Supplemental Information
Supplemental Information Figure A. Distribution of mascot (ion score) and Sequest (XCorr) scores for known and novel peptides. The mean sequest and mascot scores for known and novel peptides were calculated and were found to be similar for known and novel peptides.
Supplemental Information Figure B. Conservation analysis of known and novel genes. Evolutionary rates were calculated from OrthoDB database and were found to be similar for both known and novel genes.
Supplemental Information Figure C. Distribution of ORF length in annotated genes, predicted non-coding RNAs and non-coding RNAs with peptide evidence from proteomic data. The median length of reading frames for annotated genes and non-coding RNAs was found to be ~1,240 bp and ~153 bp, respectively. The median length of reading frames for non-coding RNAs, which were found to have peptide evidence from proteomic data was ~225 bp.
Supplemental Information Figure D. Spectral counts and sequence coverage for translated non-coding RNAs. i. The sequence coverage and spectral counts for translations from tissue restricted non-coding RNAs were lower than ubiquitously expressed non-coding RNAs. ii. Similar trend was observed for ubiquitously expressed and tissue restricted known proteins.
Experimentally Validated MS/MS spectra
AAFENEVAGESK

**Experiment**

**Validated**
AAIPVISDIQR

Experiment

Validated

Extracted from: C:\Users\Dawson Lab\Desktop\PandeyLab\Raja\An.stephensi\Antennae_in-gel\An_Steph_Antennae_IG_30.raw   #525 4   RT: 66.39
FTMS, HCD@39.00, z=+2, Mono m/z=591.84558 Da, MH+=1182.68389 Da, Match Tol.=0.05 Da

Extracted from: F:\Manish\Synthetic peptides\New Peptide\SyntheticPeptides\SyntheticPeptidesPool1.raw   #2214   RT: 22.50
FTMS, HCD@39.00, z=+2, Mono m/z=591.84558 Da, MH+=1182.68389 Da, Match Tol.=0.05 Da
AGTININEGTVR

Experiment

Validated
DVNVWSGIGGALDR
GLVWEEIGVWK

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**Experiment**

- Extracted from: D:/IOB/A.stephensi/Brain/Ingel/raw files/Ano_ste_brain_2.raw
- RT: 65.52
- FTMS, HCD@39.00, z=+2, Mono m/z=658.35706 Da, MH+=1315.70683 Da, Match Tol.=0.05 Da
- GLVWEEIGVWK

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**Validated**

- Extracted from: F:/Manish/Synthetic peptides/MS1/SyntheticPep_Pool1.raw
- RT: 43.65
- FTMS, HCD@39.00, z=+2, Mono m/z=658.35687 Da, MH+=1315.70647 Da, Match Tol.=0.05 Da
- GLVWEEIGVWK
EGTFQGLHHTFPVTIGLR

Extracted from: D:\IOB\A.stephensi\Salivarygland\Asteph_SalGland_IG_25.raw   #4282   RT: 42.51
FTMS, HCD@39.00, z=+2, Mono m/z=937.00 Da, MH+=1873.0028 Da, Match Tol.=0.05 Da

Validated
FEVPNQSDER

Experiment

Validated
FFAYPITSYYSYR

Extracted from: C:\Users\Blesson\Lab\Data\Peptide\Peaks\Anopheles\MAG\MAG_512.raw   #534   RT: 45.23
FTMS, HCD@39.00, z=+2, Mono m/z=433.4222 Da, Tol=+0.05 Da, Match Tol=+0.05 Da

Extracted from: F:\Users\Blesson\Synthetic peptides\Peptide\Peaks\Anopheles\MAG\MAG_512.raw   #534   RT: 45.23
FTMS, HCD@39.00, z=+2, Mono m/z=433.4222 Da, Tol=+0.05 Da, Match Tol=+0.05 Da

Experiment

Validated
GSYSLIEPDGSR

Experiment

Validated
GWLSPLDYSVER
HQIDSVPTVLFLR

Experiment

Validated
IELINQLSETGLR
Experiment

Validated

IFGGTDVEDGVTAPYLVALLR

Extracted from: C:\Users\Dawson\Desktop\PandeyLab\An.stephensi\MAG\MAG_Q20.raw  #7064  RT: 60.02
FTMS-HCD@39.00, z=+2, Mono m/z=1054.05933 Da, MH+=2107.11138 Da, Match Tol.=0.05 Da

Extracted from: F:\Manish\Synthetic peptides\Raw files\SyntheticPep_Pool2_190307171558.raw  #5462  RT: 49.35
FTMS-HCD@39.00, z=+2, Mono m/z=1054.05859 Da, MH+=2107.10991 Da, Match Tol.=0.05 Da
IITTPQATAIGVAGSGK
IPFHPYFIYK
DLTTDDAATLIQSVFR

Experiment

Validated
VQGGSVLHLVLALR

Experiment

Validated
| m/z     | Experiment  | Validated |
|---------|-------------|-----------|
| 585.2988 |             | LIGTNVGDLK |
| 515.3236 |             |           |
| 432.2446 |             | 147.1326  |
| 337.3188 |             | 815.4265  |
| 260.1968 |             | 199.1807  |
| 147.1326 |             | 916.5099  |
| 337.3188 |             | 260.1968  |
| 260.1968 |             | 337.3188  |
LPVKDGQYSVNLDR

Experiment

Validated
LVVSGSSDNSIR

Experiment

Validated
NLLQVDLTKR

Experiment

Validated

Extracted from: C:\Users\Dawson Lab\Desktop\Pandey_lab\Raja\An_stephensi\Maxillary Palps_in-gel\An_Steph_Max_palp_IG_11.raw   #2435   RT: 32.87
FTMS, HCD@39.00, z=+2, Mono m/z=600.36060 Da, MH+=1199.71391 Da, Match Tol.=0.05 Da

Extracted from: F:\Manish\Synthetic peptides\Raw files\SyntheticPep_Pool2.raw   #1593   RT: 17.74
FTMS, HCD@39.00, z=+2, Mono m/z=600.35925 Da, MH+=1199.71123 Da, Match Tol.=0.05 Da

Extracted from: F:\Manish\Synthetic peptides\Raw files\SyntheticPep_Pool2.raw   #1593   RT: 17.74
FTMS, HCD@39.00, z=+2, Mono m/z=600.35925 Da, MH+=1199.71123 Da, Match Tol.=0.05 Da
**INSLEEKEKDYDLEYVVKR**

**Extracted from:** C:\Users\Dawson\Lab\Dawson\Pandey\Lab\Postdoc\MAG\S19.py, #3137 RT: 26.68

**FTMS, HCD@39.00, z=+2, Mono m/z=1064.05 347 Da, MH+=2127.09966 Da, Match Tol.=0.05 Da**

**Validated**

**Extracted from:** F:\Manish\Synthetic peptides\Test\SyntheticPep_Pool1.raw, #2025 RT: 20.85

**FTMS, HCD@39.00, z=+2, Mono m/z=1064.05 505 Da, MH+=2127.10283 Da, Match Tol.=0.05 Da**

**Experiment**
The image contains two FTMS mass spectra with the following annotations:

**Experiment**
- M/z range: 100 to 900
- Intensity [counts] (10^6)
- Extracted from: C:\Users\Dawson Lab\Desktop\PandeyLab\Raja\An.stephensi\Maxillary Palps_in-gel\An_Steph_Max_palp_IG_01.raw
- RT: 19.99
- FTMS, HCD@39.00, z=+3, Mono m/z=416.54407 Da, MH+=1247.61765 Da, Match Tol.=0.05 Da

**Validated**
- M/z range: 100 to 900
- Intensity [counts] (10^3)
- Extracted from: C:\Users\Dawson Lab\Desktop\PandeyLab\Raja\An.stephensi\Maxillary Palps_in-gel\An_Steph_Max_palp_IG_01.raw
- RT: 19.99
- FTMS, HCD@39.00, z=+3, Mono m/z=416.54407 Da, MH+=1247.61765 Da, Match Tol.=0.05 Da

The spectra show the fragmentation patterns of peptides, including y ions, b ions, and [M+3H]³⁺ ions, with their respective masses and intensities.
TVTAISTESATNTEAK

Extracted from: D:\IOB\A.stephensi\Brain\Ingel\raw files\Ano_ste_brain_7Re.raw   #1283   RT: 19.31
FTMS, HCD@39.00, z=+2, Mono m/z=812.40857 Da, MH+=1623.80986 Da, Match Tol.=0.05 Da

Extracted from: F:\Manish\Synthetic peptides\Raw files\SyntheticPep_Pool2.raw   #679   RT: 10.72
FTMS, HCD@39.00, z=+2, Mono m/z=812.40857 Da, MH+=1623.80986 Da, Match Tol.=0.05 Da
GVGAIPPPPQR

Extracted from: D:\IOB\A.stephensi\Brain\A. stephensielsonco1, brain, S raw #1276 RT: 15.73
FTMS, HCD@39.00, z=+2, Mono m/z=544.81549 Da, MH+=1088.62370 Da, Match Tol.=0.05 Da

Extracted from: F:\Manish\Synthetic peptides\Raw files\SyntheticPep_Pool2_150207171656.raw #1132 RT: 16.16
FTMS, HCD@39.00, z=+2, Mono m/z=544.81506 Da, MH+=1088.62285 Da, Match Tol.=0.05 Da
TYGADPDHHLDDYR

Extracted from: F:\Manish\Synthetic peptides\Raw files\SyntheticPeptide_Pool2.raw   #634   RT: 10.42
FTMS, HCD@39.00, z=+2, Mono m/z=780.34760 Da, MH+=1559.68791 Da, Match Tol.=0.05 Da

Experiment

Validated
VGFNGLLQPFSDAVK

Experiment

Validated
VLEGTVAVGPGAR
Experiment

**VRYEPVNLEK**

Validated
YEPVNLEKER
YREGLEELVR
Experiment

Validated

ASMYFFNTNPSGR
DIPVAVATPAVAAK

Experiment

Validated
The image contains two FTMS chromatograms comparing the extracted MS/MS spectra of a synthetic peptide pool. The primary peptide sequence is FSNYGQVIR, and the experiment is validated. The chromatograms show the distribution of fragment ions across different m/z values, with intensity in counts. The spectra are extracted from two different raw files: one from a larval sample and another from a synthetic peptide pool. The raw files and their metadata are included as part of the chromatogram images.
GGTPTSAATTPPAKR
GPGDTSNFDDYEEETLR

Experiment

Validated
GQYSLLHEADGTER
GWILVDYPNIADDVK

Experiment

Validated
INVDQAFHELVR

Experiment

Validated
ITTTDTGIHFKK

Experiment

Validated
IVDYTADDVR
LFGLIPITHPEPLIFAGR

Experiment

Validated
LTSYDWENQFESFQDATR
NFLAISISAGIR
NPFGVVEHTINEEFR

Experiment

Validated
NPTNTAALDDFER
QFIDSYFSSFTNDPVR

Extracted from: D:\IOB\A.stephensi\Salivarygland\Asteph_SalGland_IG_08.raw   #6527   RT: 54.85
FTMS, HCD@39.00, z=+2, Mono m/z=961.95233 Da, MH+=1922.89739 Da, Match Tol.=0.05 Da

Extracted from: F:\Manish\Synthetic peptides\Raw files\SyntheticPep_Pool1.raw   #3791   RT: 39.71
FTMS, HCD@39.00, z=+2, Mono m/z=961.95050 Da, MH+=1922.89372 Da, Match Tol.=0.05 Da
SLASLLQTLTLDVVR

Experiment

Validated
SSLIHAILGELPLESGSIK

Experiment

Validated
**TFPALVYFR**

**Experiment**

- Extracted from: C:\Users\Dawson Lab\Desktop\PandeyLab\Raja\An.stephensi\Thorax\An_steph_thorax1.raw  #5611   RT: 55.91
- FTMS, HCD@39.00, z=+2, Mono m/z=557.30737 Da, MH+=1113.60747 Da, Match Tol.=0.05 Da

**Validated**

- Extracted from: F:\Manish\Synthetic peptides\raw files\SyntheticPepPool1.raw  #3781   RT: 39.68
- FTMS, HCD@39.00, z=+2, Mono m/z=567.30600 Da, MH+=1113.61052 Da, Match Tol.=0.05 Da
TIPDNFLSSAANLLLR

Experiment

Validated
TSDGSQATSNVVR

Experiment

Vali‌dated
VAAHYQISALNK

Experiment

Validated
VFTYDFGPHIQVPTK

Experiment

Validated
VLNHGDFWTNNILFK
VSTISQLVQQMFR

Experiment

Validated
VVEVGVPYVFQYR
Experiment

Validated

VYADVNSHKPR
YFAQGSATWENEK
DDTYTAHAGAK

Experiment

Validated
ELNELQAIVGR
FFETNPSGR
GGPTSAATTPPAK

Experiment

Validated
GSQITDDEIR

Experiment

Validated
HIEAGAEK

Experiment

Validated
**KAYAADGHEVLDR**

**Experiment**

Extracted from: C:\Users\Dawson Lab\Desktop\Pandey Lab\Raja\An_stephensi\Salivary gland_3RPLC\An_Salivary_Salivary_3RPLC_10.raw  #2592  RT: 23.59  FTMS, HCD@32.00, z=+2, Mono m/z=722.86206 Da, MH+=1444.71684 Da, Match Tol.=0.05 Da

**Validated**

Extracted from: F:\Raja\IOB\AnStephensi\Syntheticpeptides\An_Salivary_Salivary_Peptide_Pool3.raw  #2013  RT: 31.68  FTMS, HCD@32.00, z=+2, Mono m/z=722.65982 Da, MH+=1444.71233 Da, Match Tol.=0.05 Da
KIWQATK

Extracted from C:\Users\Dawson Lab\Develop\ProtScale\Replein\An.stephens\Elite_Tests\HPLCv3_step_Tests\HPLC_15.raw #9411 RT: 30.03 FTMS, HCD@32.00, z=+2, Mono m/z=437.76025 Da, MH+=874.51323 Da, Match Tol.=0.05 Da

Extracted from F:\Raja\IOB\An.Stephensi\Syntheticpeptides\An_Stephensi_SyntheticPeptide_Pool3.raw #1801 RT: 30.21 FTMS, HCD@32.00, z=+2, Mono m/z=437.76257 Da, MH+=874.51787 Da, Match Tol.=0.05 Da

Experiment

Validated
LDQTIQSIASK

[Diagram showing mass spectrometry results with peaks labeled for y- and b-series ions, indicating the presence of peptides at specific m/z values.]

Extracted from: C:\Users\Dawson Lab\Qexs\Pendley Lab\Raja_peptides\Elas_Tes6_hRPLC\Ar_steph_Tes6_hRPLC_2.raw #8472, RT: 48.51
FTMS, HCD@32.00, z=+2, Mono m/z=902.53048 Da, M+H+=1203.66014 Da, Match Tol.=0.1 Da

Extracted from: F:\Raja\IOB\An.Stephensi\Syntheticpeptides\An_Stephensi_SyntheticPeptide_Pool3.raw #6534, RT: 68.20
FTWS, HCD@32.00, z=+2, Mono m/z=902.53260 Da, M+H+=1203.65837 Da, Match Tol.=0.05 Da

Experiment

Validated
LLTPAEQEEQVSK

Experiment

Validated
LQVEHTFEQQTGAK

Extracted from: C:\Users\Dawson Lab\Desktop\PandeyLab\Raja\An.stephensi\Elite_T estis_bRPLC\An_steph_T estis_bRP_22.raw   #7133   RT : 43.72
FTMS, HCD@32.00, z=+2, Mono m/z=808.39923 Da, MH+=1615.79119 Da, Match Tol.=0.1 Da

Extracted from: F:\Raja\IOB\An.Stephensi\Syntheticpeptides\An_Stephensi_SyntheticPeptide_Pool3.raw   #2873   RT: 37.90
FTMS, HCD@32.00, z=+2, Mono m/z=808.40295 Da, MH+=1615.79863 Da, Match Tol.=0.05 Da
SSILQVLLR

Extracted from: C:\Users\Dawson Lab\Desktop\PandeyLab\Raja\An.stephensi\Elite_Salivary gland_bRPLC\An_Stephensi_Salgland_b RPLC_12.raw   #17189   RT: 96.26
FTMS, HCD@32.00, z=+2, Mono m/z=514.82782 Da, MH+=1028.64836 Da, Match Tol.=0.05 Da

Extracted from: F:\Raja\IOB\An.Stephensi\Syntheticpeptides\An_Stephensi_SyntheticPeptide_Pool3.raw   #7276   RT: 75.17
FTMS, HCD@32.00, z=+2, Mono m/z=514.82654 Da, MH+=1028.64580 Da, Match Tol.=0.05 Da

Validated

Experiment
SVVQYDSAGASNNNR

Experiment

Validated
TEELNQETR

Extracted from: C:\Users\Dawson Lab\Desktop\PandeyLab\Raja\An.stephensi\Your项目名称\An_stephensi_Your项目名称.raw   #3858   RT : 28.31
FTMS, HCD@32.00, z=+2, Mono m/z=560.26837 Da, MH+=1119.52947 Da, Match Tol.=0.1 Da

Extracted from: F:\Raja\IOB\An.Stephensi\Syntheticpeptides\An_Stephensi_SyntheticPeptide_Pool3.raw   #1467   RT: 27.74
FTMS, HCD@32.00, z=+2, Mono m/z=560.26691 Da, MH+=1119.52654 Da, Match Tol.=0.05 Da
TGDVLGFGK

Experiment

Validated
TIATIDVHAR

Experiment

Validated
Experimen validated: TLTENGYQVNLR

Extracted from: C:\Users\Dawson Lab\Desktop\PandeyLab\Raja\An.stephensi\Elite_T estis_bRPLC\An_steph_T estis_bRP_21.raw   #9725   RT : 56.00
FTMS, HCD@32.00, z=+2, Mono m/z=704.36426 Da, MH+=1407.72124 Da, Match Tol.=0.1 Da

Extracted from: F:\Raja\IOB\An.Stephensi\Syntheticpeptides\An_Stephensi_SyntheticPeptide_Pool3.raw   #4090   RT: 47.17
FTMS, HCD@32.00, z=+2, Mono m/z=704.36334 Da, MH+=1407.71941 Da, Match Tol.=0.05 Da

[Graph showing mass spectra data for TLTENGYQVNLR]
TVLEQQVTR

Experiment

Valuated
VTEPPRGFK
AKDGAATVAEDVK

Experiment

Validated
FLEVLQNDFR

Experiment

Validated
ILSEVVLRPK

Experiment

Validated
KANDTEAAVVSER

**Experiment**

**Validated**

Extracted from: C:\Users\Dawson Lab\Desktop\Pandey Lab\An.stephensi\Elite_Testis_bRPLC\An_steph_T estis_bRP_21.raw   #4330   RT : 32.14
FTMS, HCD@32.00, z=+2, Mono m/z=695.35431 Da, MH+=1389.70134 Da, Match Tol.=0.1 Da

Extracted from: F:\Raja\IOB\An.Stephensi\Syntheticpeptides\An_Stephensi_SyntheticPeptide_Pool4.raw   #1898   RT: 31.28
FTMS, HCD@32.00, z=+2, Mono m/z=695.84192 Da, MH+=1390.67656 Da, Match Tol.=0.05 Da
LAAVTSSTQK

Experiment

Validated
NAAADTVDSQIK

Extracted from: C:\Users\Dawson Lab\Desktop\PandeyLab\Raja\An.stephensi\Elite_Salivary gland_bRPLC\An_Stephensi_Salgland_bRPLC_10.raw   #3216   RT: 27.19
FTMS, HCD@32.00, z=+2, Mono m/z=616.80981 Da, MH+=1232.61235 Da, Match Tol.=0.05 Da

Extracted from: F:\Raja\IOB\An.Stephensi\Syntheticpeptides\An_Stephensi_SyntheticPeptide_Pool4.raw   #1939   RT: 31.62
FTMS, HCD@32.00, z=+2, Mono m/z=616.80841 Da, MH+=1232.60954 Da, Match Tol.=0.05 Da

Validated
NTGEIYEVAVVHR
SAAHLALENYQR

Experiment

Validated
SIFSDFIQQHR

Experiment

Validated
SPQGPASEK

Experiment

Validated

Extracted from: C:\Users\Dawson Lab\Desktop\PandeyLab\Raja\An.stephensi\Elite_Testis_bRPLC\An_steph_T estis_bRP_2.raw   #2411   RT: 20.92
FTMS, HCD@32.00, z=+2, Mono m/z=450.72488 Da, M+=[900.44249 Da, Match Tol.=+0.02 Da

Extracted from: F:\Raja\IOB\An.Stephensi\Syntheticpeptides\An_Stephensi_SyntheticPeptide_Pool4.raw   #1013   RT: 23.92
FTMS, HCD@32.00, z=+2, Mono m/z=450.72461 Da, M+=[900.44194 Da, Match Tol.=+0.05 Da
TPYFSSIPTR

Extracted from: C:\Users\Dawson Lab\Desktop\PandeyLab\Raja\An.stephensi\Elite_Testis_bRPLC\An_steph_T estis_bRP_3.raw   #12383   RT: 52.74
FTMS, HCD@32.00, z=+2, Mono m/z=584.80231 Da, MH+=1168.59734 Da, Match Tol.=0.05 Da

Extracted from: F:\Raja\IOB\An.Stephensi\Syntheticpeptides\An_Stephensi_SyntheticPeptide_Pool4.raw   #4485   RT: 51.42
FTMS, HCD@32.00, z=+2, Mono m/z=584.80231 Da, MH+=1168.59734 Da, Match Tol.=0.05 Da

Experiment

Validated
YDPTIEDFYR

Extracted from F:\Raja\Doctor\An.Stephensi\SyntheticPeptides\An.Stephensi\SyntheticPeptide pool 4.raw #5367 RT: 58.58
FTMS, HCD@32.00, z=+2, Mono m/z=659.80048 Da, MH+=1318.59368 Da, Match Tol.=0.05 Da

**Experiment**

YDPTIEDFYR

Extracted from F:\Raja\Doctor\An.Stephensi\SyntheticPeptides\An.Stephensi\SyntheticPeptide Pool 4.raw #5367 RT: 58.58
FTMS, HCD@32.00, z=+2, Mono m/z=659.80048 Da, MH+=1318.59368 Da, Match Tol.=0.05 Da

**Validated**
AVSASTATGK

Experiment

Validated
**SPYSWDLPADWK**

**Experiment**

**Validated**
YYSENNGLDGYK

Experiment

Validated

Extracted from: C:\Users\Dawson Lab\Desktop\PandeyLab\Raja\An.stephensi\Elite_Testis_bRP_1C\An_stephElite_BRP_2.raw   #7288  RT: 41.45
FTMS, HCD@32.00, z=+2, Mono m/z=711.81464 Da, fDr=+1422.61200 Da, Match Tol.=+0.05 Da

Extracted from: F:\Raja\IOB\An.Stephensi\Syntheticpeptides\An_Stephensi_SyntheticPeptide_Pool4.raw   #2641  RT: 37.19
FTMS, HCD@32.00, z=+2, Mono m/z=711.81006 Da, fDr=+1422.61284 Da, Match Tol.=+0.05 Da
QAFAIER

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**Experiment**

Extracted from: C:\Users\Dawson Lab\Desktop\PandeyLab\Raja\An.stephensi\Elite_Testis_bRP\C\An_steph_Tests_bRP\11.raw   #7592   RT: 41.92
FTMS, HCD@32.00, z=+2, Mono m/z=417.7265 Da, M+2=834.4457 Da, Match Tol.=0.05 Da

**Validated**

Extracted from: F:\Raja\IOB\An.Stephensi\SyntheticPeptides\An_Stephensi_SyntheticPeptide_Pool4.raw   #3198   RT: 41.47
FTMS, HCD@32.00, z=+2, Mono m/z=417.7262 Da, M+2=834.4451 Da, Match Tol.=0.05 Da

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**Intense**

- *y*⁺
- *b*⁺
- *y*⁺
- *b*⁺
- *y*⁺
- *y*⁺
- *y*⁺
- *y*⁺
- *y*⁺
- *y*⁺
- *y*⁺
ALLNLPK

Experiment

Validated
DYELESR

Experiment

Validated
