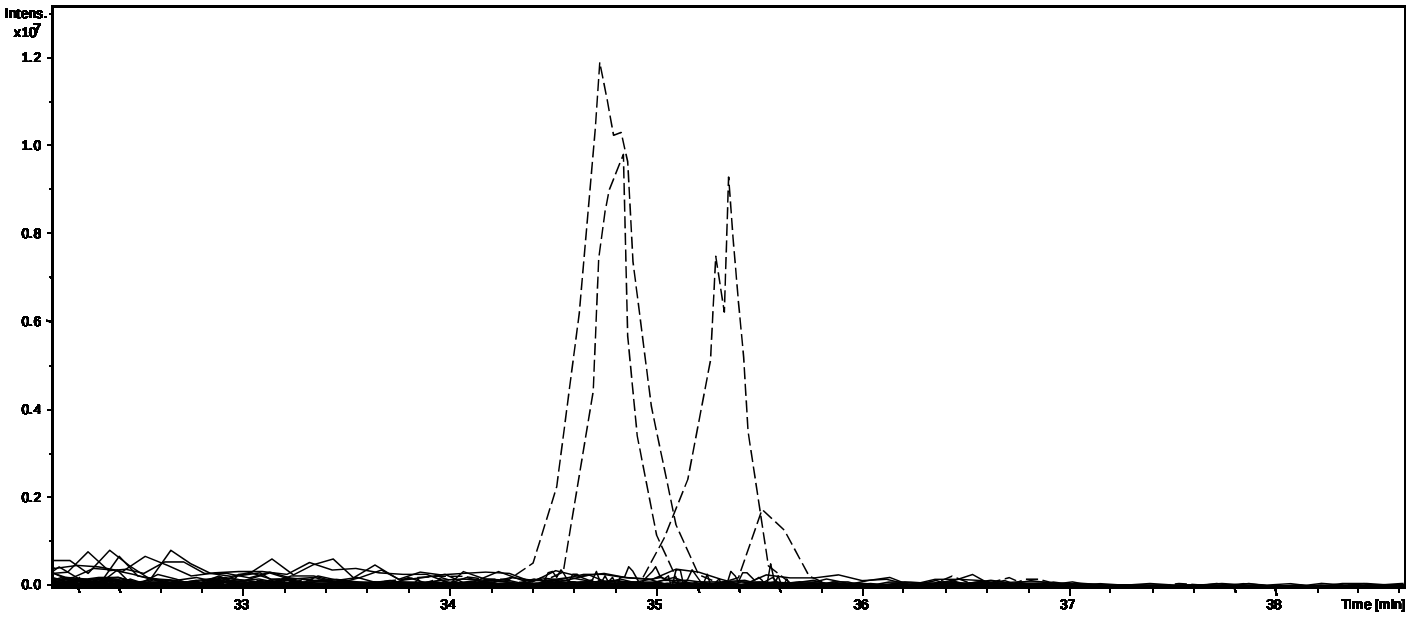
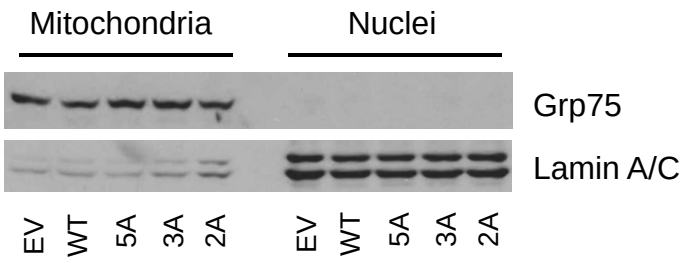


Figure S4


Mitochondria
Nuclei
Grp75
Lamin A/C
EV
WT
5A
3A
2A
EV
WT
5A
3A
2A