MALDI MSI Reveals the Spatial Distribution of Protein Markers in Tracheobronchial Lymph Nodes and Lung of Pigs after Respiratory Bacterial Infection

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Original and modified IHC images of IL-1β including cryosections from control/healthy lung and necrotic area (NA), marginal zone (MZ) and tracheobronchial lymph node (TBLN) from pigs infected by *Actinobacillus pleuropneumoniae* (APP)



Figure S1. IHC image of IL-1 β in cryosection of control/healthy lung before and after modification in GIMP 2.10.22. The length of scale bar is 1 mm. The positive IHC signal has purple color in modified image.



Figure S2. IHC image of IL-1 β in cryosection of first necrotic area (NA) from lungs obtained from pigs infected with APP, before and after modification in GIMP 2.10.22. The length of scale bar is 1 mm. The positive IHC signal has purple color in modified image.



Figure S3. IHC image of IL-1 β in cryosection of second necrotic area (NA) from lungs obtained from pigs infected with APP, before and after modification in GIMP 2.10.22. The length of scale bar is 1 mm. The positive IHC signal has light purple color in modified image.



Figure S4. IHC image of IL-1 β in cryosection of third necrotic area (NA) from lungs obtained from pigs infected with APP, before and after modification in GIMP 2.10.22. The length of scale bar is 1 mm. The positive IHC signal has purple color in modified image.



Figure S5. IHC image of IL-1 β in cryosection of tracheobronchial lymph node (TBLN) obtained from pigs infected with APP, before and after modification in GIMP 2.10.22. The length of scale bar is 1 mm. The positive IHC signal has purple color in modified image.



Figure S6. IHC image of IL-1 β in cryosection of marginal zone (MZ) from lungs obtained from pigs infected with APP, before and after modification in GIMP 2.10.22. The length of scale bar is 1 mm. The positive IHC signal has purple color in modified image.

Original and modified IHC images of CD163 including cryosections from control/healthy lung and necrotic area (NA), marginal zone (MZ) and tracheobronchial lymph node (TBLN) from pigs infected by *Actinobacillus pleuropneumoniae* (APP)



Figure S7. IHC image of IL-1 β in cryosection of control/healthy lung before and after modification in GIMP 2.10.22. The length of scale bar is 1 mm. The positive IHC signal has brown color in modified image.



Figure S8. IHC image of IL-1 β in cryosection of first necrotic area (NA) from lungs obtained from pigs infected with APP, before and after modification in GIMP 2.10.22. The length of scale bar is 1 mm. The positive IHC signal has brown color in modified image.



Figure S9. IHC image of IL-1β in cryosection of second necrotic area (NA) from lungs obtained from pigs infected with APP, before and after modification in GIMP 2.10.22. The length of scale bar is 1 mm. The positive IHC signal has brown/grey color in modified image.



Figure S10. IHC image of IL-1 β in cryosection of third necrotic area (NA) from lungs obtained from pigs infected with APP, before and after modification in GIMP 2.10.22. The length of scale bar is 1 mm. The positive IHC signal has brown color in modified image.



Figure S11. IHC image of IL-1 β in cryosection of tracheobronchial lymph node (TBLN) obtained from pigs infected with APP, before and after modification in GIMP 2.10.22. The length of scale bar is 1 mm. The positive IHC signal has brown color in modified image.



Figure S12. IHC image of IL-1 β in cryosection of marginal zone (MZ) from lungs obtained from pigs infected with APP, before and after modification in GIMP 2.10.22. The length of scale bar is 1 mm. The positive IHC signal has brown color in modified image.

Identification of protegrin-4 precursor by MALDI-LIFT-TOF/TOF and MASCOT

(MATRIX) SCIENCE/ Mascot Search Results

| User | : | | | | | |
|--------------|---|--|--|--|--|--|
| Email | : | | | | | |
| Search title | : | | | | | |
| MS data file | : | DATA.TXT | | | | |
| Database | : | NCB1prot 20190409 (198058129 sequences; 72054219131 residues) | | | | |
| Taxonomy | : | Other mammalia (2621646 sequences) | | | | |
| Timestamp | : | 10 Dec 2019 at 15:13:04 GMT | | | | |
| Warning | : | There is an error in a configuration file: Error in the configuration file\config\mascot.dat - database UniProt proteome | | | | |
| Warning | : | 2 sequences ignored because length greater than maximum configured | | | | |
| Protein hits | : | NP 999028.1 protegrin-4 precursor [Sus scrofa] | | | | |
| | | XP 022368645.1 dihydrolipoyllysine-residue acetyltransferase component of pyruvate dehydrogenase complex, mitochondrial [Enh | | | | |
| | | XP 006873889.1 PREDICTED: protein ITFG3 [Chrysochloris asiatica] | | | | |
| | XP 012782233.1 PREDICTED: centromere protein J [Ochotona princeps] XP 023392235.1 ubiquitin-associated domain-containing protein 1 [Pteropus vampyrus] XP 024436573.1 LOW QUALITY PROTEIN: sushi domain-containing protein 3 [Desmodus rotundus] BYP83234.1 hvpothetical protein CB1 000589041 [Camelus ferus] | | | | | |
| | | | | | | |
| | | | | | | |
| | | | | | | |
| | | XP 007940149.1 PREDICTED: homeobox protein Hox-B9 [Orycteropus afer afer] | | | | |
| | | XP 019837838.1 PREDICTED: sorting nexin-ll isoform X1 [Bos indicus] | | | | |
| | | XP 008685282.1 PREDICTED: xin actin-binding repeat-containing protein 2 isoform X1 [Ursus maritimus] | | | | |

Mascot Score Histogram

Ions score is -10*Log(P), where P is the probability that the observed match is a random event. Individual ions scores > 42 indicate peptides with significant homology. Individual ions scores > 47 indicate identity or extensive homology (p<0.05). Protein scores are derived from ions scores as a non-probabilistic basis for ranking protein hits.



Peptide Summary Report

| Format As | Peptide Summary | | Help |
|-----------|------------------------------------|----------------------------------|--------------------|
| | Significance threshold p< 0.05 | Max. number of hits 20 | |
| | Standard scoring MudPIT scoring O | Ions score or expect cut-off | Show sub-sets 0 |
| | Show pop-ups (Suppress pop-ups O | Sort unassigned Decreasing Score | Require bold red 🛛 |
| | Preferred taxonomy All entries | $\mathbf{\Sigma}$ | |

Overview Table

Click on column header to jump to entry in results list. Move mouse over any indicator to highlight identical peptides. Click on an indicator to see details of individual match. Use check boxes to select sub-set of queries for new search.

| Mouse over | -AccessionSequence- |
|------------------------------|--|
| ✓ <u>1125.48</u> | Hit: 1 2 3 4 5 6 7 8 2 10 <u>660</u> (1+) • • • • • • • • • • • • |
| Select All | Select None Search Selected Error tolerant Archive Report |
| 1. <u>NP</u> pro | <u>999028.1</u> Mass: 25839 Score: 54 Matches: 1(1) Sequences: 1(1) tegrin-4 precursor [Sus scrofa] |
| Che | ck to include this hit in error tolerant search or archive report |
| Quer | ry Observed Mr(expt) Mr(calc) Delta Miss Score Expect Rank Unique Peptide <u>1</u> 1125.4860 1124.4787 1124.5767 -0.0980 0 55 0.0084 1 U R.FPPPNFPGPR.F |
| Pro | teins matching the same set of peptides: |
| AAB | <u>33924.1</u> Mass: 66/5 Score: 53 Matches: 1(1) Sequences: 1(1) Denlemini Denleminimenti pontido (decemen repeate) (suito loukouttas Pontido 79 sel |
| P51 | prenint, re-taining to the second sec |
| Recl | Mame: Full=Frombenin and tritroticin precursor; Althame: Full=C6 (Contains: RecName: Full=Prombenin-1; Short=FF-1; Contains: RecName: |
| XP | 020924182.1 Mass: 29255 Score: 51 Matches: 1(1) Sequences: 1(1) |
| pro | tegrin-4 isoform X1 [Sus scrofa] |
| 2. <u>XP</u> dih □ Che | 022368645.1 Mass: 68935 Score: 27 Matches: 1(0) Sequences: 1(0) ydrolipoyllysine-residue acetyltransferase component of pyruvate dehydrogenase complex, mitochondrial [Enhydra lutris kenyoni] ck to include this hit in error tolerant search or archive report |
| Che | ck to include this hit in error tolerant search or archive report |

Query Observed Mr(expt) Mr(calc) Delta Miss Score Expect Rank Unique Peptide <u>1</u> 1125.4860 1124.4787 1124.5284 -0.0497 0 27 6.3 2 U R.EDPGAPCLAPR.A Figure S13. Results of identification of protegrin-4 precursor from MASCOT.



Figure S14. LIFT mass spectrum with annotated fragments of protegrin-4 precursor

peptide FPPPNFPGPR. BioTools 3.2 was used for annotation.

Protein sequence coverage: 4%

Matched peptides shown in **bold red**.

| 1 | METQRASLCL | GRWSLWLLLL | ALVVPSASAQ | ALSYREAVLR | AVDRLNEQSS |
|-----|------------|--------------------|--------------------|------------|------------|
| 51 | EANLYRLLEL | DQPPKADEDP | GTPKPVSFTV | KETVCPRPTR | RPPELCDFKE |
| 101 | NGRVKQCVGT | VTLDQIKDPL | DITCNEGVRR | FPWWWPFLRR | PRLRRQAFPP |
| 151 | PNVPGPRFPP | PNVPGPR FPP | PNFPGPR FPP | PNFPGPRFPP | PNFPGPPFPP |
| 201 | PIFPGPWFPP | PPPFRPPPFG | PPRFPGRR | | |

Figure S15. Protegrin-4 precursor protein sequence coverage covered by FPPPNFPGPR. The sequence coverage is low, but the identified peptide is unique for this protein family.

Figure S16. The LIFT spectrum analysis report for protegrin-4 precursor peptide FPPPNFPGPR. BioTools 3.2 was used for analysis.

MALDI MSI mean mass spectra



Figure S17. The MALDI MSI mean mass spectrum created from all measured tissue sections. SCiLS Lab 2014b was used for processing the spectra applying baseline removal and normalization.



Figure S18. Zoomed MALDI MSI mean mass spectrum created from all measured tissue sections showing the peak of IL-1 β peptide NLYLSCVLKDDKPTLQLESVDPKNYPK. SCiLS Lab 2014b was used for processing the spectra applying baseline removal and normalization.



Figure S19. Zoomed MALDI MSI mean mass spectrum created from all measured tissue sections showing the peak of protegrin-4 precursor peptide FPPPNFPGPR. SCiLS Lab 2014b was used for processing the spectra applying baseline removal and normalization.



Figure S20. Zoomed MALDI MSI mean mass spectrum created from all measured tissue sections showing the peak of CD163 peptide TSYQVYSK. SCiLS Lab 2014b was used for processing the spectra applying baseline removal and normalization.



Figure S21. The preparation of humid box used for *on-tissue* trypsinization. (**A**) A magnetic stirrer with hotplate used for heating the humid box. (**B**) A humid box prepared from used tip rack, which was filled with water and on top was placed Kimwipe tissue soaked in LC-MS water. The ITO glass slides with applied trypsin solution were placed on the soaked Kimwipe tissue and the humid box/tip rack was closed. (**C**) A final set up used for 18h trypsinization.