Supporting Information for Publication

Mapping citrullinated sites in multiple organs of mice using hyper-citrullinated library.

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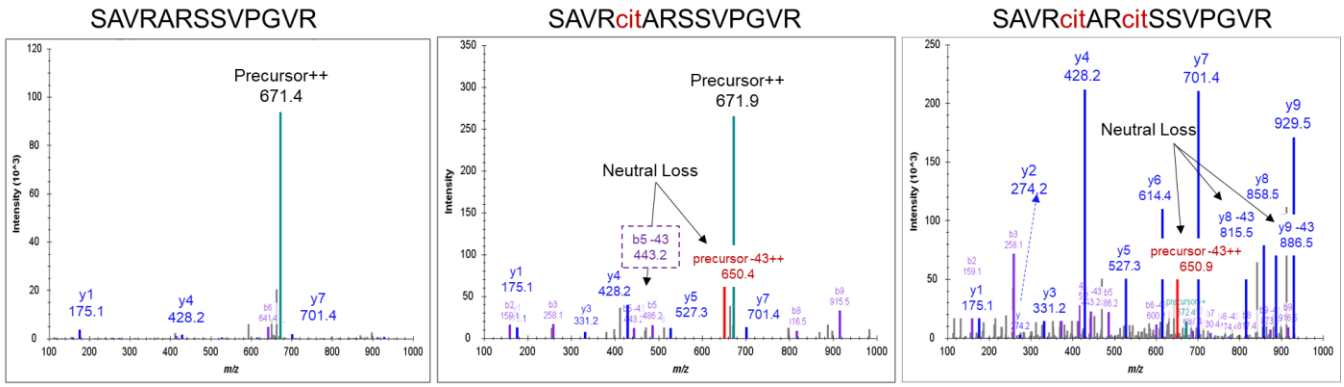
Supplementary Figures.

Supplementary Tables.

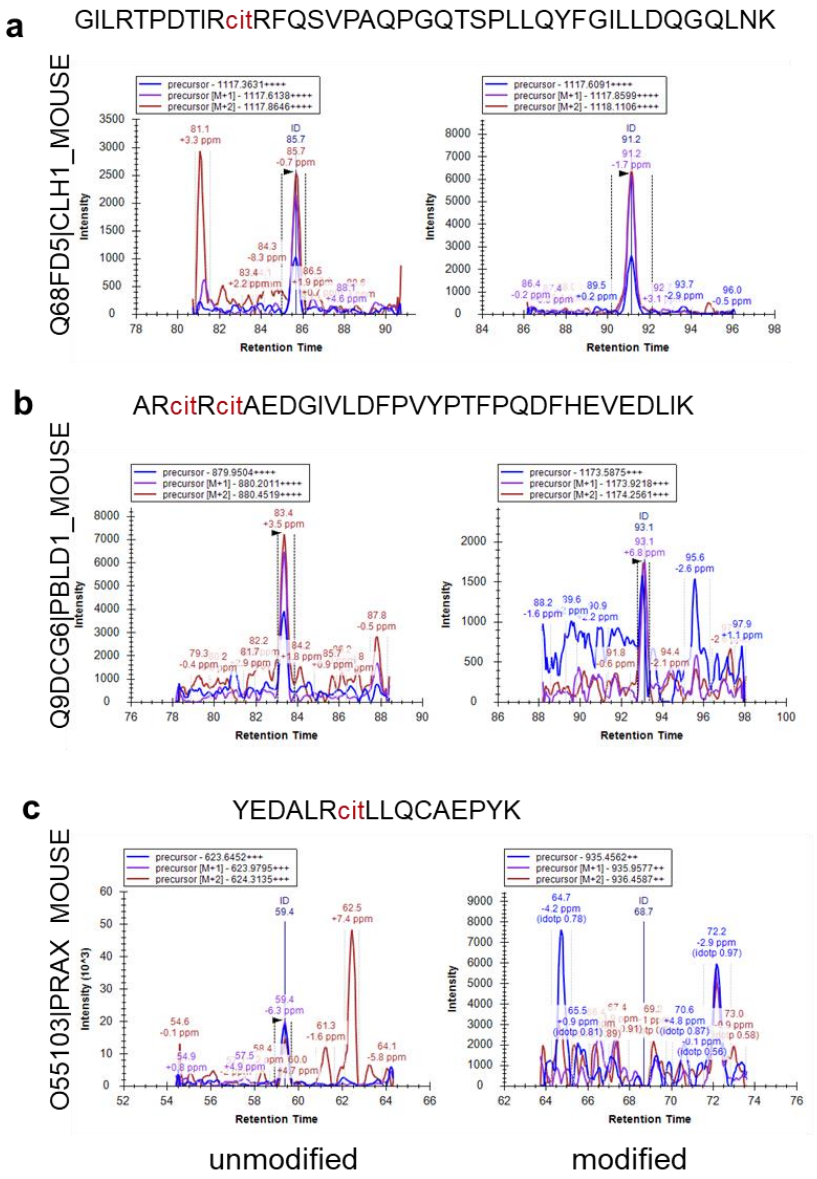
Table S1. List of detected citrullinated sites across the mouse organs. Attached separately.

A table showing citrullinated protein and peptide information identified crossed mouse organs by DDA on TripleTof 6600. The lists include the following information:1) Protein ID and 2) Protein Name, 3) Alternative IDs, 4)Modified Peptide, 5) Modification Site, 6) 10 Amino Acid around the modified site, 7)Unmodified Peptide Pair, 8) RT of Modified Peptide, 9) RT of Unmodified Peptide, 10) RT Shift, 11) Charge State of Modified Peptide, 12) Charge State of Unmodified Peptide, 13) Charge Shift, 14) Total Neutral Loss Ions,15) Neutral Loss Ions with Intensity, 16) Organ: Heart, 17) Organ: Skeletal Muscle, 18) Organ: Kidney, 19) Organ: Liver, 20) Organ: Lung, 21) Organ: Brian. Skyline Validation was performed using the peptide Identification represented by MS/MS spectrum with median retention time (columns 22-29). In cases where confident chromatographic peak groups could not be confirmed using this representative peptide identity, alternative peptide identity from other MS files was chosen to confirm peptide identification as indicated by "*" symbol along with the associated charge state next to the file name in column "Mod-Unmod File Pair for Skyline Validation"

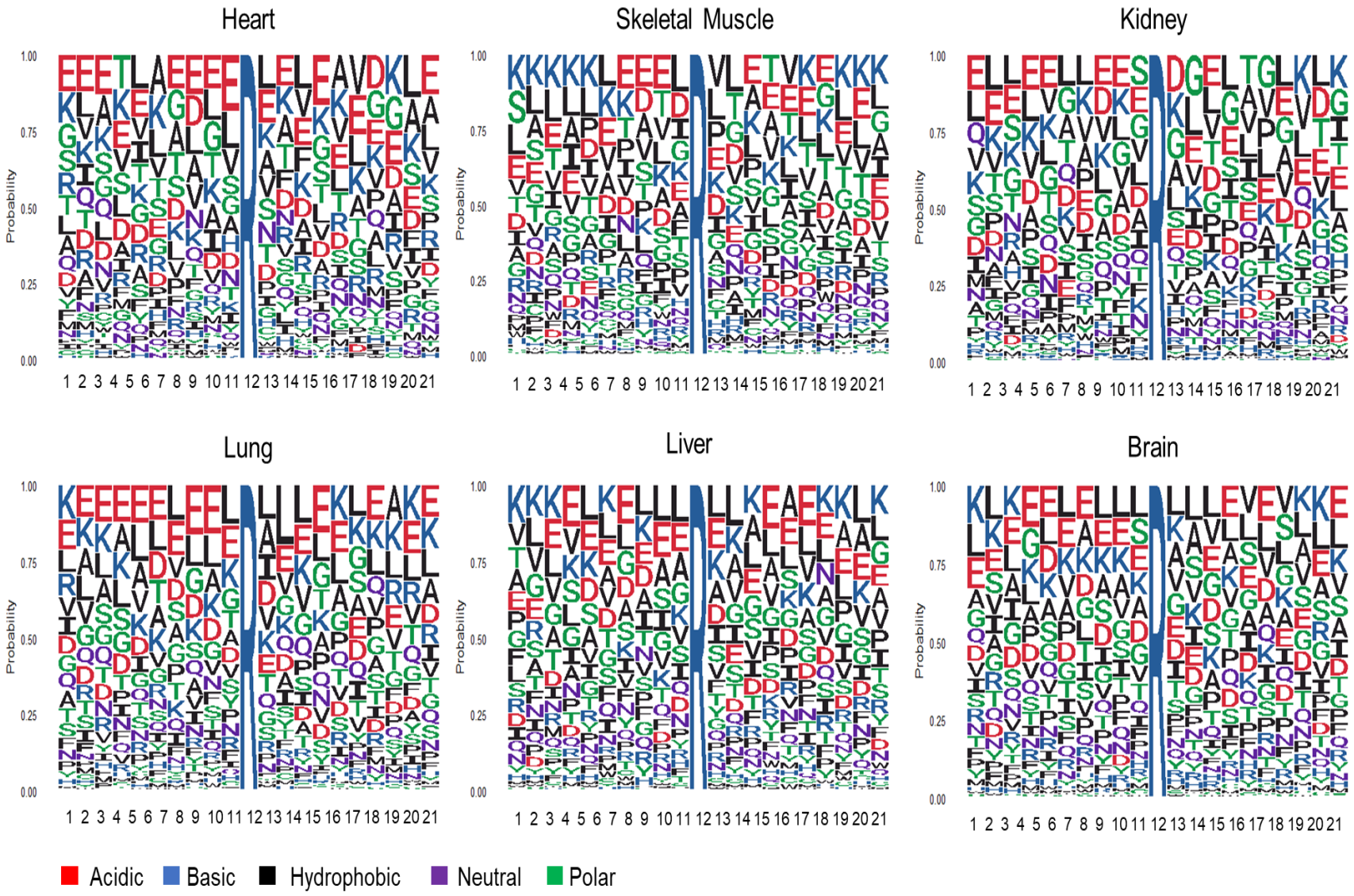
Table S2. The UniProt list of genes that have at least one SNP at arginine residue that match residue identified in our hyper-citrullinated library.



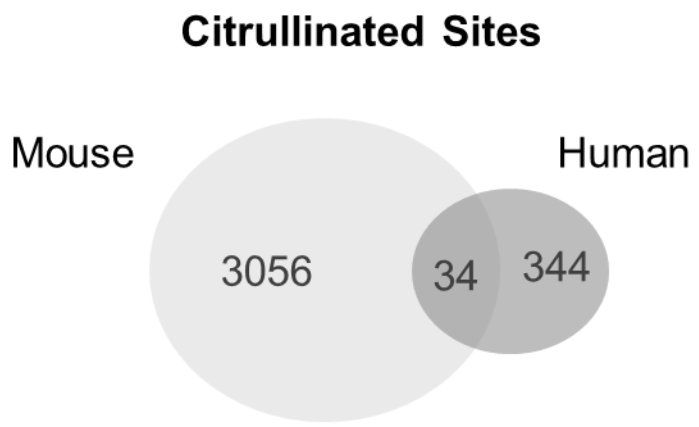
Supplemental figure 1. Neutral loss of HNCO from model citrullinated peptides. MS/MS/MS spectra of SAVRcitARSSVPGVR, SAVRcitARcitSSVPGVR (m/z 650.4 and 650.9 respectively) and SAVRARSSVPGVR (m/z 671.4) showed strong presence of the neutral loss peak specific to citrullination in single and double citrullinated peptide.



Supplemental figure 2. Schematic demonstration on how to define citrullinated and non citrullinated peptide sequence in the Skyline. (a) Chromatographic Peak Groups with Confirmed Peptide Identity, Isotope Dot Product >=0.9, Retention Time Drift <= +/- 0.2, Total Area >= 100000, TotalArea/FWHM>=1000000 and Average Mass Error PPM <= +/- 5 were defined as “Good”; (b) Chromatographic Peak Groups with Confirmed Peptide Identity and meeting 4 of the following 5 quality measures: Isotope Dot Product >=0.7, Retention Time Drift <= +/- 0.4, Total Area >= 10000, TotalArea/FWHM>=100000 and Average Mass Error PPM <= +/- 10 were defined as “Okay”; (c) All other Peak Groups that did not meet the above thresholds or had poor peak quality measures such as Isotope Dot Product <0.7 or Retention Time Drift >= +/- 1 were automatically flagged as “Bad” and discarded from any further analyses.



Supplemental figure 3. Properties of arginine citrullinated sites. Sequence probability logos of significantly enriched citrullinated site motifs for ±10 amino-acid sequences flanking the arginine sites across studied mouse organs



Supplemental figure 4. Comparison of two citrullinated datasets in human and mouse. Data are representative of results from the hyper-citrullinated mouse library and Chien-Yun Lee *et al.* human data.