Supplemental Material

Comprehensive identification of proteins from MALDI imaging

Stefan K. Maier, Hannes Hahne, Amin Moghaddas Gholami, Benjamin Balluff, Stephan Meding, Cédrik Schoene, Axel K. Walch & Bernhard Kuster

Supplemental Figures:

Supplemental Figure 1	MALDI imaging specimen from normal tissue of the stomach during the sample preparation workflow and the resulting SDS PAGE lanes
Supplemental Figure 2	SDS PAGE lanes of the 10 tissue samples and matrix extractions
Supplemental Figure 3	Completely annotated HCD tandem mass spectrum of full length thymosin beta-4
Supplemental Figure 4	Comparison of Top Down search results on peptide and spectrum level

Supplemental Tables:

Supplemental Table 1	Protein summary for bottom up protein identification of ten tissue entities and matrix extracts
Supplemental Table 2	Peptide summary for bottom up protein identification of ten tissue entities and matrix extracts
Supplemental Table 3	Protein identifications based on single peptide identifications
Supplemental Table 4	List of masses identified in MALDI protein imaging studies from humans and rodents
Supplemental Table 5	List of 72 proteins identified in MALDI IMS studies in rodents including respective references
Supplemental Table 6	Protein summary for top down protein identification of matrix extracts (oesophagus and colon)
Supplemental Table 7	Peptide/proteoform summary for top down protein identification of matrix extracts (oesophagus and colon)
Supplemental Table 8	Results for ‘canonical pathway’ from IPA

Supplemental Files

Supplemental File 1: Subset database (matrix and tissue proteome)

This fasta file comprises all sequences of proteins identified in the bottom up proteomics experiments from the matrix and tissue extracts of the ten different tissue entities shown in Figure 2a of the main manuscript. This fasta file can be used by all search engines commonly used in proteomics (including but not limited to Mascot, Sequest, Andromeda, XTandem, OMSSA etc.)

Supplemental File 2: Subset database (matrix proteome only)

Fasta file containing only proteins identified from the two matrix extracts.

Supplemental File 3: Bottom-up dataset

Scaffold file containing the results of the bottom-up protein identification experiments of ten tissue entities (compare Fig. 2a) searched against the IPI human database.

Supplemental File 4: Top-down dataset

Scaffold file containing the results of the top-down protein identification experiments (colon and esophagus matrix extracts searched against the IPI human database and the MaTisseDB).

Supplemental files 3 and 4 are available via proteomeXchange (accession PXD000125) and can be viewed using the Scaffold viewer available at:

http://www.proteomesoftware.com/Proteome_software_prod_Scaffold3_download-main.html

Detailed method for matrix coating of imaging specimen with the Bruker ImagePrep

	Matrix thickness	Nebulization	Incubation	dry
Phase 1	10 cycles	25% power 30% modulation 2.2 s spray	5 s	60 s
Phase 2	1 cycle	-	-	60 s
Phase 3	Matrix layer thickness 0.1 V 4-18 cycles	25% power 30% modulation Sensor 0.1 V	30 s	20% Complete dry every 2nd cycle Safe dry 20 s
Phase 4	Matrix layer thickness 0.3 V 8-40 cycles	25% power 30% modulation Sensor 0.2 V	30 s	20% Complete dry every 4th cycle Safe dry 50 s
Phase 5	Matrix layer thickness 0.35 V 4-64 cycles	25% power 35% modulation Sensor 0.3 V	30 s	20% Complete dry every 4th cycle Safe dry 60 s