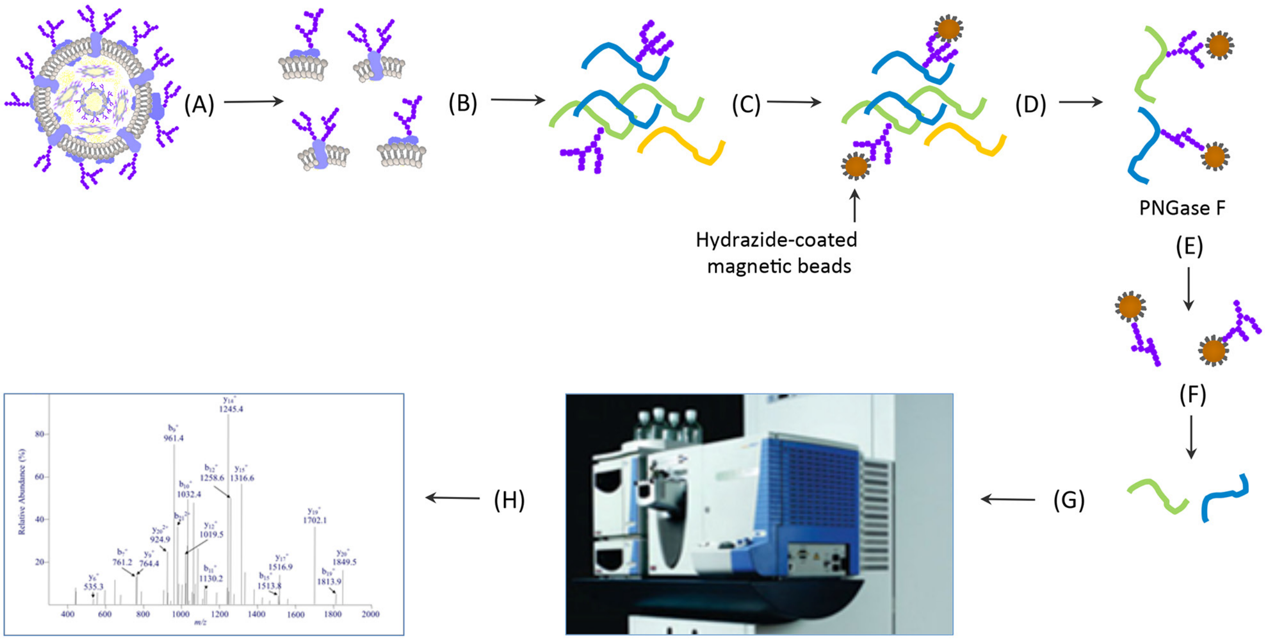
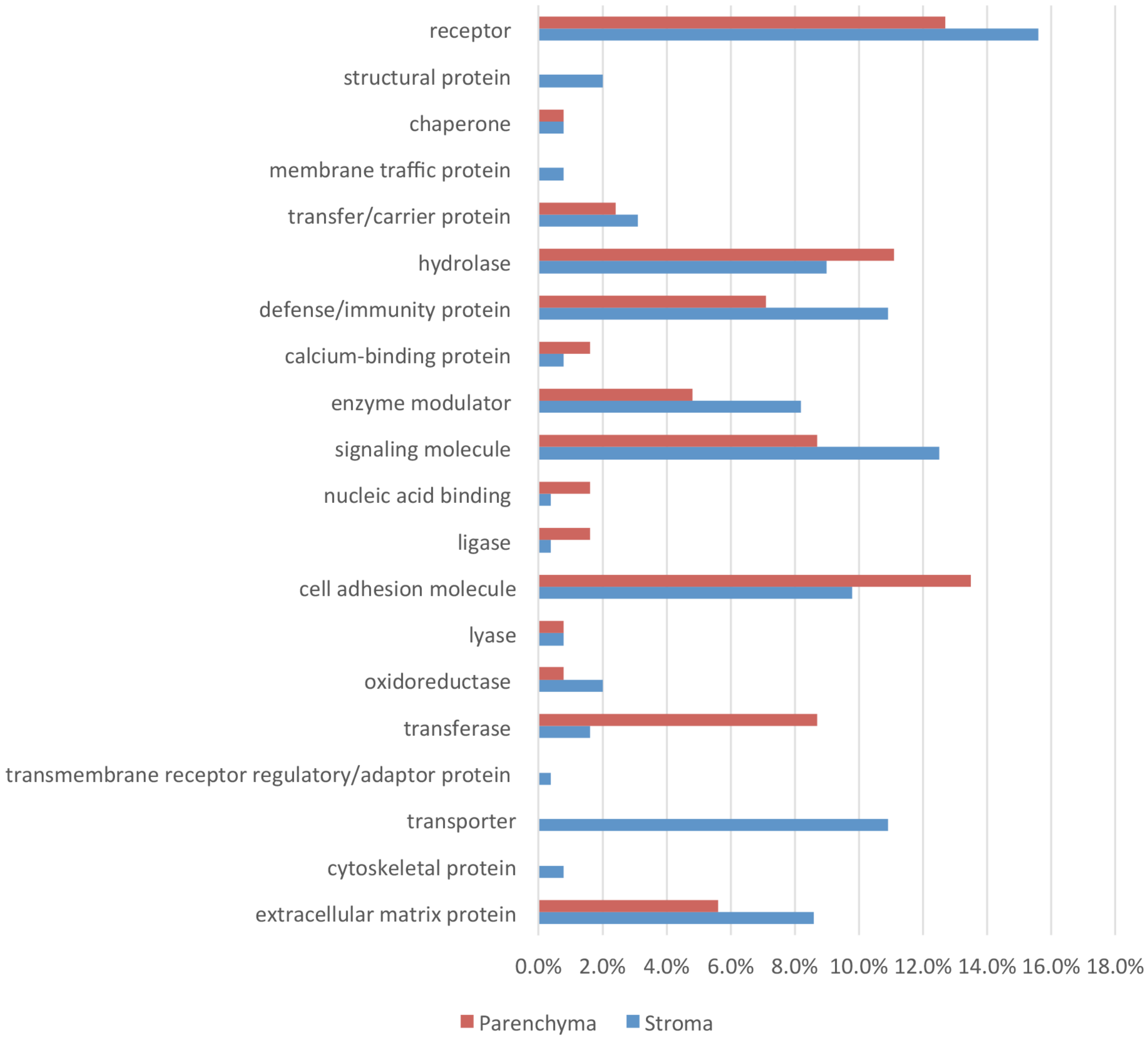
Direct molecular dissection of tumor parenchyma from tumor stroma in tumor xenograft using mass spectrometry-based glycoproteomics

SUPPLEMENTARY MATERIALS

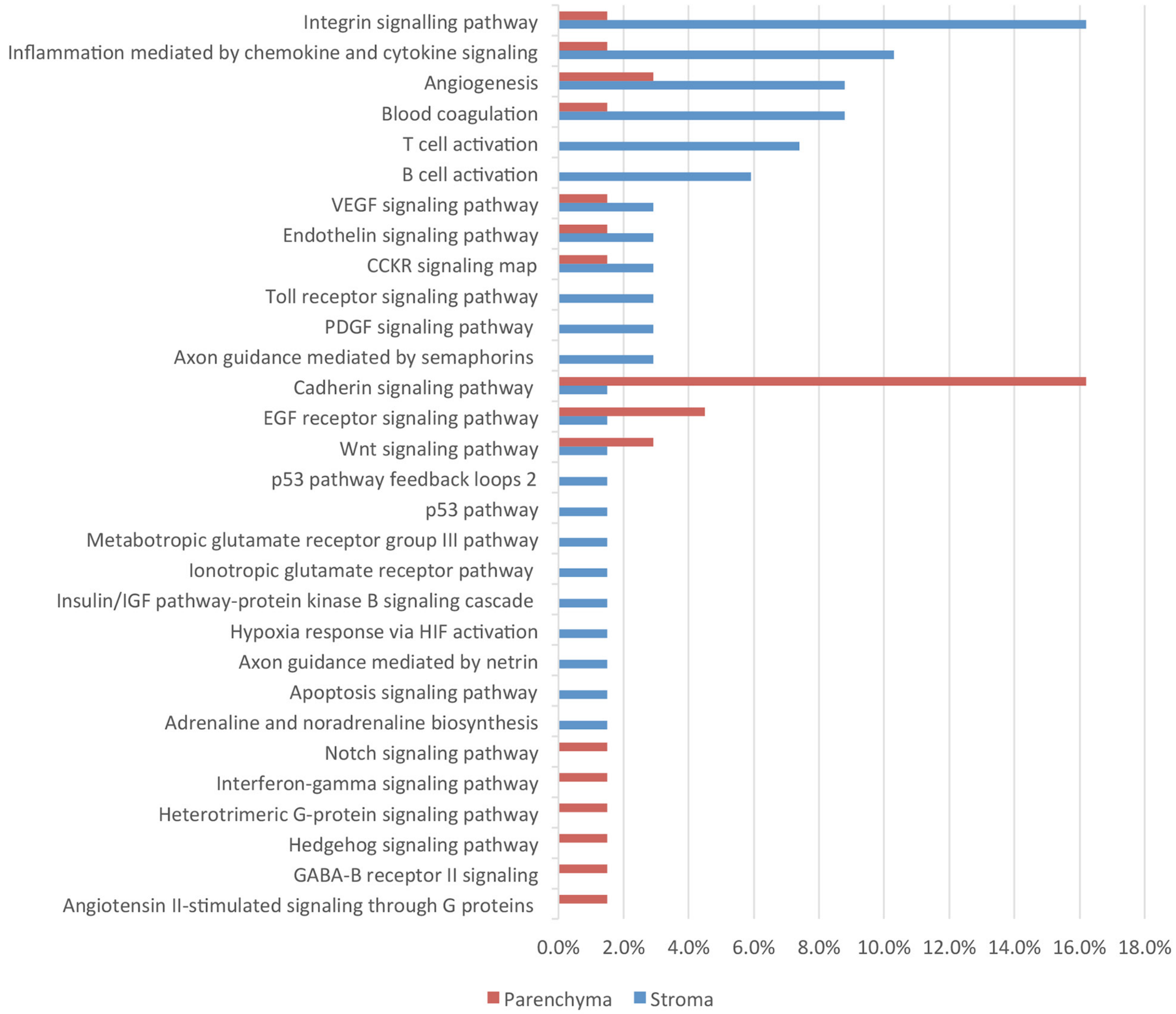


Supplementary Figure 1: Glycoproteomic workflow. (A) Tissue homogenization. **(B)** Tryptic digestion. **(C)** Oxidation and hydrazide-beads conjugation. **(D)** non-glycopeptide removal. **(E)** PNGase F based glycan removal. **(F)** N-glycopeptides collection. **(G)** LC-MS analysis. **(H)** Protein identification.

Supplementary Figure 2: Distribution of N-glycoproteins proteins identified by at least one protein and species specific N-glycopeptide.

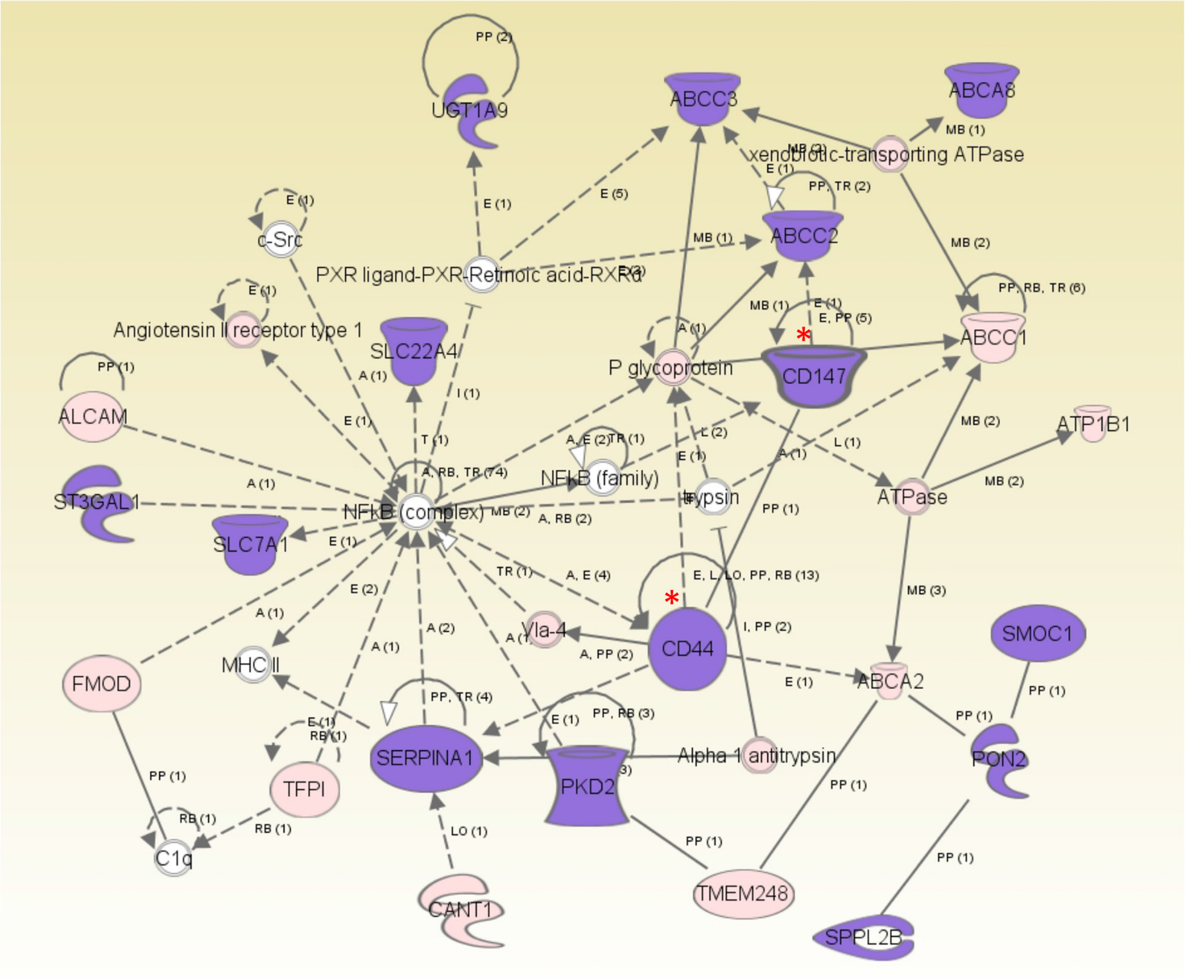


Supplementary Figure 3: Protein class analysis using the PANTHER software.

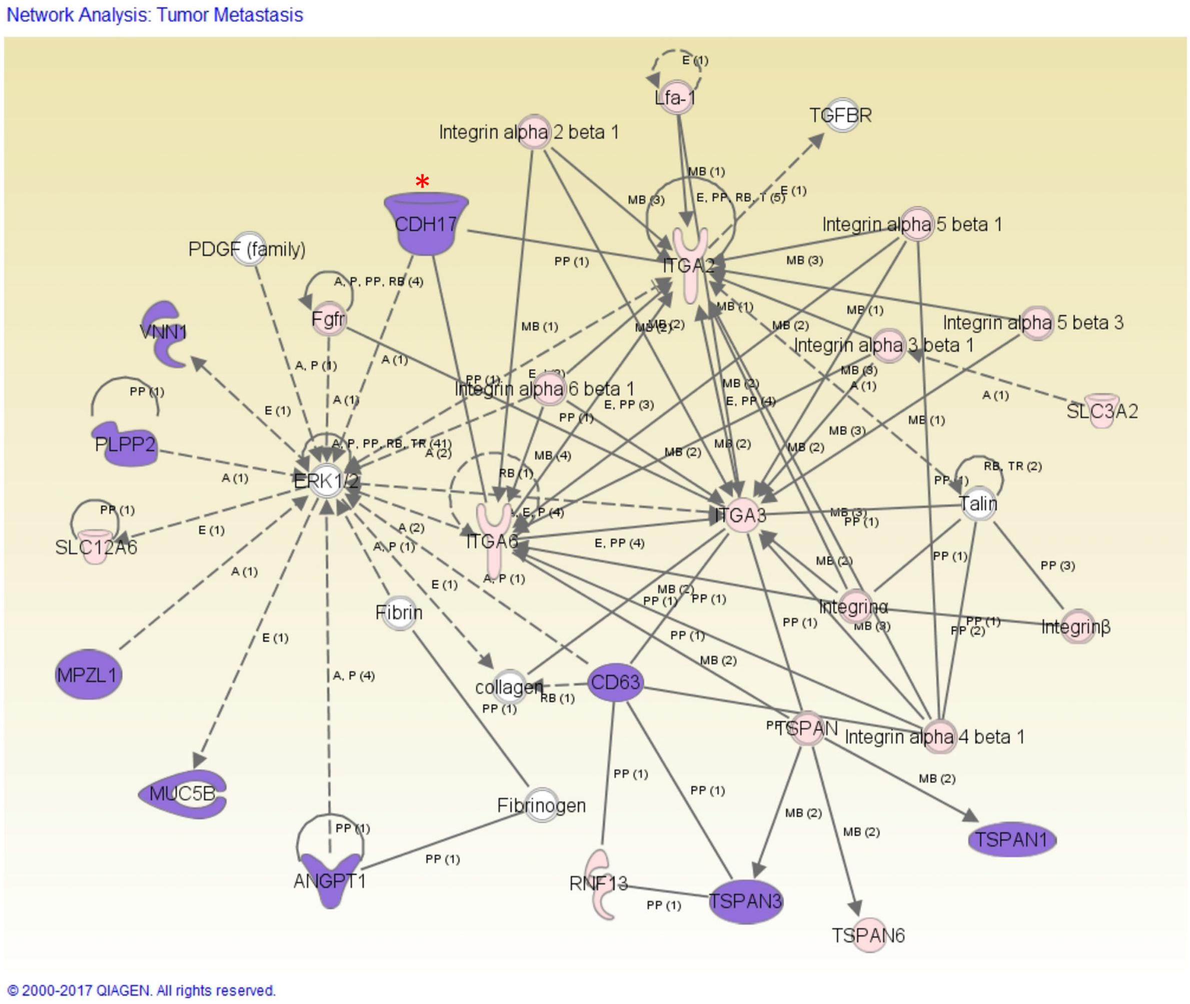


Supplementary Figure 4: Pathway analysis using the PANTHER software.

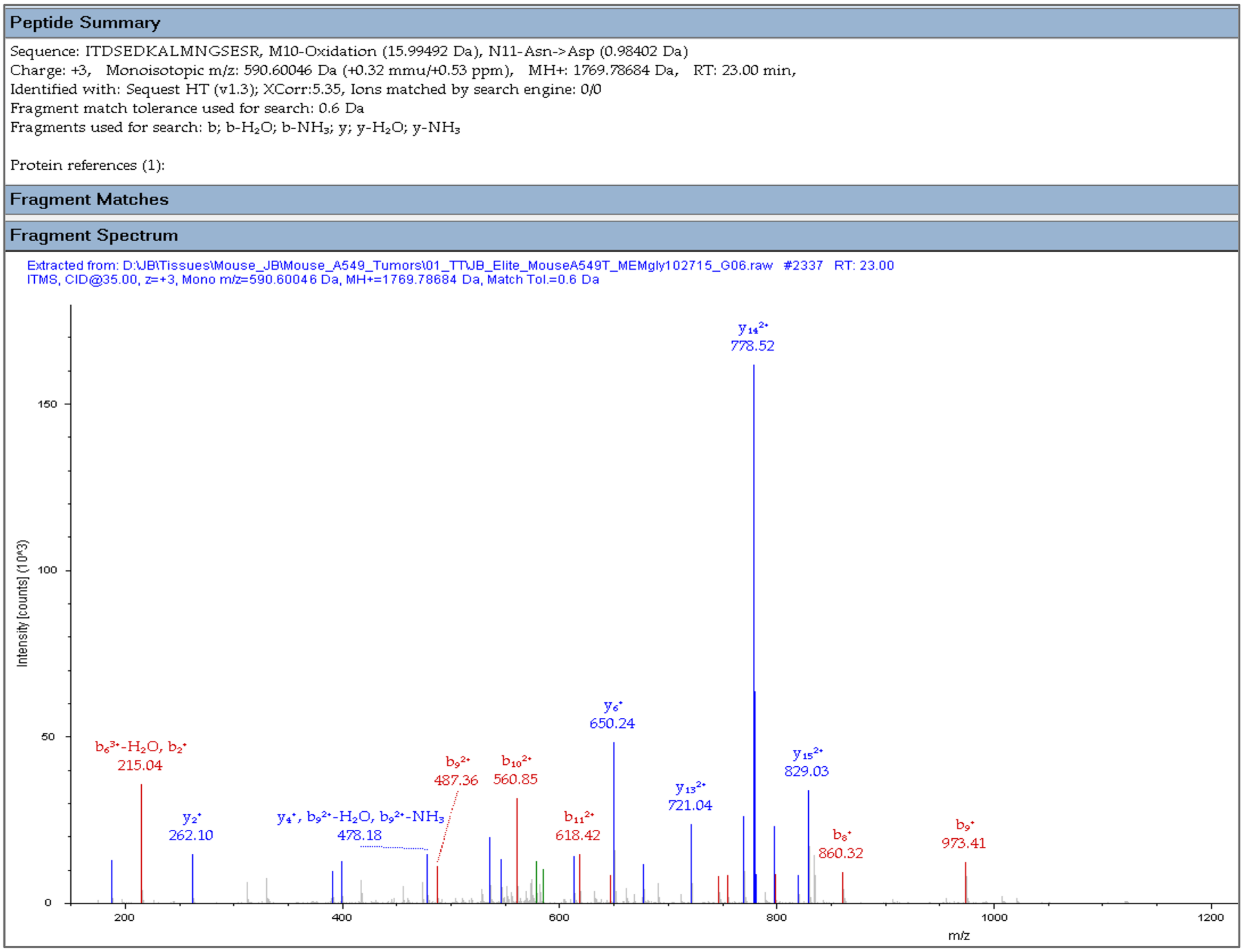
Network Analysis: Malignant Tumor Invasion



Supplementary Figure 5: IPA® analysis reveals malignant tumor invasion as one of top N-glycosylated protein networks activated in tumor parenchyma. Molecules selected for orthogonal cross-validation (i.e., CD147 and CD44) are marked with red asterisk.



Supplementary Figure 6: IPA® analysis reveals tumor metastasis as one of top N-glycosylated protein networks activated in tumor parenchyma. Molecule selected for orthogonal cross-validation (i.e., CDH17) is marked with red asterisk.



Peptide Summary

Sequence: ITDSEDKALMNGSESR, M10-Oxidation (15.99492 Da), N11-Asn->Asp (0.98402 Da)
Charge: +3, Monoisotopic m/z: 590.60046 Da (+0.32 mmu/+0.53 ppm), MH+: 1769.78684 Da, RT: 23.00 min,
Identified with: Sequest HT (v1.3); XCorr:5.35, Ions matched by search engine: 0/0
Fragment match tolerance used for search: 0.6 Da
Fragments used for search: b; b-H_2O; b-NH_3; y; y-H_2O; y-NH_3

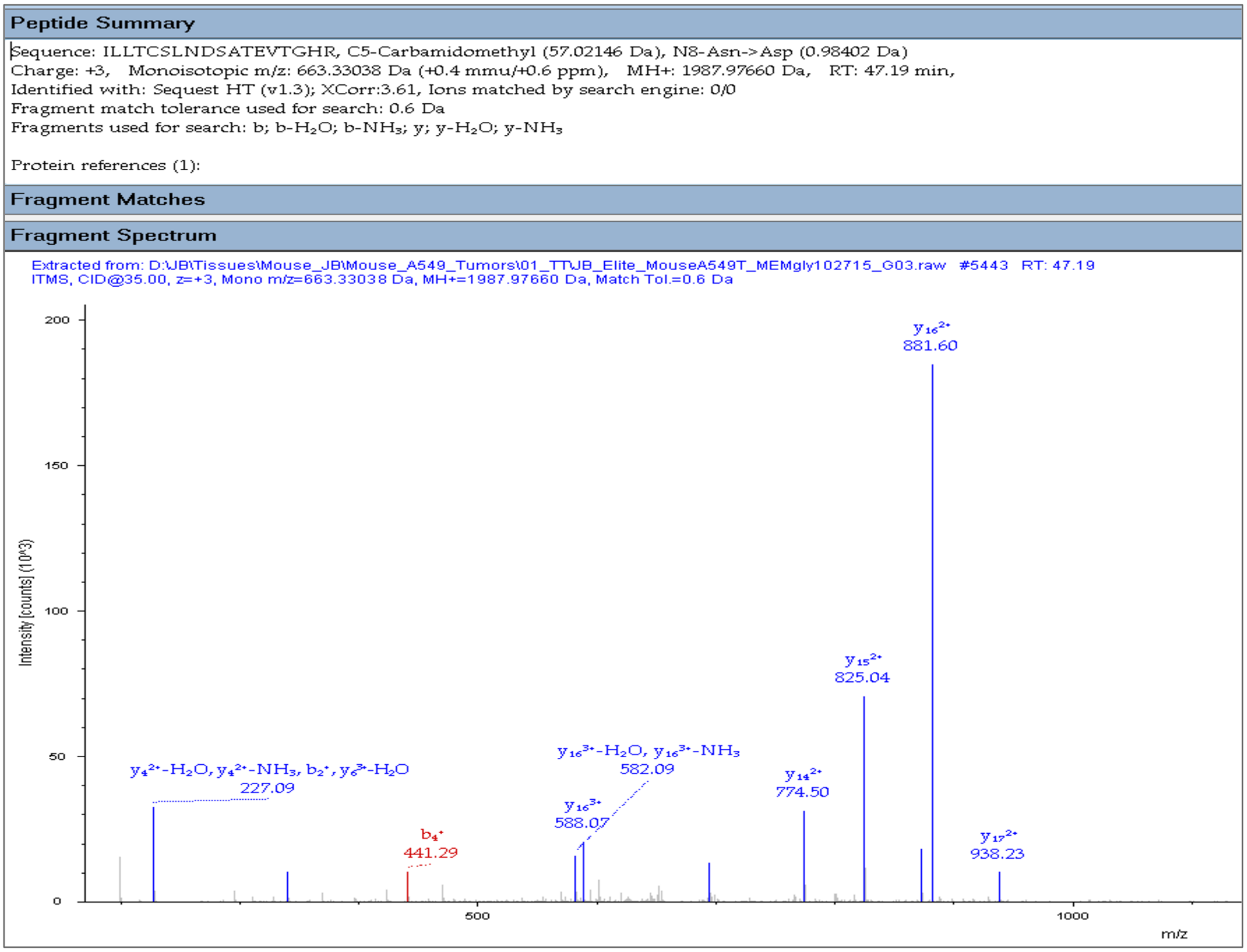
Protein references (1):

Fragment Matches

Fragment Spectrum

Extracted from: D:\JB\Tissues\Mouse_JB\Mouse_A549_Tumors\01_TT\JB_Elite_MouseA549T_MEMgly102715_G06.raw #2337 RT: 23.00
ITMS, CID@35.00, z=+3, Mono m/z=590.60046 Da, MH+=1769.78684 Da, Match Tol.=0.6 Da

Supplementary Figure 7: MS^2 spectrum depicting unambiguous identification of the human-parenchymal CD147 ortholog in tumor tissue by detection of protein- and species-specific N-glycopeptide.



Peptide Summary

Sequence: ILLTCSLNDSATEVTGHR, C5-Carbamidomethyl (57.02146 Da), N8-Asn->Asp (0.98402 Da)
Charge: +3, Monoisotopic m/z: 663.33038 Da (+0.4 mmu/+0.6 ppm), MH+: 1987.97660 Da, RT: 47.19 min,
Identified with: Sequest HT (v1.3); XCorr:3.61, Ions matched by search engine: 0/0
Fragment match tolerance used for search: 0.6 Da
Fragments used for search: b; b-H_2O; b-NH_3; y; y-H_2O; y-NH_3

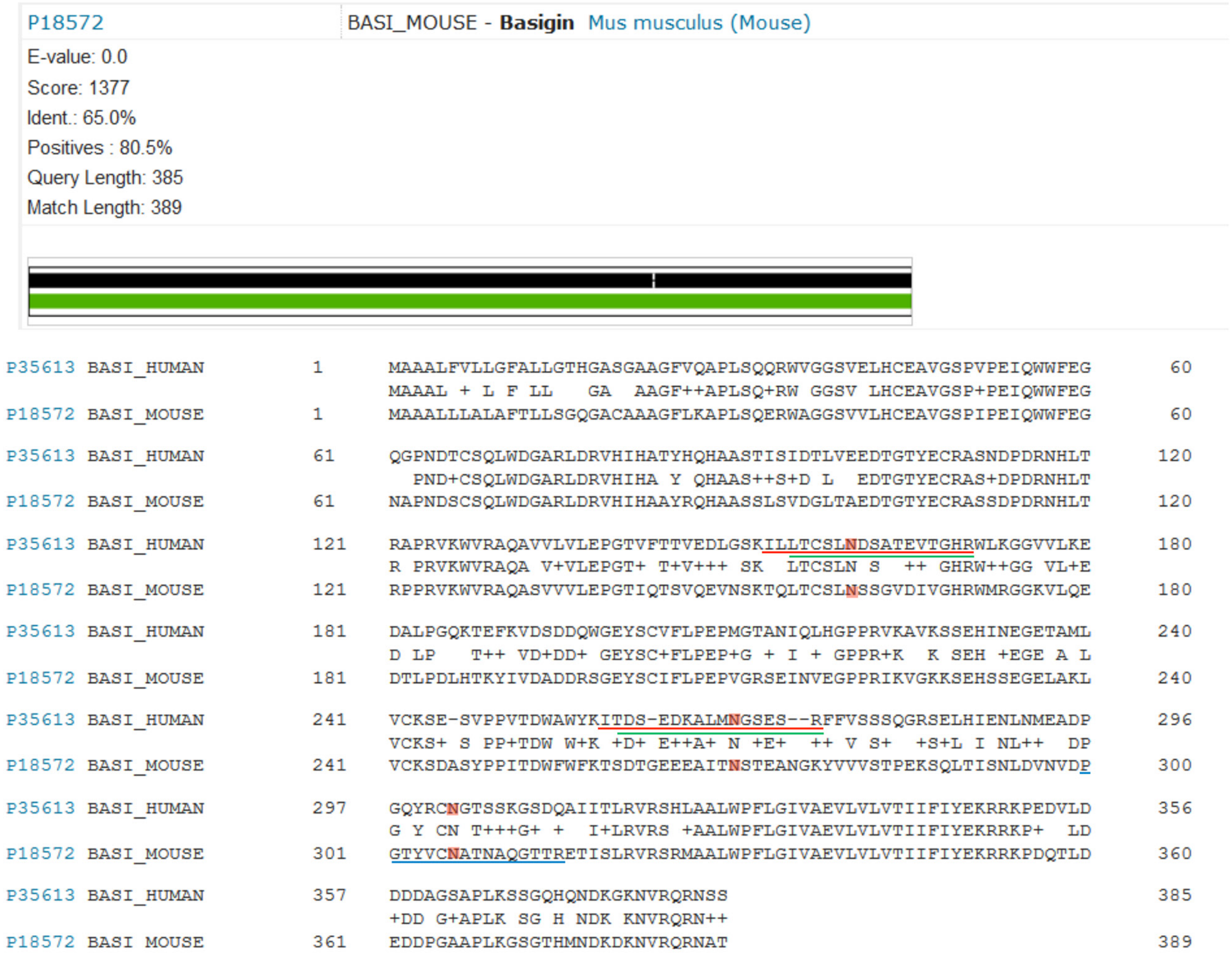
Protein references (1):

Fragment Matches

Fragment Spectrum

Extracted from: D:\JB\Tissues\Mouse_JB\Mouse_A549_Tumors\01_TTJB_Elite_MouseA549T_MEMgly102715_G03.raw #5443 RT: 47.19
ITMS, CID@35.00, z=+3, Mono m/z=663.33038 Da, MH+=1987.97660 Da, Match Tol.=0.6 Da

Supplementary Figure 8: MS^2 spectrum depicting unambiguous identification of the human-parenchymal CD147 ortholog in tumor tissue by detection of protein- and species-specific N-glycopeptide.



P18572 BASI_MOUSE - **Basigin** Mus musculus (Mouse)

E-value: 0.0
Score: 1377
Ident.: 65.0%
Positives : 80.5%
Query Length: 385
Match Length: 389

```
P35613 BASI_HUMAN       1      MAAALFVLLGFALLGTHGASGAAGFVQAPLSQQRWVGGSVELHCEAVGSPVPEIQWWFEG     60
                               MAAAL + L F LL   GA  AAGF++APLSQ+RW GGSV LHCEAVGSP+PEIQWWFEG
P18572 BASI_MOUSE       1      MAAALLLALAFTLLSGQGACAAAGFLKAPLSQERWAGGSVVLHCEAVGSPIPEIQWWFEG     60

P35613 BASI_HUMAN       61     QGPNDTCSQLWDGARLDRVHIHATYHQHAASTISIDTLVEEDTGTYECRASNDPDRNHLT     120
                                 PND+CSQLWDGARLDRVHIHA Y QHAAS++S+D L  EDTGTYECRAS+DPDRNHLT
P18572 BASI_MOUSE       61     NAPNDSCSQLWDGARLDRVHIHAAYRQHAASSLSVDGLTAEDTGTYECRASSDPDRNHLT     120

P35613 BASI_HUMAN       121    RAPRVKWVRAQAVVLVLEPGTVFTTVEDLGSKILLTCSLNDSATEVTGHRWLKGGVVLKE     180
                               R PRVKWVRAQA V+VLEPGT+ T+V+++ SK  LTCSLN S  ++ GHRW++GG VL+E
P18572 BASI_MOUSE       121    RPPRVKWVRAQASVVVLEPGTIQTSVQEVNSKTQLTCSLNSSGVDIVGHRWMRGGKVLQE     180

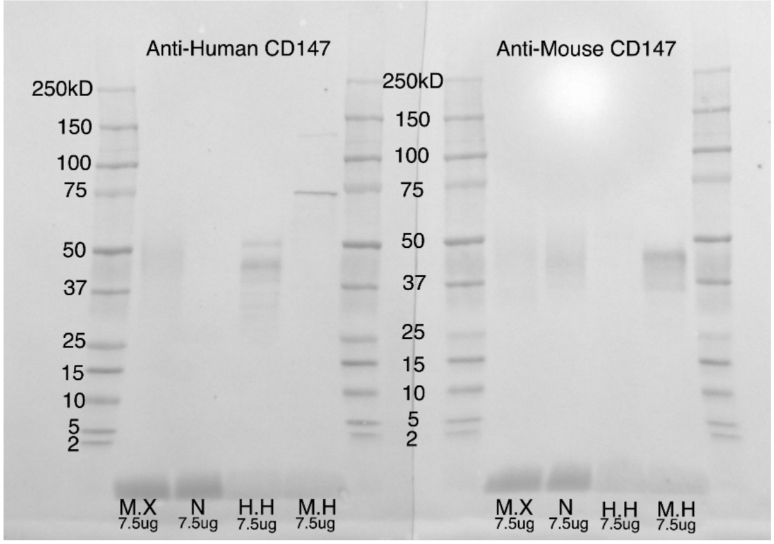
P35613 BASI_HUMAN       181    DALPGQKTEFKVDSDDQWGEYSCVFLPEPMGTANIQLHGPPRVKAVKSSEHINEGETAML     240
                               D LP   T++ VD+DD+ GEYSC+FLPEP+G + I + GPPR+K  K SEH +EGE A L
P18572 BASI_MOUSE       181    DTLPDLHTKYIVDADDRSGEYSCIFLPEPVGRSEINVEGPPRIKVGKKSEHSSEGELAKL     240

P35613 BASI_HUMAN       241    VCKSE-SVPPVTDWAWYKITDS-EDKALMNGSES--RFFVSSSQGRSELHIENLNMEADP     296
                               VCKS+ S PP+TDW W+K +D+ E++A+ N +E+  ++ V S+  +S+L I NL++  DP
P18572 BASI_MOUSE       241    VCKSDASYPPITDWFWFKTSDTGEEEAITNSTEANGKYVVVSTPEKSQLTISNLDVNVDP     300

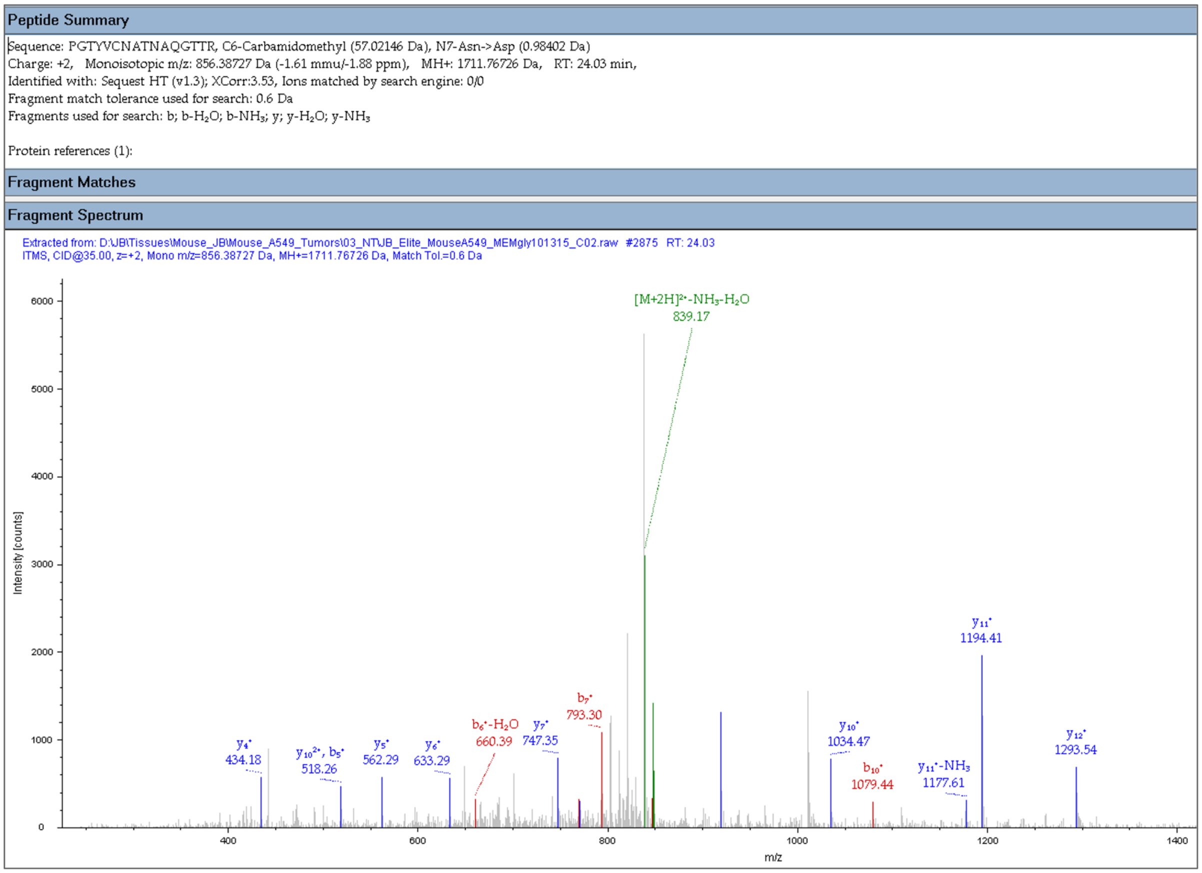
P35613 BASI_HUMAN       297    GQYRCNGTSSKGSDQAIITLRVRSHLAALWPFLGIVAEVLVLVTIIFIYEKRRKPEDVLD     356
                               G Y CN T+++G+ +  I+LRVRS +AALWPFLGIVAEVLVLVTIIFIYEKRRKP+  LD
P18572 BASI_MOUSE       301    GTYVCNATNAQGTTRETISLRVRSRMAALWPFLGIVAEVLVLVTIIFIYEKRRKPDQTLD     360

P35613 BASI_HUMAN       357    DDDAGSAPLKSSGQHQNDKGKNVRQRNSS                                    385
                               +DD G+APLK SG H NDK KNVRQRN++
P18572 BASI_MOUSE       361    EDDPGAAPLKGSGTHMNDKDKNVRQRNAT                                    389
```

Supplementary Figure 9: BLAST analysis confirming protein and species specificity of identified N-glycopeptides from CD147 orthologs. Underlined in red are human-specific peptides identified in tumor parenchyma. Underlined in green are human-specific peptides identified in 2D-grown A549 cells. Underlined in blue is mouse-specific N-glycopeptide identified in normal mouse lungs. NxST motif is highlighted in red.



Supplementary Figure 10: WB analysis of tumor tissue homogenate. Using anti-human CD147 antibody the WB analysis verified expression of human-derived CD147 in tumor homogenate (MX) and human hart (HH) homogenate (i.e., positive control). The WB analysis using the same antibody was negative in normal (N) mouse lungs and mouse hart (MH) used as negative control.


Peptide Summary

Sequence: PGTYVCNATNAQGTTR, C6-Carbamidomethyl (57.02146 Da), N7-Asn->Asp (0.98402 Da)
Charge: +2, Monoisotopic m/z: 856.38727 Da (-1.61 mmu/-1.88 ppm), MH+: 1711.76726 Da, RT: 24.03 min,
Identified with: Sequest HT (v1.3); XCorr:3.53, Ions matched by search engine: 0/0
Fragment match tolerance used for search: 0.6 Da
Fragments used for search: b; b-H_2O; b-NH_3; y; y-H_2O; y-NH_3

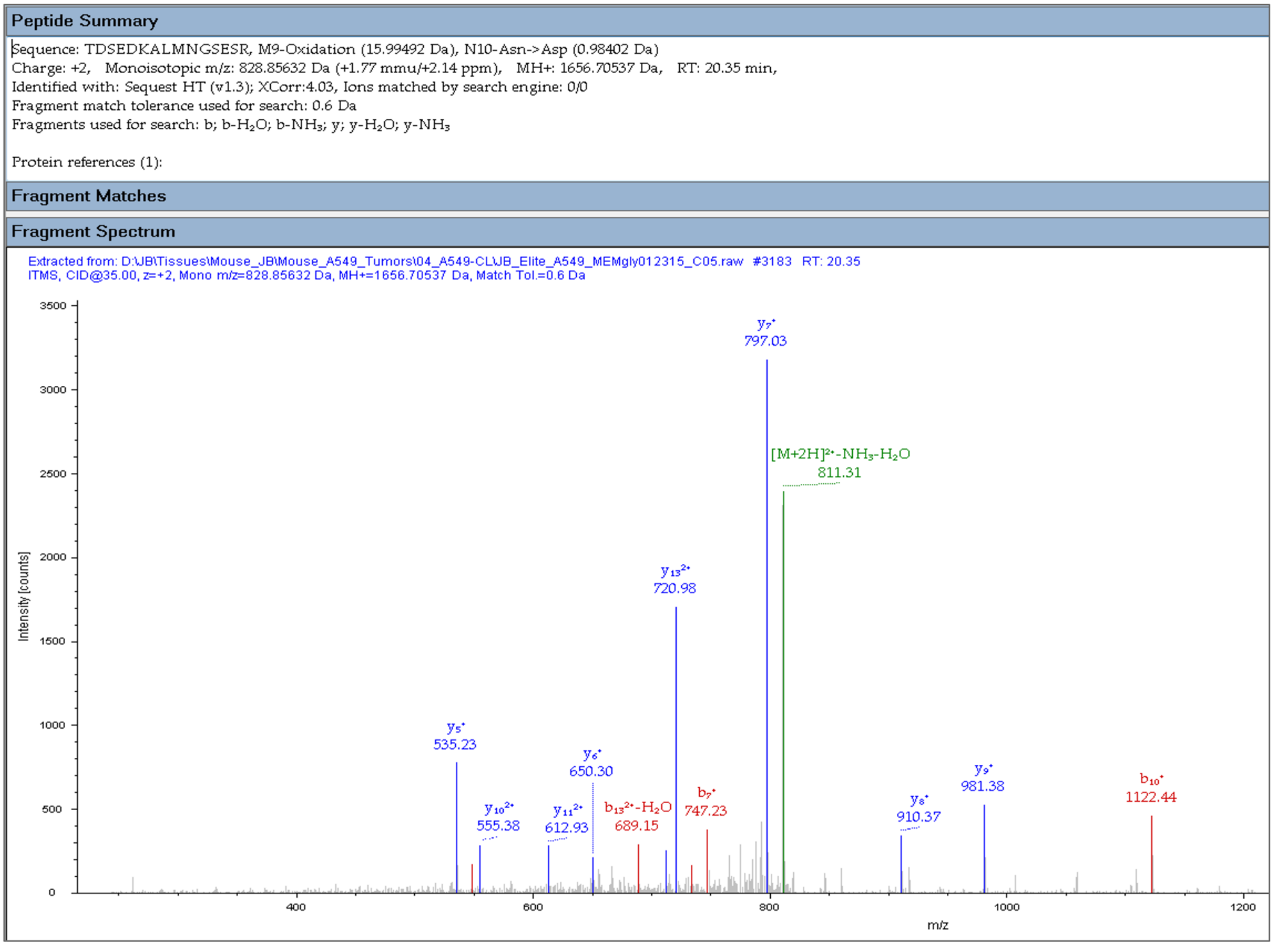
Protein references (1):

Fragment Matches

Fragment Spectrum

Extracted from: D:\JB\Tissues\Mouse_JB\Mouse_A549_Tumors\03_NTJB_Elite_MouseA549_MEMgly101315_C02.raw #2875 RT: 24.03
ITMS, CID@35.00, z=+2, Mono m/z=856.38727 Da, MH+=1711.76726 Da, Match Tol.=0.6 Da

Supplementary Figure 11: MS^2 spectrum depicting unambiguous identification of the mouse CD147 ortholog in normal lungs by detection of protein- and species-specific N-glycopeptide.



Peptide Summary

Sequence: TDSEDKALMNGSESR, M9-Oxidation (15.99492 Da), N10-Asn->Asp (0.98402 Da)
Charge: +2, Monoisotopic m/z: 828.85632 Da (+1.77 mmu/+2.14 ppm), MH+: 1656.70537 Da, RT: 20.35 min,
Identified with: Sequest HT (v1.3); XCorr:4.03, Ions matched by search engine: 0/0
Fragment match tolerance used for search: 0.6 Da
Fragments used for search: b; b-H_2O; b-NH_3; y; y-H_2O; y-NH_3

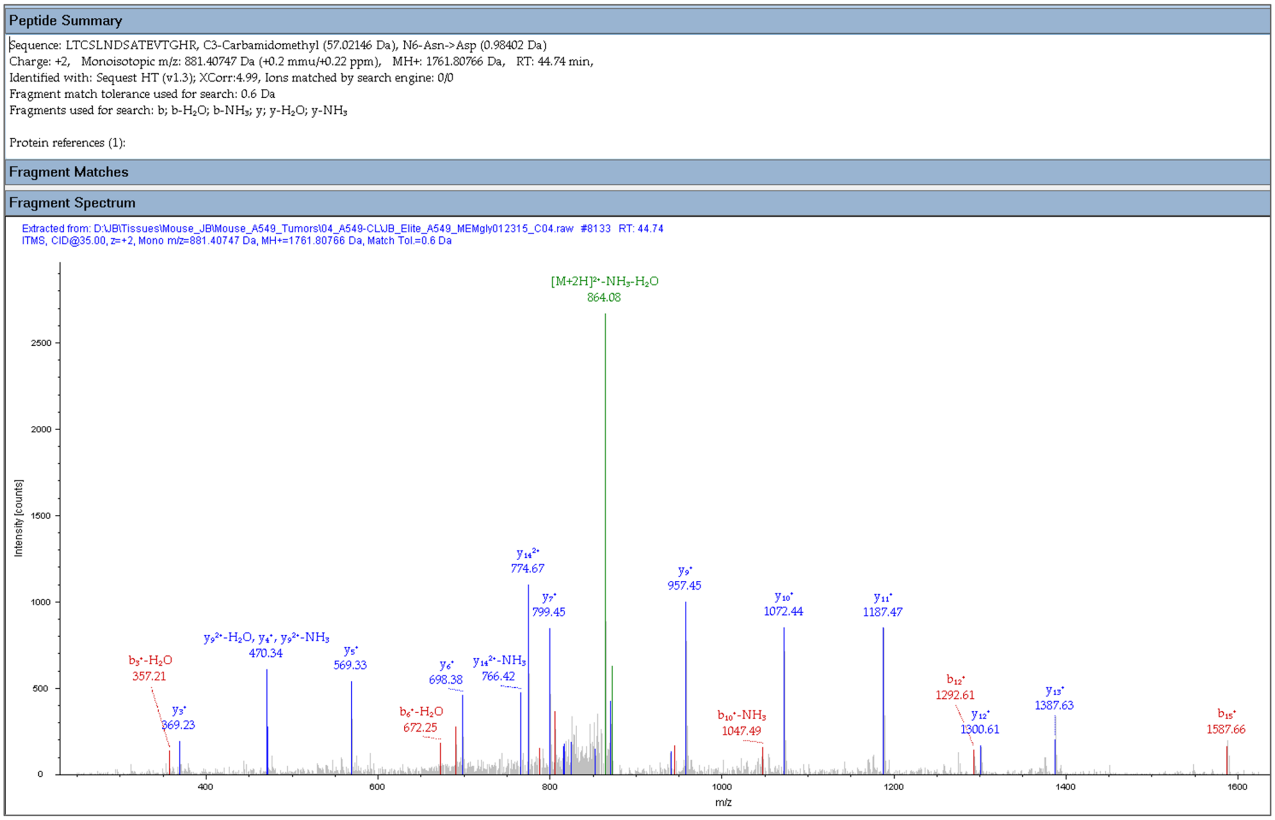
Protein references (1):

Fragment Matches

Fragment Spectrum

Extracted from: D:\JB\Tissues\Mouse_JB\Mouse_A549_Tumors\04_A549-CL\JB_Elite_A549_MEMgly012315_C05.raw #3183 RT: 20.35
ITMS, CID@35.00, z=+2, Mono m/z=828.85632 Da, MH+=1656.70537 Da, Match Tol.=0.6 Da

Supplementary Figure 12: MS^2 spectrum depicting unambiguous identification of the human CD147 ortholog in 2D-grown A549 cells by detection of protein- and species-specific N-glycopeptide.



Peptide Summary

Sequence: LTCSLNDSATEVTGHR, C3-Carbamidomethyl (57.02146 Da), N6-Asn->Asp (0.98402 Da)
Charge: +2, Monoisotopic m/z: 881.40747 Da (+0.2 mmu/+0.22 ppm), MH+: 1761.80766 Da, RT: 44.74 min,
Identified with: Sequest HT (v1.3); XCorr:4.99, Ions matched by search engine: 0/0
Fragment match tolerance used for search: 0.6 Da
Fragments used for search: b; b-H_2O; b-NH_3; y; y-H_2O; y-NH_3

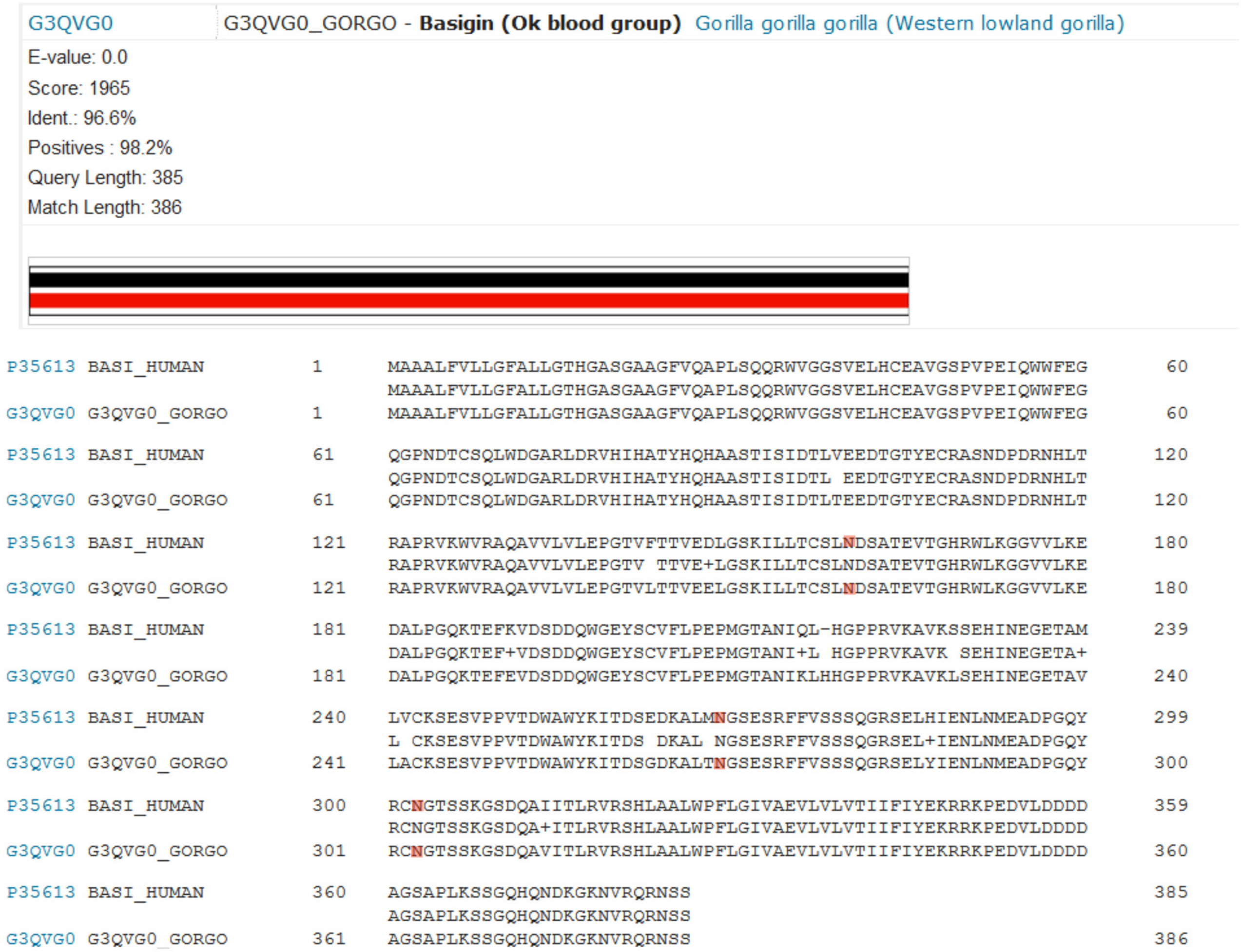
Protein references (1):

Fragment Matches

Fragment Spectrum

Extracted from: D:\JB\Tissues\Mouse_JB\Mouse_A549_Tumors\04_A549-CL\JB_Elite_A549_MEMgly012315_C04.raw #8133 RT: 44.74
ITMS, CID@35.00, z=+2, Mono m/z=881.40747 Da, MH+=1761.80766 Da, Match Tol.=0.6 Da

Supplementary Figure 13: MS^2 spectrum depicting unambiguous identification of the human CD147 ortholog in 2D-grown A549 cells by detection of protein- and species-specific N-glycopeptide.



G3QVG0 | G3QVG0_GORGO - **Basigin (Ok blood group)** Gorilla gorilla gorilla (Western lowland gorilla)

E-value: 0.0
Score: 1965
Ident.: 96.6%
Positives : 98.2%
Query Length: 385
Match Length: 386

```
P35613 BASI_HUMAN        1      MAAALFVLLGFALLGTHGASGAAGFVQAPLSQQRWVGGSVELHCEAVGSPVPEIQWWFEG      60
                                MAAALFVLLGFALLGTHGASGAAGFVQAPLSQQRWVGGSVELHCEAVGSPVPEIQWWFEG
G3QVG0 G3QVG0_GORGO      1      MAAALFVLLGFALLGTHGASGAAGFVQAPLSQQRWVGGSVELHCEAVGSPVPEIQWWFEG      60

P35613 BASI_HUMAN        61     QGPNDTCSQLWDGARLDRVHIHATYHQHAASTISIDTLVEEDTGTYECRASNDPDRNHLT      120
                                QGPNDTCSQLWDGARLDRVHIHATYHQHAASTISIDTL EEDTGTYECRASNDPDRNHLT
G3QVG0 G3QVG0_GORGO      61     QGPNDTCSQLWDGARLDRVHIHATYHQHAASTISIDTLTEEDTGTYECRASNDPDRNHLT      120

P35613 BASI_HUMAN        121    RAPRVKWVRAQAVVLVLEPGTVFTTVEDLGSKILLTCSLNDSATEVTGHRWLKGGVVLKE      180
                                RAPRVKWVRAQAVVLVLEPGTV TTVE+LGSKILLTCSLNDSATEVTGHRWLKGGVVLKE
G3QVG0 G3QVG0_GORGO      121    RAPRVKWVRAQAVVLVLEPGTVLTTVEELGSKILLTCSLNDSATEVTGHRWLKGGVVLKE      180

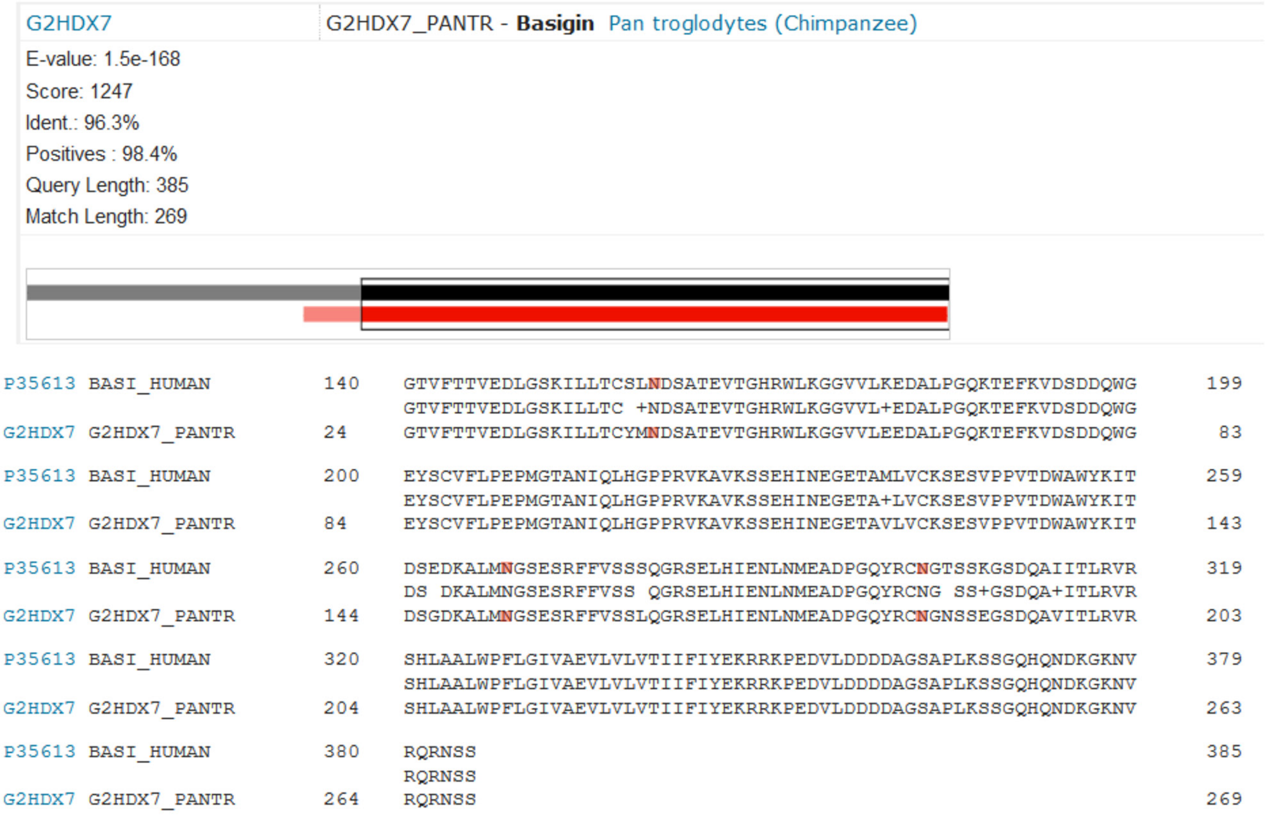
P35613 BASI_HUMAN        181    DALPGQKTEFKVDSDDQWGEYSCVFLPEPMGTANIQL-HGPPRVKAVKSSEHINEGETAM      239
                                DALPGQKTEF+VDSDDQWGEYSCVFLPEPMGTANI+L HGPPRVKAVK SEHINEGETA+
G3QVG0 G3QVG0_GORGO      181    DALPGQKTEFEVDSDDQWGEYSCVFLPEPMGTANIKLHHGPPRVKAVKLSEHINEGETAV      240

P35613 BASI_HUMAN        240    LVCKSESVPPVTDWAWYKITDSEDKALMNGSESRFFVSSSQGRSELHIENLNMEADPGQY      299
                                L CKSESVPPVTDWAWYKITDS DKAL NGSESRFFVSSSQGRSEL+IENLNMEADPGQY
G3QVG0 G3QVG0_GORGO      241    LACKSESVPPVTDWAWYKITDSGDKALTNGSESRFFVSSSQGRSELYIENLNMEADPGQY      300

P35613 BASI_HUMAN        300    RCNGTSSKGSDQAIITLRVRSHLAALWPFLGIVAEVLVLVTIIFIYEKRRKPEDVLDDDD      359
                                RCNGTSSKGSDQA+ITLRVRSHLAALWPFLGIVAEVLVLVTIIFIYEKRRKPEDVLDDDD
G3QVG0 G3QVG0_GORGO      301    RCNGTSSKGSDQAVITLRVRSHLAALWPFLGIVAEVLVLVTIIFIYEKRRKPEDVLDDDD      360

P35613 BASI_HUMAN        360    AGSAPLKSSGQHQNDKGKNVRQRNSS                                        385
                                AGSAPLKSSGQHQNDKGKNVRQRNSS
G3QVG0 G3QVG0_GORGO      361    AGSAPLKSSGQHQNDKGKNVRQRNSS                                        386
```

Supplementary Figure 14: BLAST analysis depicting comparison of human's and gorilla's CD147 orthologs. Highlighted in red are predicted N-glycosylated sites showing protein and species-specific AA sequence differences of MS-identifiable N-glycopeptides.



G2HDX7 | G2HDX7_PANTR - **Basigin** Pan troglodytes (Chimpanzee)

E-value: 1.5e-168
Score: 1247
Ident.: 96.3%
Positives : 98.4%
Query Length: 385
Match Length: 269

```
P35613 BASI_HUMAN        140    GTVFTTVEDLGSKILLTCSLNDSATEVTGHRWLKGGVVLKEDALPGQKTEFKVDSDDQWG      199
                                GTVFTTVEDLGSKILLTC +NDSATEVTGHRWLKGGVVL+EDALPGQKTEFKVDSDDQWG
G2HDX7 G2HDX7_PANTR      24     GTVFTTVEDLGSKILLTCYMNDSATEVTGHRWLKGGVVLEEDALPGQKTEFKVDSDDQWG       83

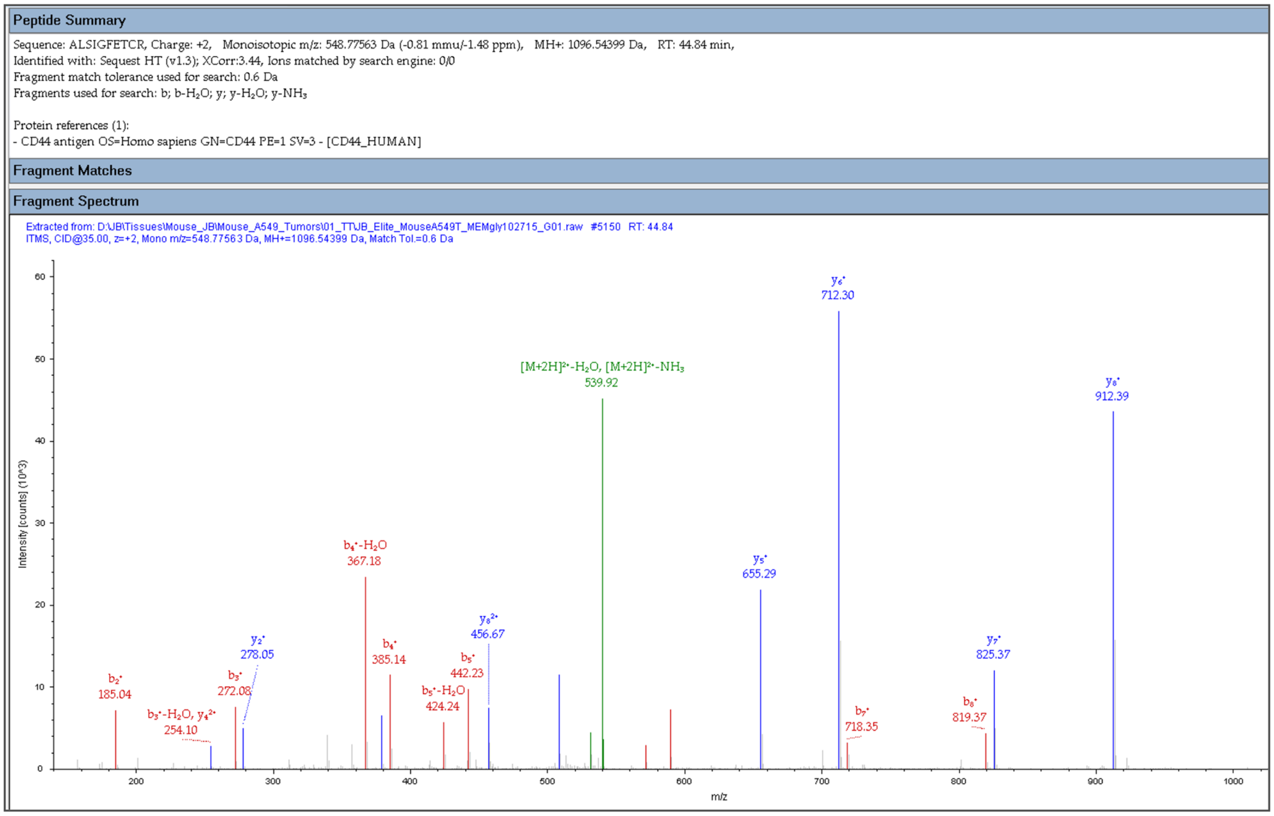
P35613 BASI_HUMAN        200    EYSCVFLPEPMGTANIQLHGPPRVKAVKSSEHINEGETAMLVCKSESVPPVTDWAWYKIT      259
                                EYSCVFLPEPMGTANIQLHGPPRVKAVKSSEHINEGETA+LVCKSESVPPVTDWAWYKIT
G2HDX7 G2HDX7_PANTR      84     EYSCVFLPEPMGTANIQLHGPPRVKAVKSSEHINEGETAVLVCKSESVPPVTDWAWYKIT      143

P35613 BASI_HUMAN        260    DSEDKALMNGSESRFFVSSSQGRSELHIENLNMEADPGQYRCNGTSSKGSDQAIITLRVR      319
                                DS DKALMNGSESRFFVSS QGRSELHIENLNMEADPGQYRCNG SS+GSDQA+ITLRVR
G2HDX7 G2HDX7_PANTR      144    DSGDKALMNGSESRFFVSSLQGRSELHIENLNMEADPGQYRCNGNSSEGSDQAVITLRVR      203

P35613 BASI_HUMAN        320    SHLAALWPFLGIVAEVLVLVTIIFIYEKRRKPEDVLDDDDAGSAPLKSSGQHQNDKGKNV      379
                                SHLAALWPFLGIVAEVLVLVTIIFIYEKRRKPEDVLDDDDAGSAPLKSSGQHQNDKGKNV
G2HDX7 G2HDX7_PANTR      204    SHLAALWPFLGIVAEVLVLVTIIFIYEKRRKPEDVLDDDDAGSAPLKSSGQHQNDKGKNV      263

P35613 BASI_HUMAN        380    RQRNSS                                                            385
                                RQRNSS
G2HDX7 G2HDX7_PANTR      264    RQRNSS                                                            269
```

Supplementary Figure 15: BLAST analysis depicting comparison of human's and chimpanzee's CD147 orthologs. Highlighted in red are predicted N-glycosylated sites showing protein and species-specific AA sequence differences of MS-identifiable N-glycopeptides.



Peptide Summary

Sequence: ALSIGFETCR, Charge: +2, Monoisotopic m/z: 548.77563 Da (-0.81 mmu/-1.48 ppm), MH+: 1096.54399 Da, RT: 44.84 min,
Identified with: Sequest HT (v1.3); XCorr:3.44, Ions matched by search engine: 0/0
Fragment match tolerance used for search: 0.6 Da
Fragments used for search: b; b-H_2O; y; y-H_2O; y-NH_3

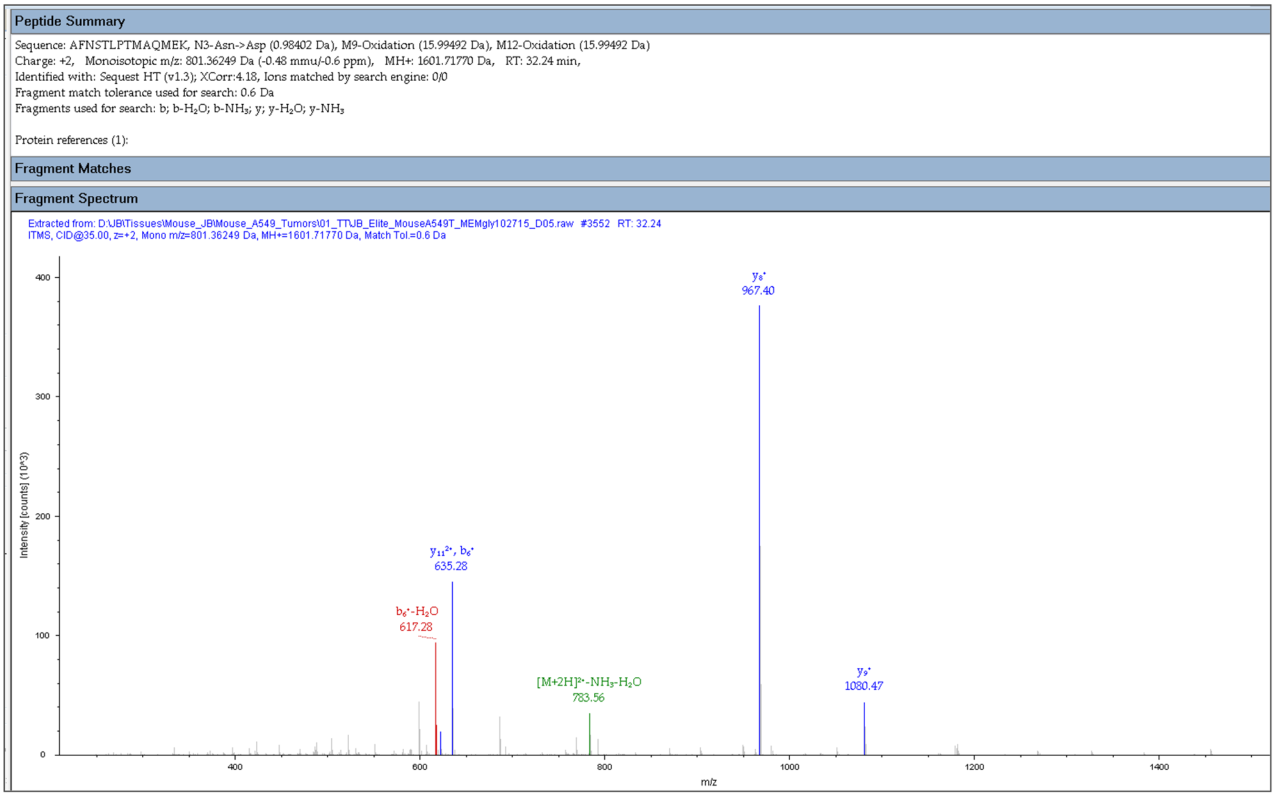
Protein references (1):
- CD44 antigen OS=Homo sapiens GN=CD44 PE=1 SV=3 - [CD44_HUMAN]

Fragment Matches

Fragment Spectrum

Extracted from: D:\JB\Tissues\Mouse_JB\Mouse_A549_Tumors\01_TT\JB_Elite_MouseA549T_MEMgly102715_G01.raw #5150 RT: 44.84
ITMS, CID@35.00, z=+2, Mono m/z=548.77563 Da, MH+=1096.54399 Da, Match Tol.=0.6 Da

Supplementary Figure 16: MS^2 spectrum depicting unambiguous identification of the human-derived CD44 ortholog in tumor parenchyma by detection of protein- and species-specific N-glycopeptide.



Peptide Summary

Sequence: AFNSTLPTMAQMEK, N3-Asn->Asp (0.98402 Da), M9-Oxidation (15.99492 Da), M12-Oxidation (15.99492 Da)
Charge: +2, Monoisotopic m/z: 801.36249 Da (-0.48 mmu/-0.6 ppm), MH+: 1601.71770 Da, RT: 32.24 min,
Identified with: Sequest HT (v1.3); XCorr:4.18, Ions matched by search engine: 0/0
Fragment match tolerance used for search: 0.6 Da
Fragments used for search: b; b-H_2O; b-NH_3; y; y-H_2O; y-NH_3

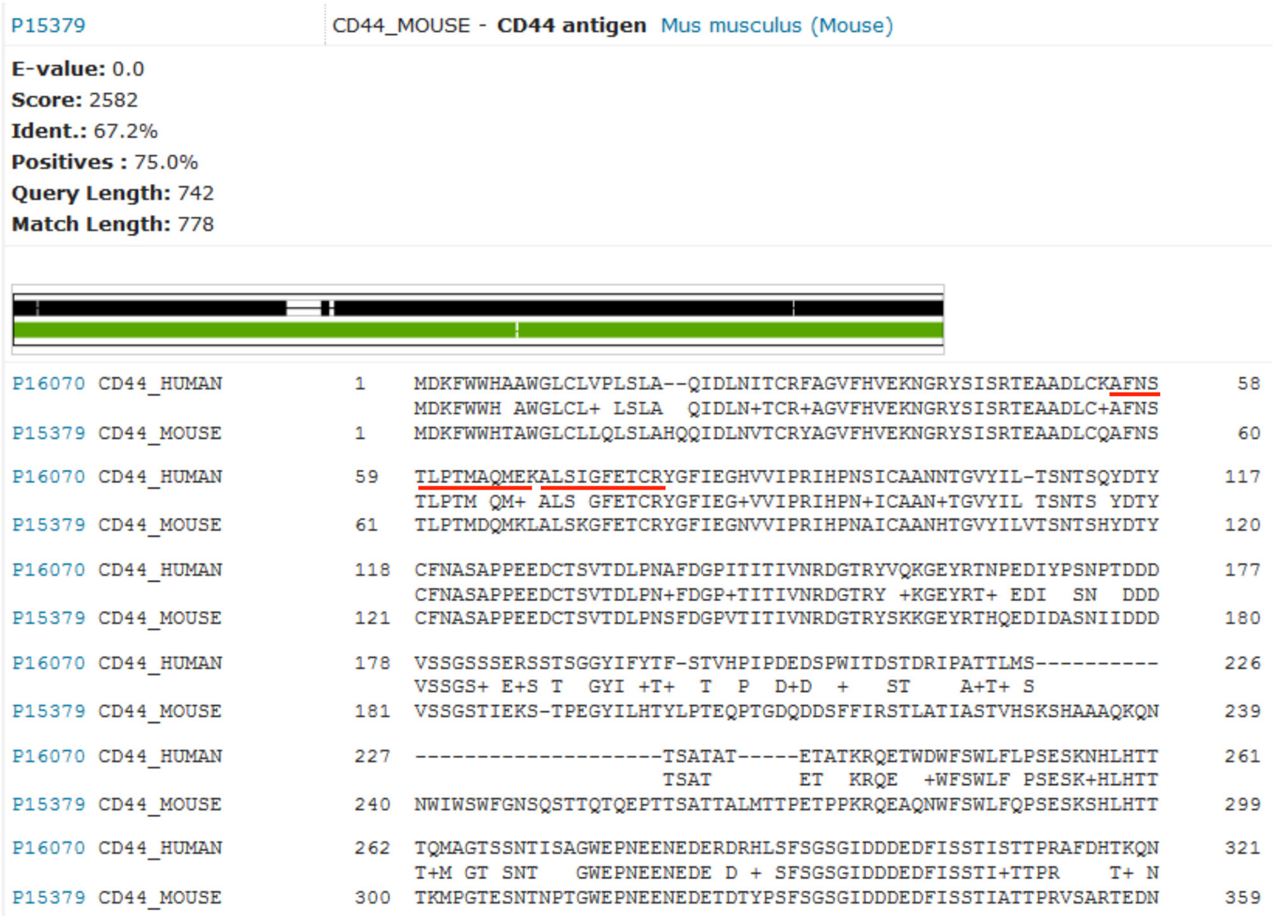
Protein references (1):

Fragment Matches

Fragment Spectrum

Extracted from: D:\JB\Tissues\Mouse_JB\Mouse_A549_Tumors\01_TTJB_Elite_MouseA549T_MEMgly102715_D05.raw #3552 RT: 32.24
ITMS, CID@35.00, z=+2, Mono m/z=801.36249 Da, MH+=1601.71770 Da, Match Tol.=0.6 Da

Supplementary Figure 17: MS^2 spectrum depicting unambiguous identification of the human-derived CD44 ortholog in tumor parenchyma by detection of protein- and species-specific N-glycopeptide.



P15379 CD44_MOUSE - **CD44 antigen** Mus musculus (Mouse)

E-value: 0.0
Score: 2582
Ident.: 67.2%
Positives : 75.0%
Query Length: 742
Match Length: 778

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P16070 CD44_HUMAN      1    MDKFWWHAAWGLCLVPLSLA--QIDLNITCRFAGVFHVEKNGRYSISRTEAADLCKAFNS    58
                            MDKFWWH AWGLCL+ LSLA  QIDLN+TCR+AGVFHVEKNGRYSISRTEAADLC+AFNS
P15379 CD44_MOUSE      1    MDKFWWHTAWGLCLLQLSLAHQQIDLNVTCRYAGVFHVEKNGRYSISRTEAADLCQAFNS    60

P16070 CD44_HUMAN      59   TLPTMAQMEKALSIGFETCRYGFIEGHVVIPRIHPNSICAANNTGVYIL-TSNTSQYDTY   117
                            TLPTM QM+ ALS GFETCRYGFIEG+VVIPRIHPN+ICAAN+TGVYIL TSNTS YDTY
P15379 CD44_MOUSE      61   TLPTMDQMKLALSKGFETCRYGFIEGNVVIPRIHPNAICAANHTGVYILVTSNTSHYDTY   120

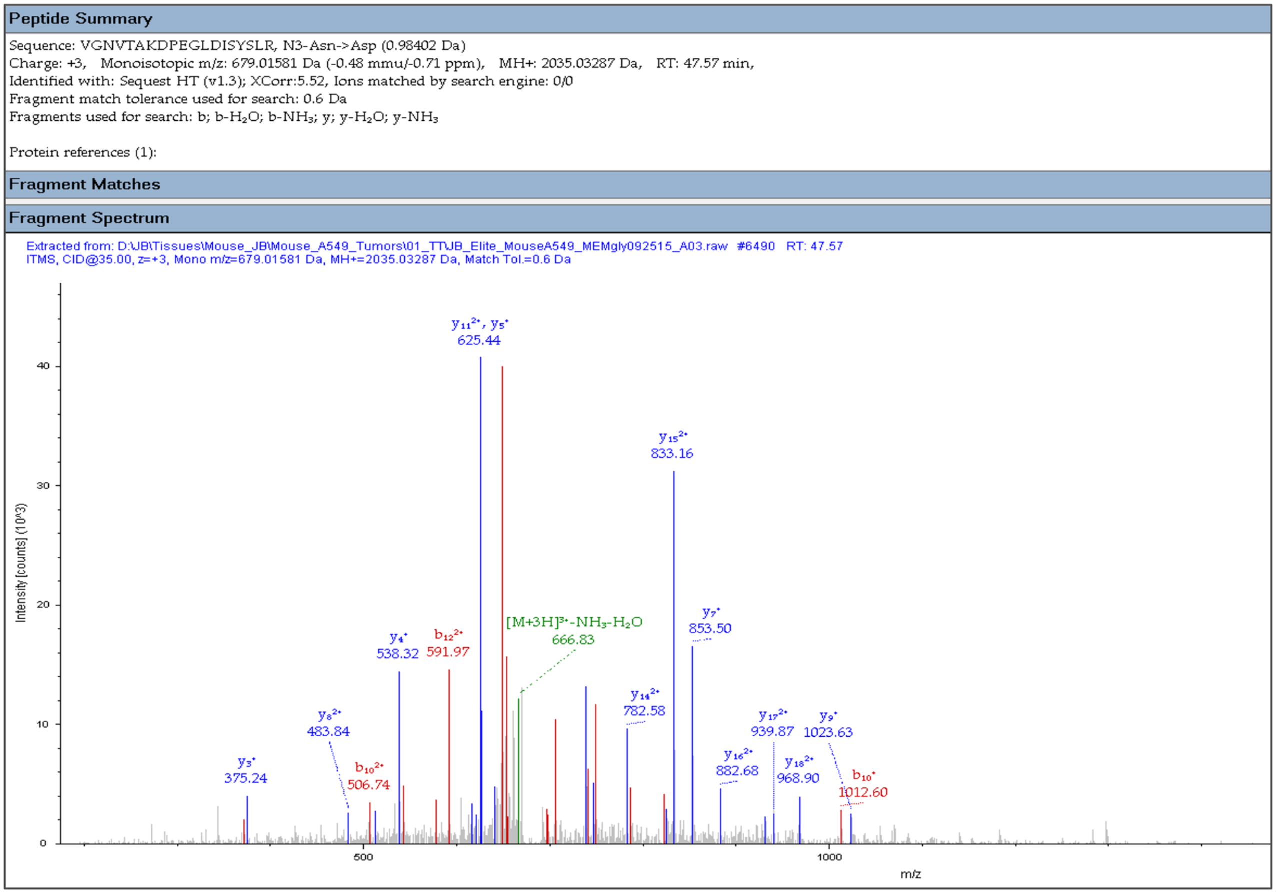
P16070 CD44_HUMAN      118  CFNASAPPEEDCTSVTDLPNAFDGPITITIVNRDGTRYVQKGEYRTNPEDIYPSNPTDDD   177
                            CFNASAPPEEDCTSVTDLPN+FDGP+TITIVNRDGTRY +KGEYRT+ EDI  SN  DDD
P15379 CD44_MOUSE      121  CFNASAPPEEDCTSVTDLPNSFDGPVTITIVNRDGTRYSKKGEYRTHQEDIDASNIIDDD   180

P16070 CD44_HUMAN      178  VSSGSSSERSSTSGGYIFYTF-STVHPIPDEDSPWITDSTDRIPATTLMS----------   226
                            VSSGS+ E+S T  GYI +T+  T  P  D+D  +   ST    A+T+ S
P15379 CD44_MOUSE      181  VSSGSTIEKS-TPEGYILHTYLPTEQPTGDQDDSFFIRSTLATIASTVHSKSHAAAQKQN   239

P16070 CD44_HUMAN      227  --------------------TSATAT-----ETATKRQETWDWFSWLFLPSESKNHLHTT   261
                                                TSAT       ET  KRQE  +WFSWLF PSESK+HLHTT
P15379 CD44_MOUSE      240  NWIWSWFGNSQSTTQTQEPTTSATTALMTTPETPPKRQEAQNWFSWLFQPSESKSHLHTT   299

P16070 CD44_HUMAN      262  TQMAGTSSNTISAGWEPNEENEDERDRHLSFSGSGIDDDEDFISSTISTTPRAFDHTKQN   321
                            T+M GT SNT   GWEPNEENEDE D + SFSGSGIDDDEDFISSTI+TTPR    T+ N
P15379 CD44_MOUSE      300  TKMPGTESNTNPTGWEPNEENEDETDTYPSFSGSGIDDDEDFISSTIATTPRVSARTEDN   359
```

Supplementary Figure 18: BLAST analysis confirming protein and species specificity of identified N-glycopeptides from human-derived CD44. Underlined in red are protein and species-specific peptides identified solely in tumor parenchyma.



Peptide Summary

Sequence: VGNVTAKDPEGLDISYSLR, N3-Asn->Asp (0.98402 Da)
Charge: +3, Monoisotopic m/z: 679.01581 Da (-0.48 mmu/-0.71 ppm), MH+: 2035.03287 Da, RT: 47.57 min,
Identified with: Sequest HT (v1.3); XCorr:5.52, Ions matched by search engine: 0/0
Fragment match tolerance used for search: 0.6 Da
Fragments used for search: b; b-H_2O; b-NH_3; y; y-H_2O; y-NH_3

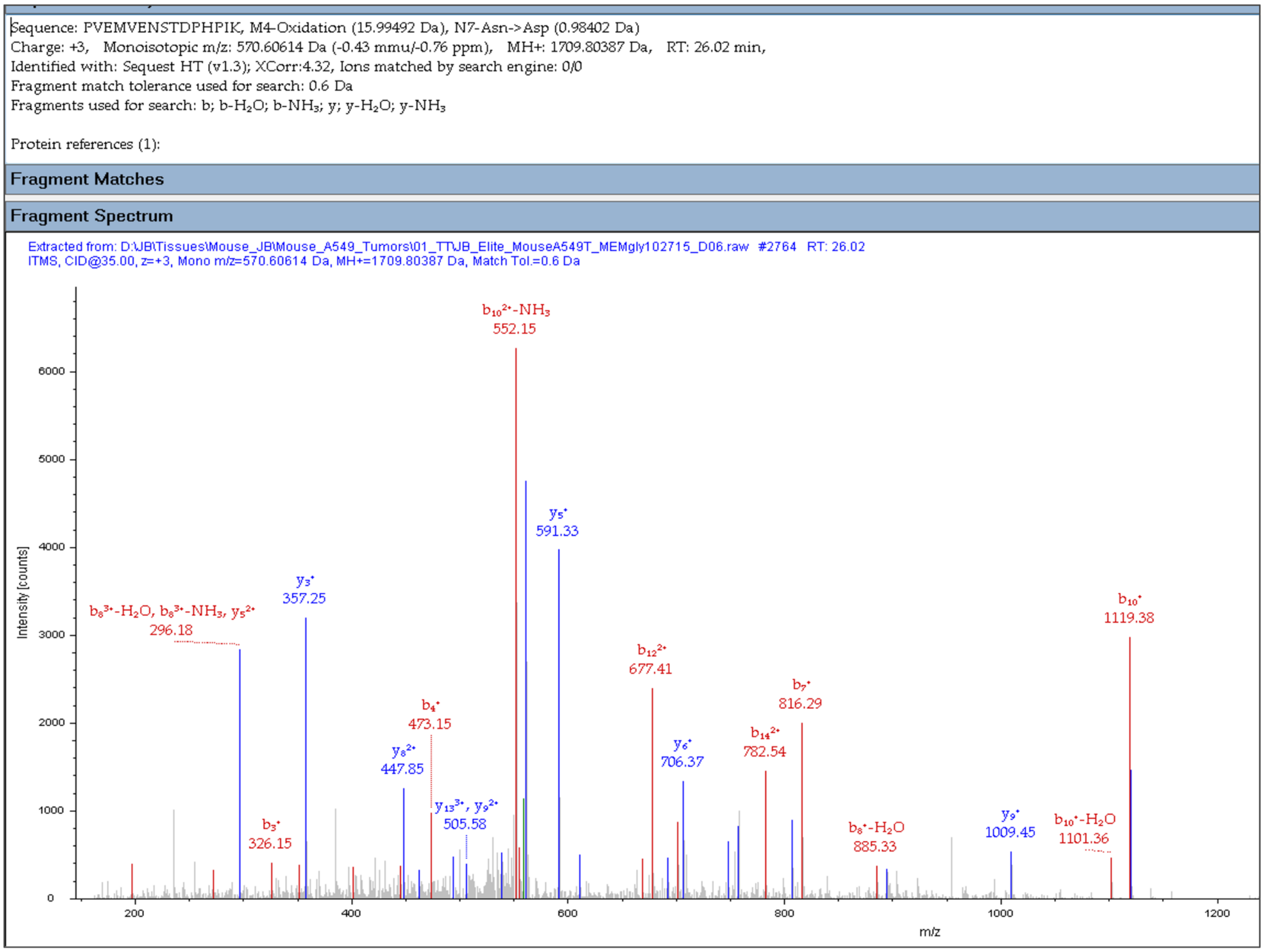
Protein references (1):

Fragment Matches

Fragment Spectrum

Extracted from: D:\JB\Tissues\Mouse_JB\Mouse_A549_Tumors\01_TTJB_Elite_MouseA549_MEMgly092515_A03.raw #6490 RT: 47.57
ITMS, CID@35.00, z=+3, Mono m/z=679.01581 Da, MH+=2035.03287 Da, Match Tol.=0.6 Da

Supplementary Figure 19: MS^2 spectrum depicting unambiguous identification of the human-derived CDH17 ortholog in tumor parenchyma by detection of protein- and species-specific N-glycopeptide.



Sequence: PVEMVENSTDPHPIK, M4-Oxidation (15.99492 Da), N7-Asn->Asp (0.98402 Da)
Charge: +3,   Monoisotopic m/z: 570.60614 Da (-0.43 mmu/-0.76 ppm),   MH+: 1709.80387 Da,   RT: 26.02 min,
Identified with: Sequest HT (v1.3); XCorr:4.32, Ions matched by search engine: 0/0
Fragment match tolerance used for search: 0.6 Da
Fragments used for search: b; b-H_2O; b-NH_3; y; y-H_2O; y-NH_3

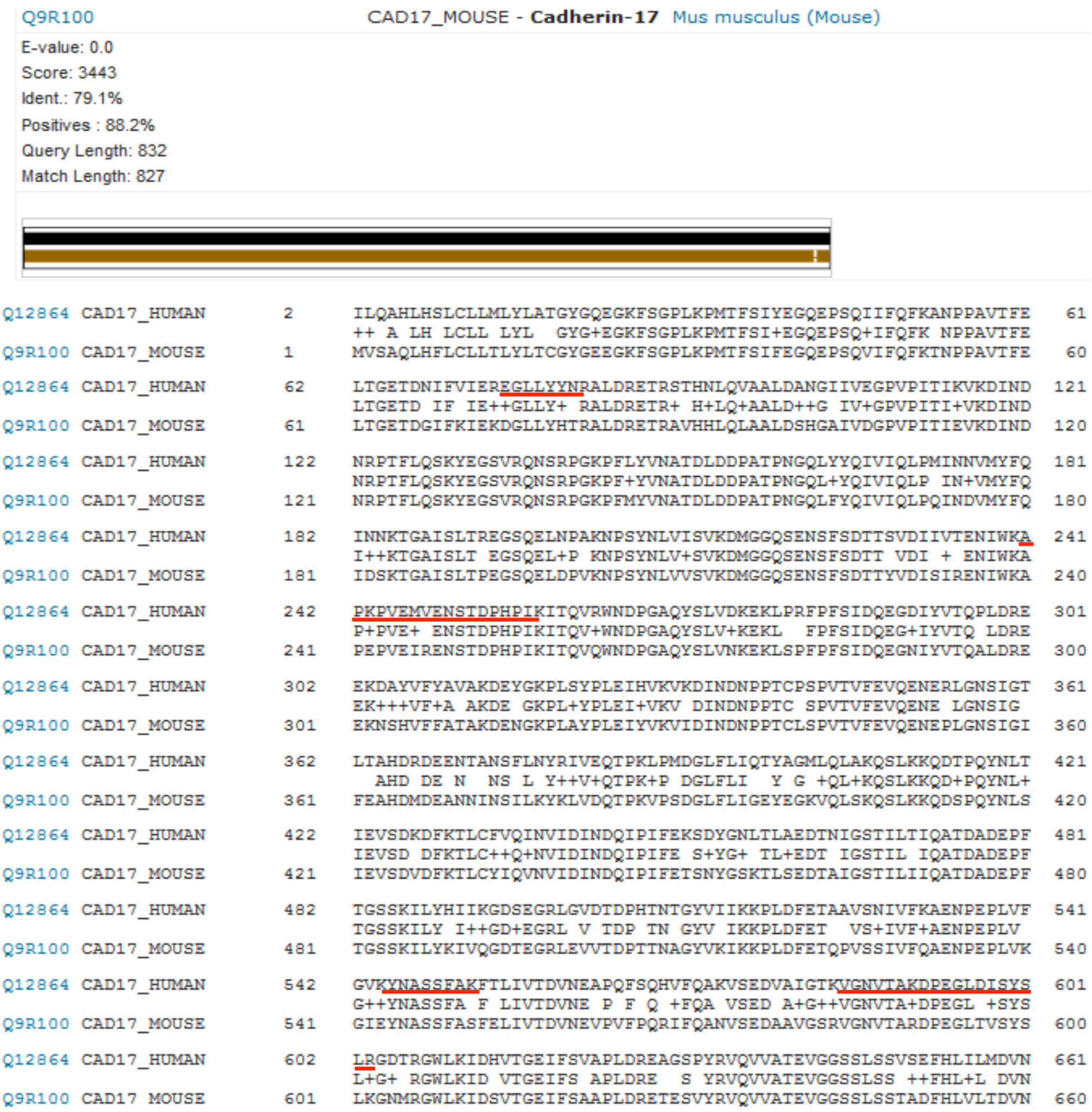
Protein references (1):

Fragment Matches

Fragment Spectrum

Extracted from: D:\JB\Tissues\Mouse_JB\Mouse_A549_Tumors\01_TTJB_Elite_MouseA549T_MEMgly102715_D06.raw #2764 RT: 26.02
ITMS, CID@35.00, z=+3, Mono m/z=570.60614 Da, MH+=1709.80387 Da, Match Tol.=0.6 Da

Supplementary Figure 20: MS^2 spectrum depicting unambiguous identification of the human-derived CDH17 ortholog in tumor parenchyma by detection of protein- and species-specific N-glycopeptide.



Q9R100 CAD17_MOUSE - **Cadherin-17** Mus musculus (Mouse)

E-value: 0.0
Score: 3443
Ident.: 79.1%
Positives : 88.2%
Query Length: 832
Match Length: 827

```
Q12864 CAD17_HUMAN       2       ILQAHLHSLCLLMLYLATGYGQEGKFSGPLKPMTFSIYEGQEPSQIIFQFKANPPAVTFE   61
                                 ++ A LH LCLL LYL  GYG+EGKFSGPLKPMTFSI+EGQEPSQ+IFQFK NPPAVTFE
Q9R100 CAD17_MOUSE       1       MVSAQLHFLCLLTLYLTCGYGEEGKFSGPLKPMTFSIFEGQEPSQVIFQFKTNPPAVTFE   60

Q12864 CAD17_HUMAN       62      LTGETDNIFVIEREGLLYYNRALDRETRSTHNLQVAALDANGIIVEGPVPITIKVKDIND  121
                                 LTGETD IF IE++GLLY+ RALDRETR+ H+LQ+AALD++G IV+GPVPITI+VKDIND
Q9R100 CAD17_MOUSE       61      LTGETDGIFKIEKDGLLYHTRALDRETRAVHHLQLAALDSHGAIVDGPVPITIEVKDIND  120

Q12864 CAD17_HUMAN       122     NRPTFLQSKYEGSVRQNSRPGKPFLYVNATDLDDPATPNGQLYYQIVIQLPMINNVMYFQ  181
                                 NRPTFLQSKYEGSVRQNSRPGKPF+YVNATDLDDPATPNGQL+YQIVIQLP IN+VMYFQ
Q9R100 CAD17_MOUSE       121     NRPTFLQSKYEGSVRQNSRPGKPFMYVNATDLDDPATPNGQLFYQIVIQLPQINDVMYFQ  180

Q12864 CAD17_HUMAN       182     INNKTGAISLTREGSQELNPAKNPSYNLVISVKDMGGQSENSFSDTTSVDIIVTENIWKA  241
                                 I++KTGAISLT EGSQEL+P KNPSYNLV+SVKDMGGQSENSFSDTT VDI + ENIWKA
Q9R100 CAD17_MOUSE       181     IDSKTGAISLTPEGSQELDPVKNPSYNLVVSVKDMGGQSENSFSDTTYVDISIRENIWKA  240

Q12864 CAD17_HUMAN       242     PKPVEMVENSTDPHPIKITQVRWNDPGAQYSLVDKEKLPRFPFSIDQEGDIYVTQPLDRE  301
                                 P+PVE+ ENSTDPHPIKITQV+WNDPGAQYSLV+KEKL  FPFSIDQEG+IYVTQ LDRE
Q9R100 CAD17_MOUSE       241     PEPVEIRENSTDPHPIKITQVQWNDPGAQYSLVNKEKLSPFPFSIDQEGNIYVTQALDRE  300

Q12864 CAD17_HUMAN       302     EKDAYVFYAVAKDEYGKPLSYPLEIHVKVKDINDNPPTCPSPVTVFEVQENERLGNSIGT  361
                                 EK+++VF+A AKDE GKPL+YPLEI+VKV DINDNPPTC SPVTVFEVQENE LGNSIG
Q9R100 CAD17_MOUSE       301     EKNSHVFFATAKDENGKPLAYPLEIYVKVIDINDNPPTCLSPVTVFEVQENEPLGNSIGI  360

Q12864 CAD17_HUMAN       362     LTAHDRDEENTANSFLNYRIVEQTPKLPMDGLFLIQTYAGMLQLAKQSLKKQDTPQYNLT  421
                                   AHD DE N  NS L Y++V+QTPK+P DGLFLI  Y G +QL+KQSLKKQD+PQYNL+
Q9R100 CAD17_MOUSE       361     FEAHDMDEANNINSILKYKLVDQTPKVPSDGLFLIGEYEGKVQLSKQSLKKQDSPQYNLS  420

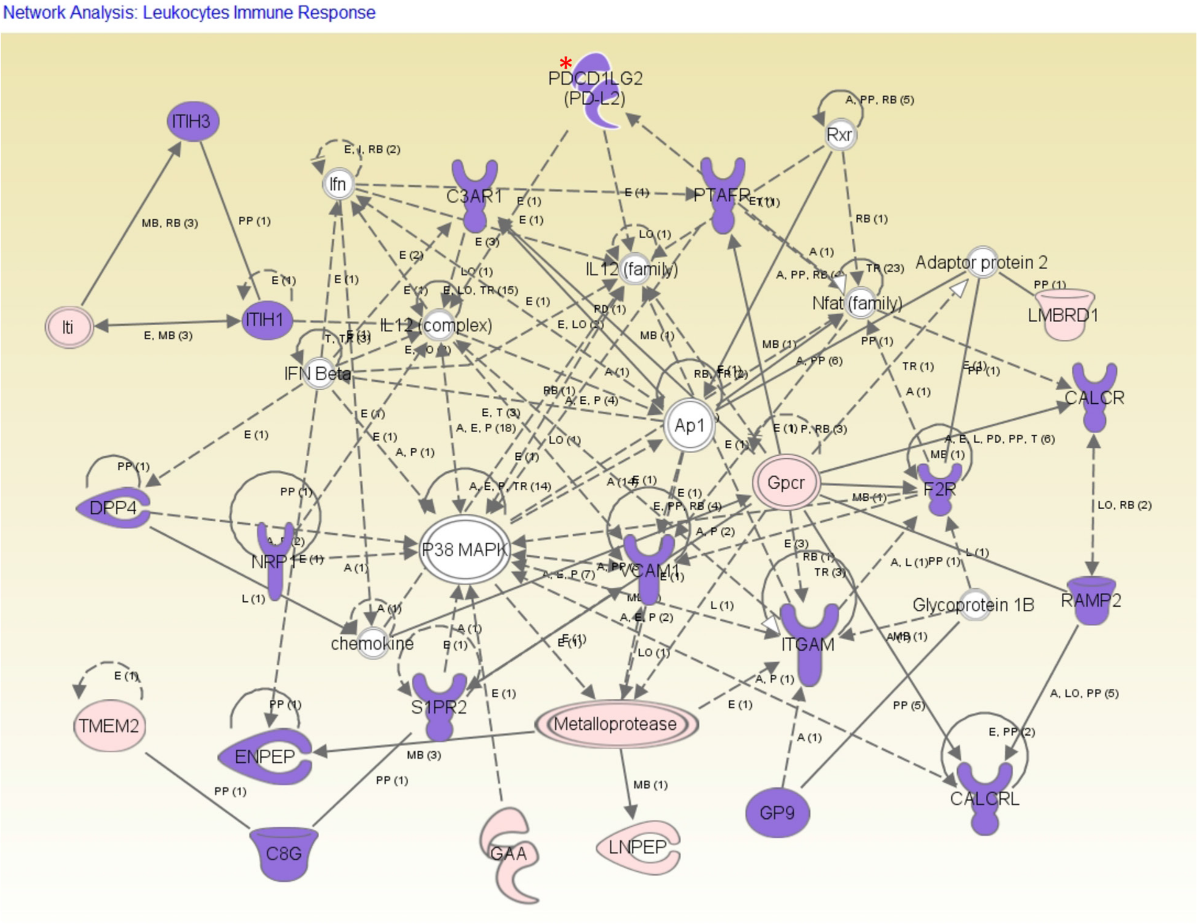
Q12864 CAD17_HUMAN       422     IEVSDKDFKTLCFVQINVIDINDQIPIFEKSDYGNLTLAEDTNIGSTILTIQATDADEPF  481
                                 IEVSD DFKTLC++Q+NVIDINDQIPIFE S+YG+ TL+EDT IGSTIL IQATDADEPF
Q9R100 CAD17_MOUSE       421     IEVSDVDFKTLCYIQVNVIDINDQIPIFETSNYGSKTLSEDTAIGSTILIIQATDADEPF  480

Q12864 CAD17_HUMAN       482     TGSSKILYHIIKGDSEGRLGVDTDPHTNTGYVIIKKPLDFETAAVSNIVFKAENPEPLVF  541
                                 TGSSKILY I++GD+EGRL V TDP TN GYV IKKPLDFET  VS+IVF+AENPEPLV
Q9R100 CAD17_MOUSE       481     TGSSKILYKIVQGDTEGRLEVVTDPTTNAGYVKIKKPLDFETQPVSSIVFQAENPEPLVK  540

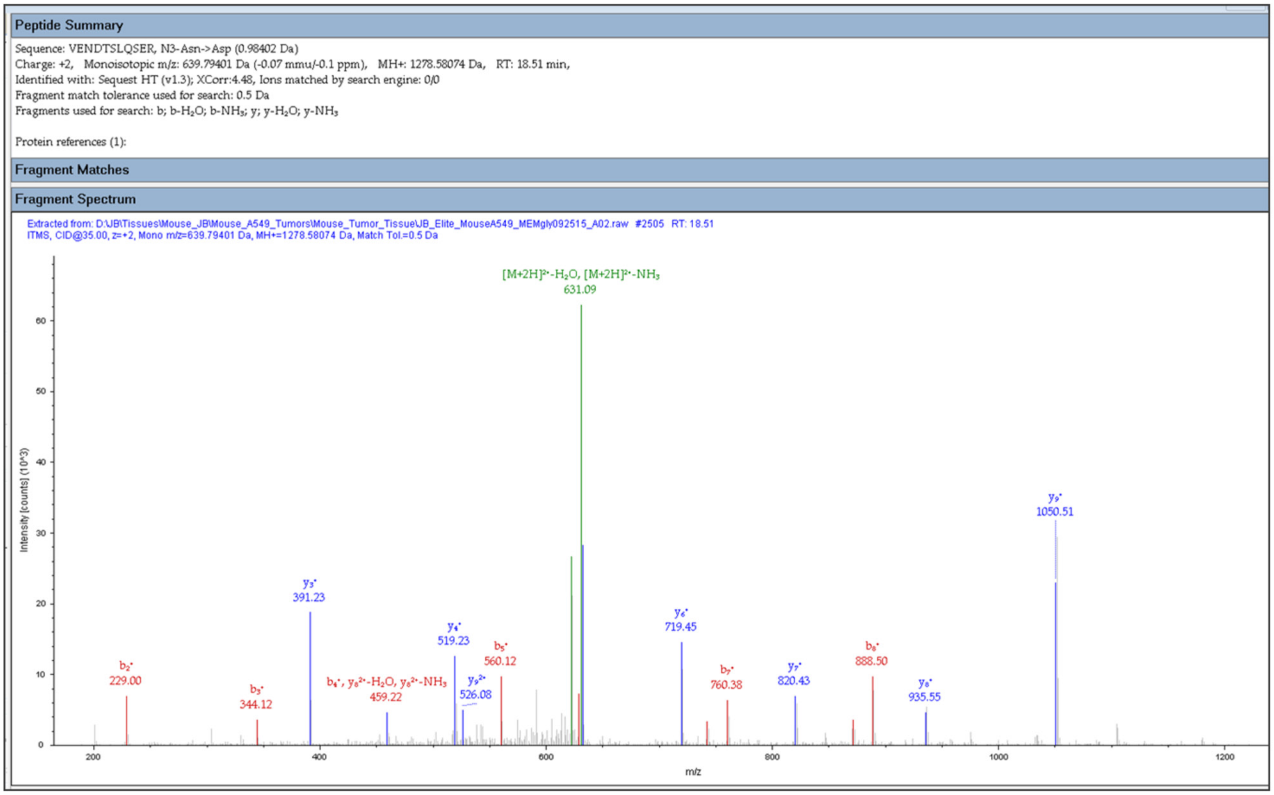
Q12864 CAD17_HUMAN       542     GVKYNASSFAKFTLIVTDVNEAPQFSQHVFQAKVSEDVAIGTKVGNVTAKDPEGLDISYS  601
                                 G++YNASSFA F LIVTDVNE P F Q +FQA VSED A+G++VGNVTA+DPEGL +SYS
Q9R100 CAD17_MOUSE       541     GIEYNASSFASFELIVTDVNEVPVFPQRIFQANVSEDAAVGSRVGNVTARDPEGLTVSYS  600

Q12864 CAD17_HUMAN       602     LRGDTRGWLKIDHVTGEIFSVAPLDREAGSPYRVQVVATEVGGSSLSSVSEFHLILMDVN  661
                                 L+G+ RGWLKID VTGEIFS APLDRE  S YRVQVVATEVGGSSLSS ++FHL+L DVN
Q9R100 CAD17 MOUSE       601     LKGNMRGWLKIDSVTGEIFSAAPLDRETESVYRVQVVATEVGGSSLSSTADFHLVLTDVN  660
```

Supplementary Figure 21: BLAST analysis confirming protein and species specificity of identified N-glycopeptides from parenchymal human-derived CDH17. Underlined in red are protein and species-specific peptides identified in tumor parenchyma.



Supplementary Figure 22: IPA® analysis reveals leukocyte immune response as one of top N-glycosylated protein networks activated in tumor stroma. Molecule selected for orthogonal cross-validation (i.e., PD-L2) is marked with red asterisk.



Peptide Summary

Sequence: VENDTSLQSER, N3-Asn->Asp (0.98402 Da)
Charge: +2, Monoisotopic m/z: 639.79401 Da (-0.07 mmu/-0.1 ppm), MH+: 1278.58074 Da, RT: 18.51 min,
Identified with: Sequest HT (v1.3); XCorr:4.48, Ions matched by search engine: 0/0
Fragment match tolerance used for search: 0.5 Da
Fragments used for search: b; b-H_2O; b-NH_3; y; y-H_2O; y-NH_3

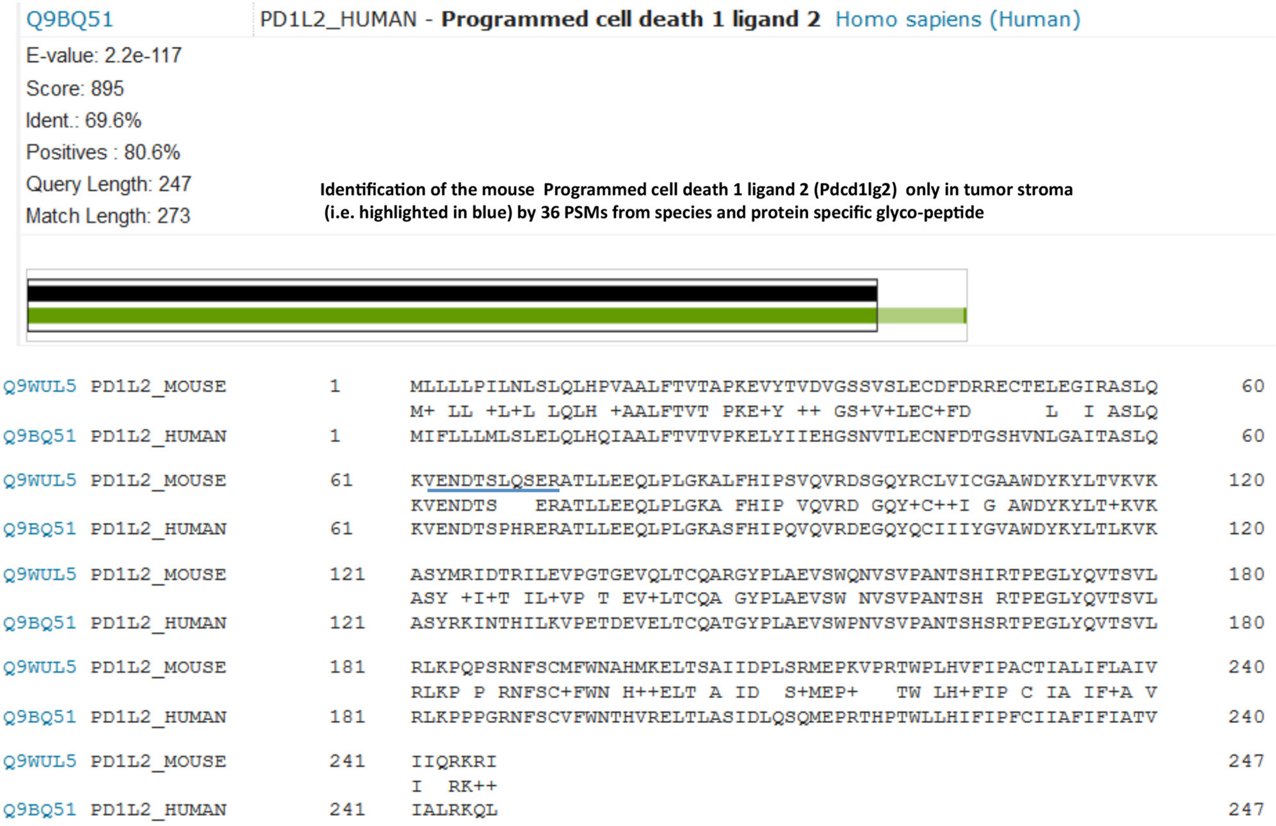
Protein references (1):

Fragment Matches

Fragment Spectrum

Extracted from: D:\JB\Tissues\Mouse_JB\Mouse_A549_Tumors\Mouse_Tumor_Tissue\JB_Elite_MouseA549_MEMgly092515_A02.raw #2505 RT: 18.51
ITMS, CID@35.00, z=+2, Mono m/z=639.79401 Da, MH+=1278.58074 Da, Match Tol.=0.5 Da

Supplementary Figure 23: MS^2 spectrum depicting unambiguous identification of the mouse-derived PD-L2 ortholog in tumor stroma by detection of protein- and species-specific N-glycopeptide.



Q9BQ51 PD1L2_HUMAN - **Programmed cell death 1 ligand 2** Homo sapiens (Human)

E-value: 2.2e-117
Score: 895
Ident.: 69.6%
Positives : 80.6%
Query Length: 247
Match Length: 273

Identification of the mouse Programmed cell death 1 ligand 2 (Pdcd1lg2) only in tumor stroma (i.e. highlighted in blue) by 36 PSMs from species and protein specific glyco-peptide

```
Q9WUL5 PD1L2_MOUSE    1    MLLLLPILNLSLQLHPVAALFTVTAPKEVYTVDVGSSVSLECDFDRRECTELEGIRASLQ    60
                           M+ LL +L+L LQLH +AALFTVT PKE+Y ++ GS+V+LEC+FD      L  I ASLQ
Q9BQ51 PD1L2_HUMAN    1    MIFLLLMLSLELQLHQIAALFTVTVPKELYIIEHGSNVTLECNFDTGSHVNLGAITASLQ    60

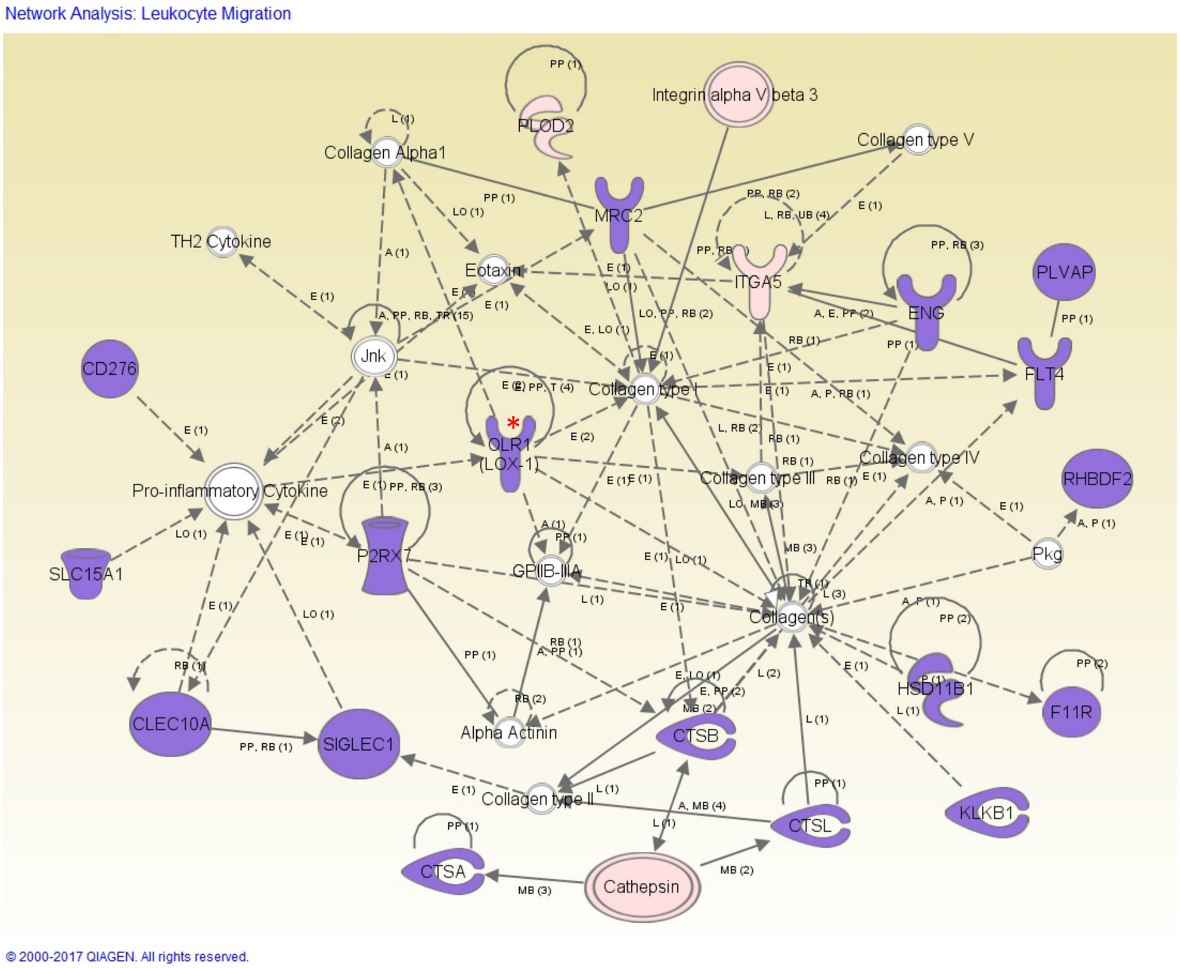
Q9WUL5 PD1L2_MOUSE    61   KVENDTSLQSERATLLEEQLPLGKALFHIPSVQVRDSGQYRCLVICGAAWDYKYLTVKVK   120
                           KVENDTS   ERATLLEEQLPLGKA FHIP VQVRD GQY+C++I G AWDYKYLT+KVK
Q9BQ51 PD1L2_HUMAN    61   KVENDTSPHRERATLLEEQLPLGKASFHIPQVQVRDEGQYQCIIIYGVAWDYKYLTLKVK   120

Q9WUL5 PD1L2_MOUSE    121  ASYMRIDTRILEVPGTGEVQLTCQARGYPLAEVSWQNVSVPANTSHIRTPEGLYQVTSVL   180
                           ASY +I+T IL+VP T EV+LTCQA GYPLAEVSW NVSVPANTSH RTPEGLYQVTSVL
Q9BQ51 PD1L2_HUMAN    121  ASYRKINTHILKVPETDEVELTCQATGYPLAEVSWPNVSVPANTSHSRTPEGLYQVTSVL   180

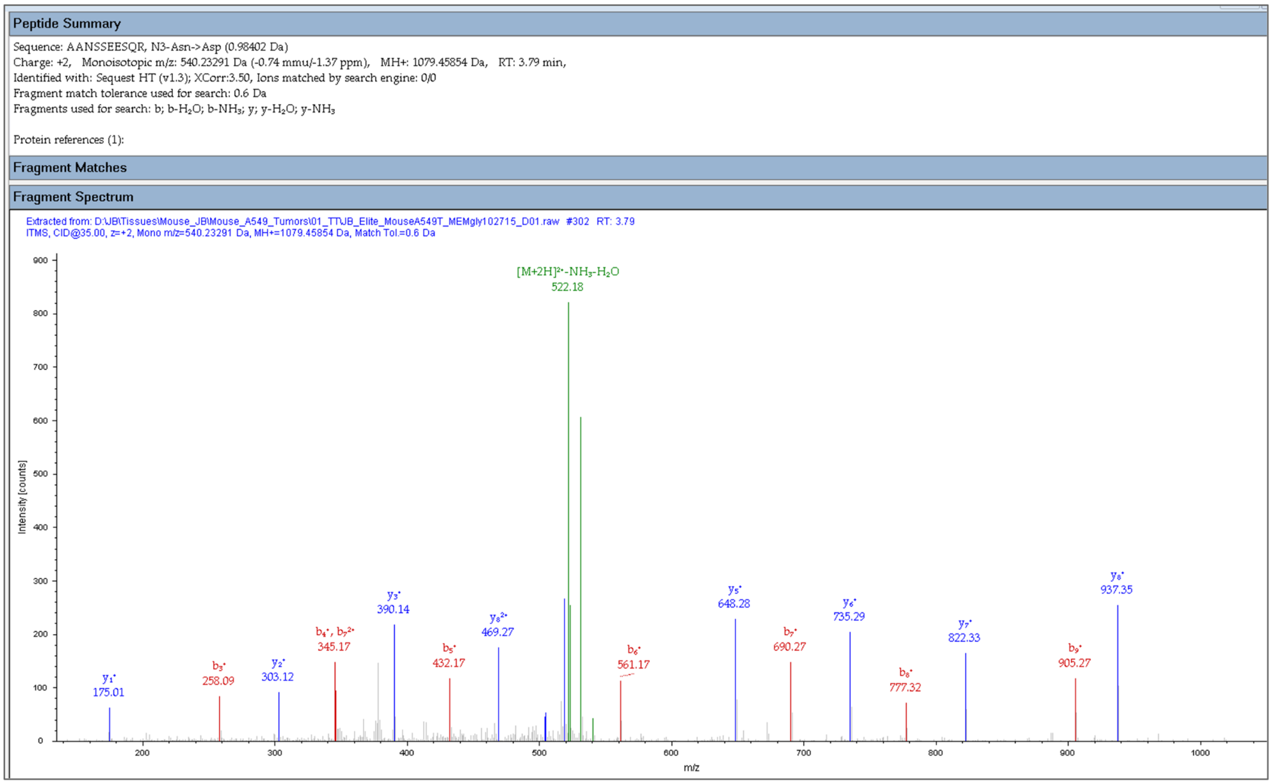
Q9WUL5 PD1L2_MOUSE    181  RLKPQPSRNFSCMFWNAHMKELTSAIIDPLSRMEPKVPRTWPLHVFIPACTIALIFLAIV   240
                           RLKP P RNFSC+FWN H++ELT A ID  S+MEP+   TW LH+FIP C IA IF+A V
Q9BQ51 PD1L2_HUMAN    181  RLKPPPGRNFSCVFWNTHVRELTLASIDLQSQMEPRTHPTWLLHIFIPFCIIAFIFIATV   240

Q9WUL5 PD1L2_MOUSE    241  IIQRKRI                                                        247
                           I  RK++
Q9BQ51 PD1L2_HUMAN    241  IALRKQL                                                        247
```

Supplementary Figure 24: BLAST analysis confirming protein and species specificity of identified N-glycopeptides from stromal mouse-derived PD-L2, Pdcd1lg2 gene product. Underlined in blue is protein and species-specific peptides identified solely in tumor stroma.



Supplementary Figure 25: IPA® analysis reveals leukocyte migration as one of top N-glycosylated protein networks activated in tumor stroma. Molecule selected for orthogonal cross-validation (i.e., LOX-1) is marked with red asterisk.



Peptide Summary

Sequence: AANSSEESQR, N3-Asn->Asp (0.98402 Da)
Charge: +2,   Monoisotopic m/z: 540.23291 Da (-0.74 mmu/-1.37 ppm),   MH+: 1079.45854 Da,   RT: 3.79 min,
Identified with: Sequest HT (v1.3); XCorr:3.50, Ions matched by search engine: 0/0
Fragment match tolerance used for search: 0.6 Da
Fragments used for search: b; b-H_2O; b-NH_3; y; y-H_2O; y-NH_3

Protein references (1):

Fragment Matches

Fragment Spectrum

Extracted from: D:\JB\Tissues\Mouse_JB\Mouse_A549_Tumors\01_TT\JB_Elite_MouseA549T_MEMgly102715_D01.raw #302 RT: 3.79
ITMS, CID@35.00, z=+2, Mono m/z=540.23291 Da, MH+=1079.45854 Da, Match Tol.=0.6 Da

Supplementary Figure 26: MS^2 spectrum depicting unambiguous identification of the mouse-derived LOX-1 ortholog in tumor stroma by detection of protein- and species-specific N-glycopeptide.

P78380 OLR1_HUMAN - **Oxidized low-density lipoprotein receptor 1** Homo sapiens (Human)

E-value: 2.2e-87
Score: 707
Ident.: 44.9%
Positives : 54.6%
Query Length: 363
Match Length: 273

```
Q9EQ09 OLR1_MOUSE           1    MTFDD-KMKPANDEPDQKSCGKKPKGLHLLSSPWWFPAAMTLVILCLVLSVTLIVQWTQL      59
                                  MTFDD K++   D+PD+KS GKK KGL  L SPWW  AA TL +LCL L VT++V   QL
P78380 OLR1_HUMAN           1    MTFDDLKIQTVKDQPDEKSNGKKAKGLQFLYSPWWCLAAATLGVLCLGLVVTIMVLGMQL      60

Q9EQ09 OLR1_MOUSE          60    RQVSDLLKQYQANLTQQDRILEGQMLAQQKAENTSQESKKELKGKIDTLTQKLNEKSKEQ     119
                                   QVSDLL Q QANLT Q                     KK+L+G+I
P78380 OLR1_HUMAN          61    SQVSDLLTQEQANLTHQ--------------------KKKLEGQIS--------------      86

Q9EQ09 OLR1_MOUSE         120    EELLQKNQNLQEALQRAANSSEESQRELKGKIDTITRKLDEKSKEQEELLQMIQNLQEAL     179
                                              A Q+A  +S+ES+ ELK  I+T+ RKL+EKSKEQ EL     NLQE L
P78380 OLR1_HUMAN          87    ------------ARQQAEEASQESENELKEMIETLARKLNEKSKEQMELHHQNLNLQETL     134

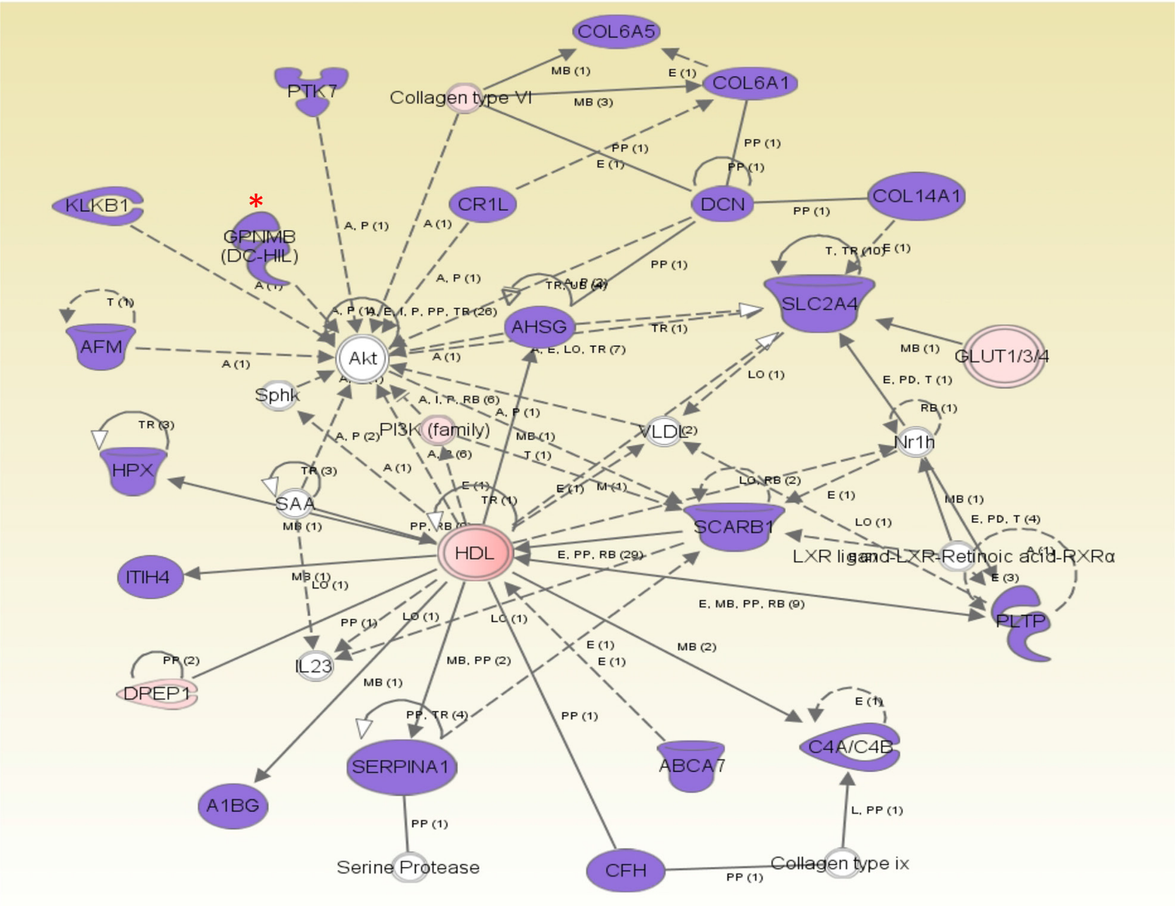
Q9EQ09 OLR1_MOUSE         180    QRAANSSEESQRELKGKIDTLTLKLNEKSKEQEELLQKNQNLQEALQRAANFSGPCPQDW     239
                                  +R AN                                               S PCPQDW
P78380 OLR1_HUMAN         135    KRVANC----------------------------------------------SAPCPQDW     148

Q9EQ09 OLR1_MOUSE         240    LWHKENCYLF-HGPFSWEKNRQTCQSLGGQLLQINGADDLTFILQAISHTTSPFWIGLHR     298
                                  +WH ENCYLF  G F+WEK+++ C SL  +LL+IN   DL FI QAIS+++ PFW+GL R
P78380 OLR1_HUMAN         149    IWHGENCYLFSSGSFNWEKSQEKCLSLDAKLLKINSTADLDFIQQAISYSSFPFWMGLSR     208

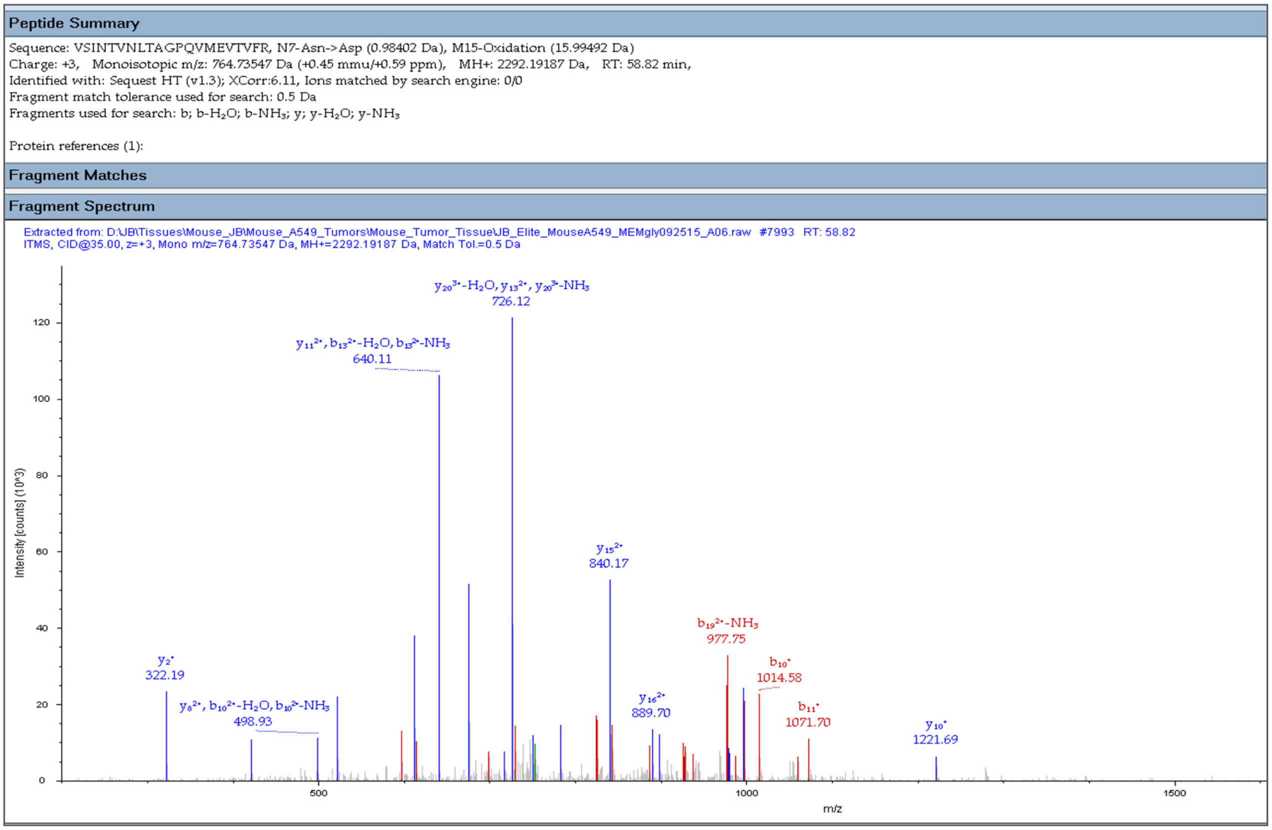
Q9EQ09 OLR1_MOUSE         299    KKPGQPWLWENGTPLNFQFFKTRGVSLQLYSSGNCAYLQDGAVFAENCILIAFSICQKKT     358
                                  + P  PWLWE+G+PL    F+ RG   Q Y SG CAY+Q GAV+AENCIL AFSICQKK
P78380 OLR1_HUMAN         209    RNPSYPWLWEDGSPLMPHLFRVRGAVSQTYPSGTCAYIQRGAVYAENCILAAFSICQKKA     268
```

Supplementary Figure 27: BLAST analysis confirming protein and species specificity of identified N-glycopeptides from stromal mouse-derived LOX-1, Olr1 gene product. Underlined in blue is protein and species-specific peptides identified solely in tumor stroma.

Network Analysis: Humoral Immune Response



Supplementary Figure 28: IPA® analysis reveals humoral immune response as one of top N-glycosylated protein networks activated in tumor stroma. Molecule selected for orthogonal cross-validation (i.e., DC-HIL) is marked with red asterisk.



Peptide Summary

Sequence: VSINTVNLTAGPQVMEVTVFR, N7-Asn->Asp (0.98402 Da), M15-Oxidation (15.99492 Da)
Charge: +3, Monoisotopic m/z: 764.73547 Da (+0.45 mmu/+0.59 ppm), MH+: 2292.19187 Da, RT: 58.82 min,
Identified with: Sequest HT (v1.3); XCorr:6.11, Ions matched by search engine: 0/0
Fragment match tolerance used for search: 0.5 Da
Fragments used for search: b; b-H_2O; b-NH_3; y; y-H_2O; y-NH_3

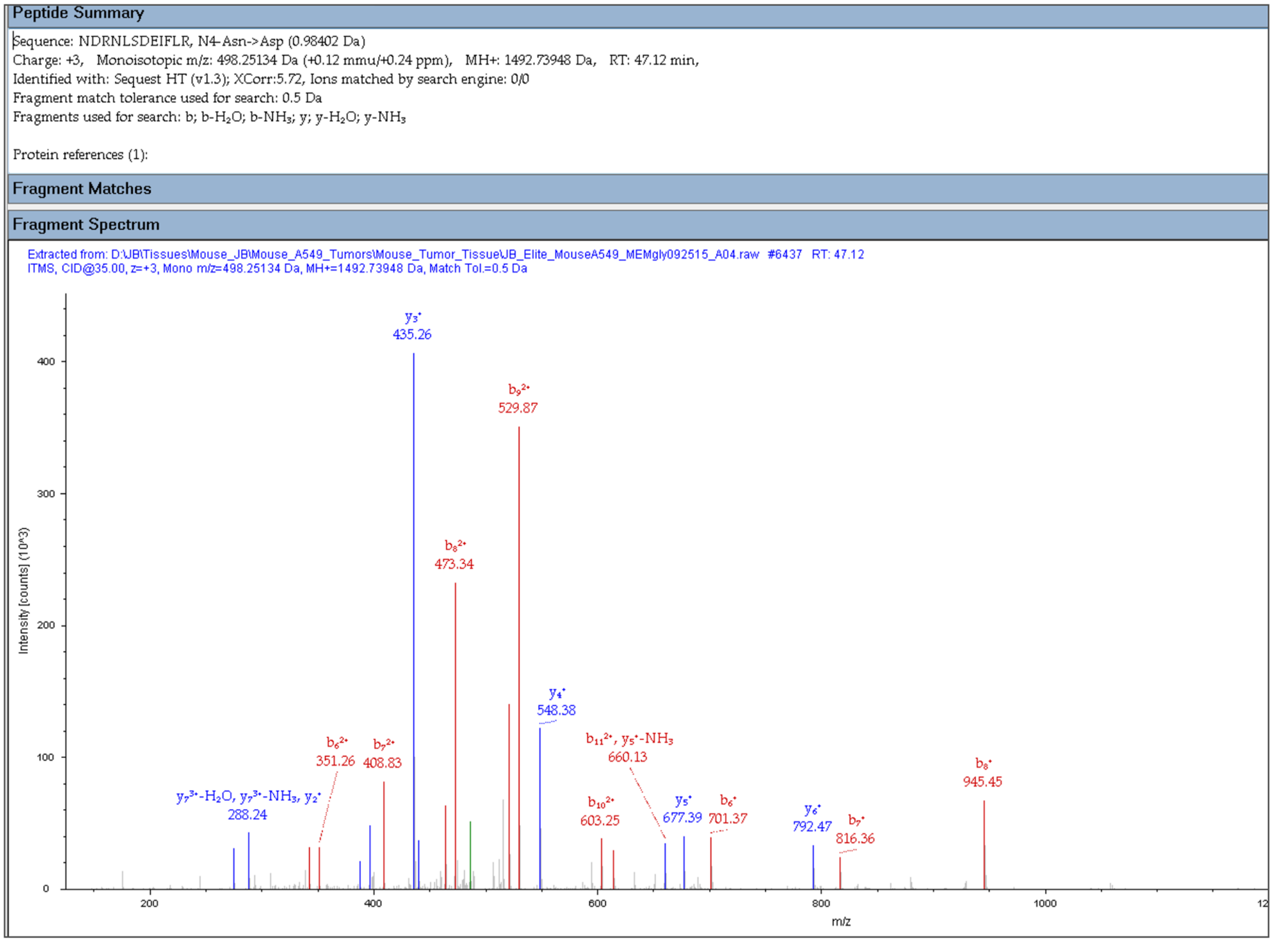
Protein references (1):

Fragment Matches

Fragment Spectrum

Extracted from: D:\JB\Tissues\Mouse_JB\Mouse_A549_Tumors\Mouse_Tumor_Tissue\JB_Elite_MouseA549_MEMgly092515_A06.raw #7993 RT: 58.82
ITMS, CID@35.00, z=+3, Mono m/z=764.73547 Da, MH+=2292.19187 Da, Match Tol.=0.5 Da

Supplementary Figure 29: MS^2 spectrum depicting unambiguous identification of the mouse-derived DC-HIL ortholog (i.e., Gpnmb gene product) in tumor stroma by detection of protein- and species-specific N-glycopeptide.



Peptide Summary

Sequence: NDRNLSDEIFLR, N4-Asn->Asp (0.98402 Da)
Charge: +3, Monoisotopic m/z: 498.25134 Da (+0.12 mmu/+0.24 ppm), MH+: 1492.73948 Da, RT: 47.12 min,
Identified with: Sequest HT (v1.3); XCorr:5.72, Ions matched by search engine: 0/0
Fragment match tolerance used for search: 0.5 Da
Fragments used for search: b; b-H_2O; b-NH_3; y; y-H_2O; y-NH_3

Protein references (1):

Fragment Matches

Fragment Spectrum

Extracted from: D:\JB\Tissues\Mouse_JB\Mouse_A549_Tumors\Mouse_Tumor_Tissue\JB_Elite_MouseA549_MEMgly092515_A04.raw #6437 RT: 47.12
ITMS, CID@35.00, z=+3, Mono m/z=498.25134 Da, MH+=1492.73948 Da, Match Tol.=0.5 Da

Supplementary Figure 30: MS^2 spectrum depicting unambiguous identification of the mouse-derived DC-HIL ortholog (i.e., Gpnmb gene product) in tumor stroma by detection of protein- and species-specific N-glycopeptide.

```
Q99P91 GPNMB_MOUSE          1    MESLCGVLGFLLLAAGLPLQAAKRFRDVLGHEQYPDHMREHNQLRGWSSDENEWDEHLYP      60
                                 ME L   LGFLLLAA LPL AAKRF DVLG+E+   +MREHNQL GWSSDEN+W+E LYP
Q14956 GPNMB_HUMAN          1    MECLYYFLGFLLLAARLPLDAAKRFHDVLGNERPSAYMREHNQLNGWSSDENDWNEKLYP      60

Q99P91 GPNMB_MOUSE          61   VWRRGDGRWKDSWEGGRVQAVLTSDSPALVGSNITFVVNLVFPRCQKEDANGNIVYEKNC      120
                                 VW+RGD RWK+SW+GGRVQAVLTSDSPALVGSNITF VNL+FPRCQKEDANGNIVYEKNC
Q14956 GPNMB_HUMAN          61   VWKRGDMRWKNSWKGGRVQAVLTSDSPALVGSNITFAVNLIFPRCQKEDANGNIVYEKNC      120

Q99P91 GPNMB_MOUSE          121  RNDLGLTSDLHVYNWTAGADDGDWEDGTSRSQHLRFPDRRPFPRPHGWKKWSFVYVFHTL      180
                                 RN+ GL++D +VYNWTA ++D D E+GT +S H  FPD +PFP   GW++W+F+YVFHTL
Q14956 GPNMB_HUMAN          121  RNEAGLSADPYVYNWTAWSEDSDGENGTGQSHHNVFPDGKPFPHHPGWRRWNFIYVFHTL      180

Q99P91 GPNMB_MOUSE          181  GQYFQKLGRCSARVSINTVNLTAGPQVMEVTVFRRYGRAYIPISKVKDVYVITDQIPVFV      240
                                 GQYFQKLGRCS RVS+NT N+T GPQ+MEVTV+RR+GRAY+PI++VKDVYV+TDQIPVFV
Q14956 GPNMB_HUMAN          181  GQYFQKLGRCSVRVSVNTANVTLGPQLMEVTVYRRHGRAYVPIAQVKDVYVVTDQIPVFV      240

Q99P91 GPNMB_MOUSE          241  TMSQKNDRNLSDEIFLRDLPIVFDVLIHDPSHFLNDSAISYKWNFGDNTGLFVSNNHTLN      300
                                 TM QKNDRN SDE FL+DLPI+FDVLIHDPSHFLN S I+YKW+FGDNTGLFVS NHT+N
Q14956 GPNMB_HUMAN          241  TMFQKNDRNSSDETFLKDLPIMFDVLIHDPSHFLNYSTINYKWSFGDNTGLFVSTNHTVN      300

Q99P91 GPNMB_MOUSE          301  HTYVLNGTFNLNLTVQTAVPGPCPPPSPSTPPPPSTPPSPPPSPLPTL-STPSPSLMPTG      359
                                 HTYVLNGTF+LNLTV+ A PGPCPP     PPPP  P  P PS   TL S  S +  P G
Q14956 GPNMB_HUMAN          301  HTYVLNGTFSLNLTVKAAAPGPCPP-----PPPPPRPSKPTPSLATTLKSYDSNTPGPAG      355

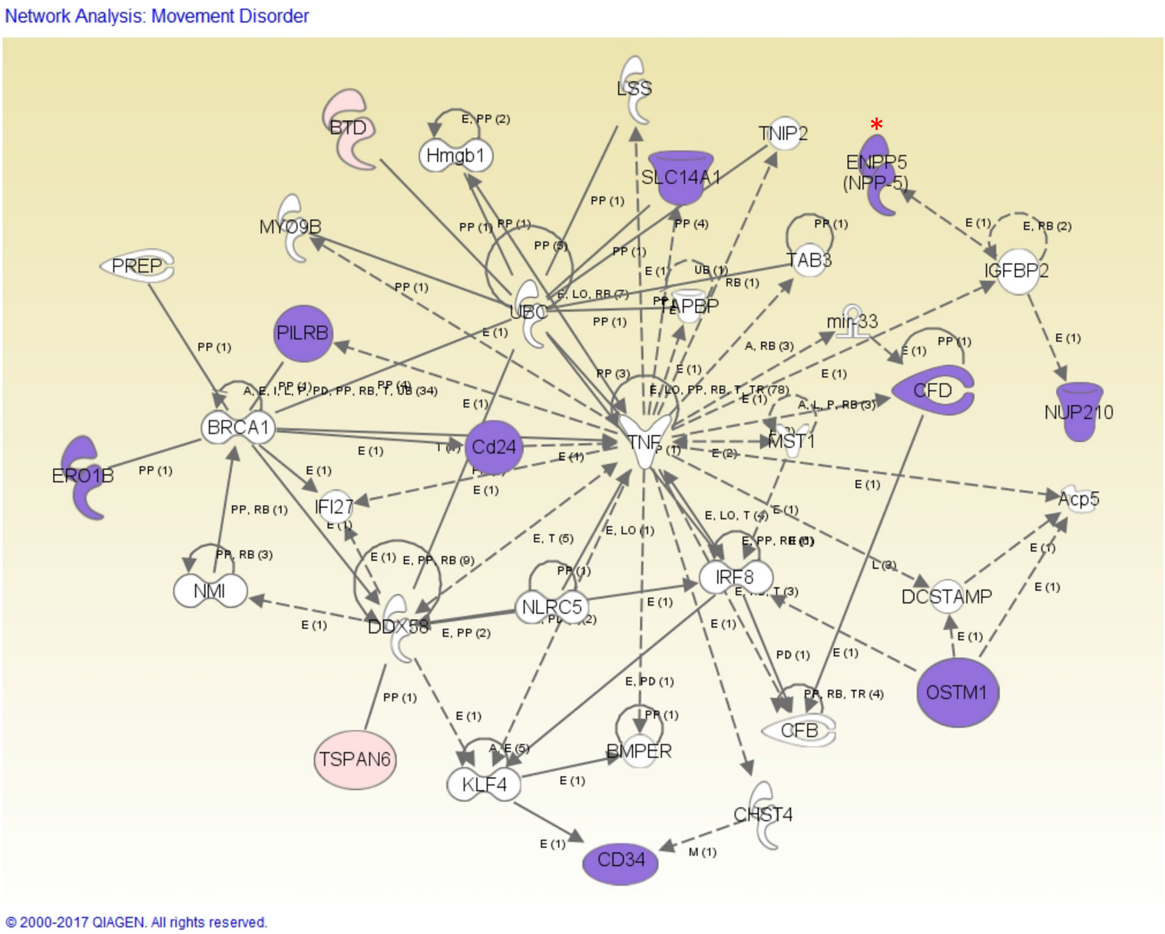
Q99P91 GPNMB_MOUSE          360  YKSMELSDISNENCRINRYGYFRATITIVEGILEVSIMQIADVPMPTPQPANSLMDFTVT      419
                                    +ELS I +ENC+INRYG+F+ATITIVEGILEV+I+Q+ DV MP P P +SL+DF VT
Q14956 GPNMB_HUMAN          356  DNPLELSRIPDENCQINRYGHFQATITIVEGILEVNIIQMTDVLMPVPWPESSLIDFVVT      415

Q99P91 GPNMB_MOUSE          420  CKGATPMEACTIISDPTCQIAQNRVCSPVAVDGLCLLSVRRAFNGSGTYCVNFTLGDDAS      479
                                 C+G+ P E CTIISDPTC+I QN VCSPV VD +CLL+VRR FNGSGTYCVN TLGDD S
Q14956 GPNMB_HUMAN          416  CQGSIPTEVCTIISDPTCEITQNTVCSPVDVDEMCLLTVRRTFNGSGTYCVNLTLGDDTS      475

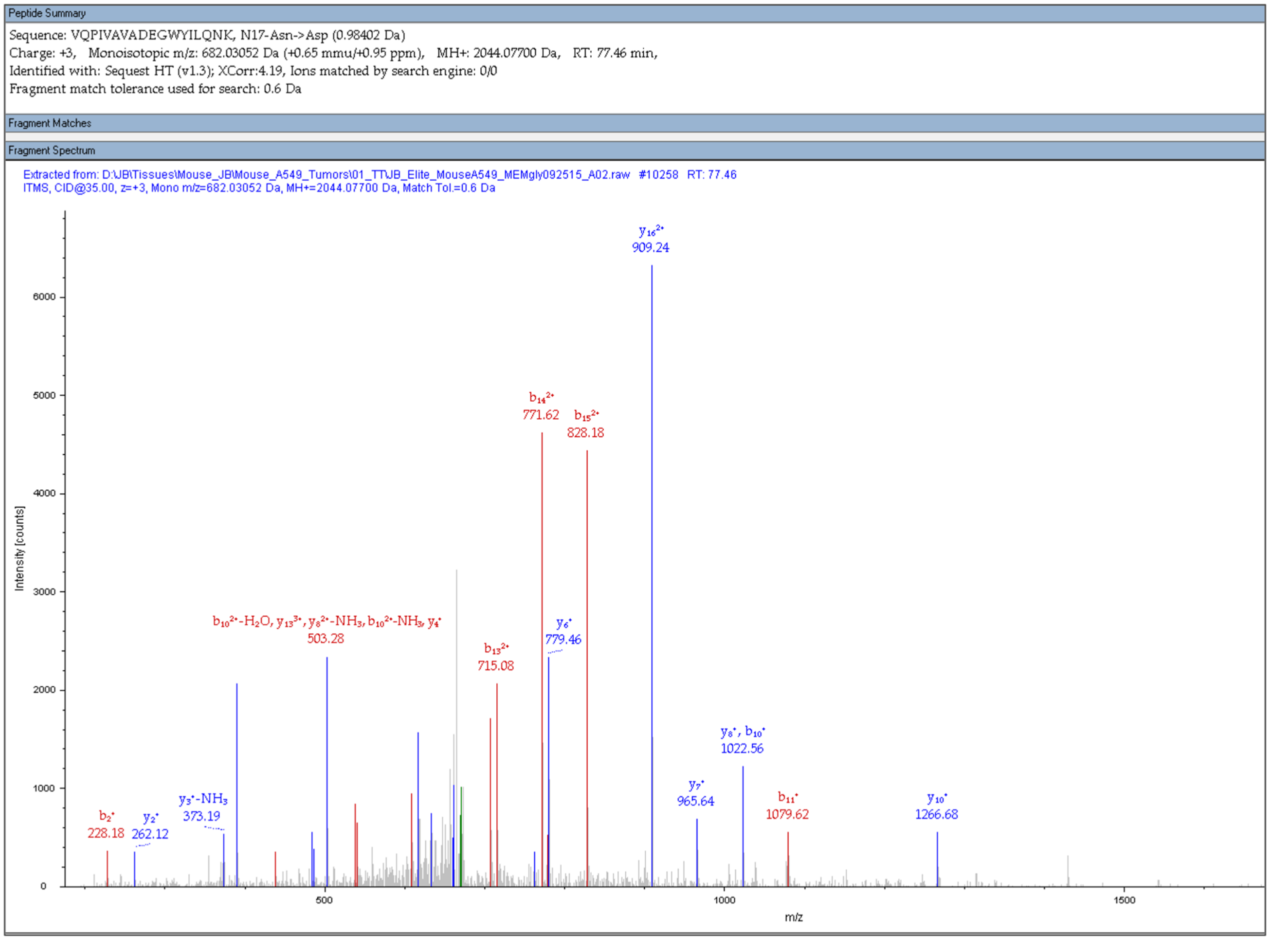
Q99P91 GPNMB_MOUSE          480  LALTSTLISIPGKDPDSPLRAVNGVLISIGCLAVLVTMVTILLYKKHKAYKPIGNCPRNT      539
                                 LALTSTLIS+P +DP SPLR  N  LIS+GCLA+ VT++++L+YKKHK Y PI N P N
Q14956 GPNMB_HUMAN          476  LALTSTLISVPDRDPASPLRMANSALISVGCLAIFVTVISLLVYKKHKEYNPIENSPGNV      535

Q99P91 GPNMB_MOUSE          540  VKGKGLSVLLSHAKAPFFRGDQEKDPLLQDK                                   570
                                 V+ KGLSV L+ AKA FF G+QEKDPLL+++
Q14956 GPNMB_HUMAN          536  VRSKGLSVFLNRAKAVFFPGNQEKDPLLKNQ                                   566
```

Supplementary Figure 31: BLAST analysis confirming protein and species specificity of identified N-glycopeptides from stromal mouse-derived DC-HIL, Gpnmb gene product. Underlined in blue are protein and species-specific peptides identified solely in tumor stroma.



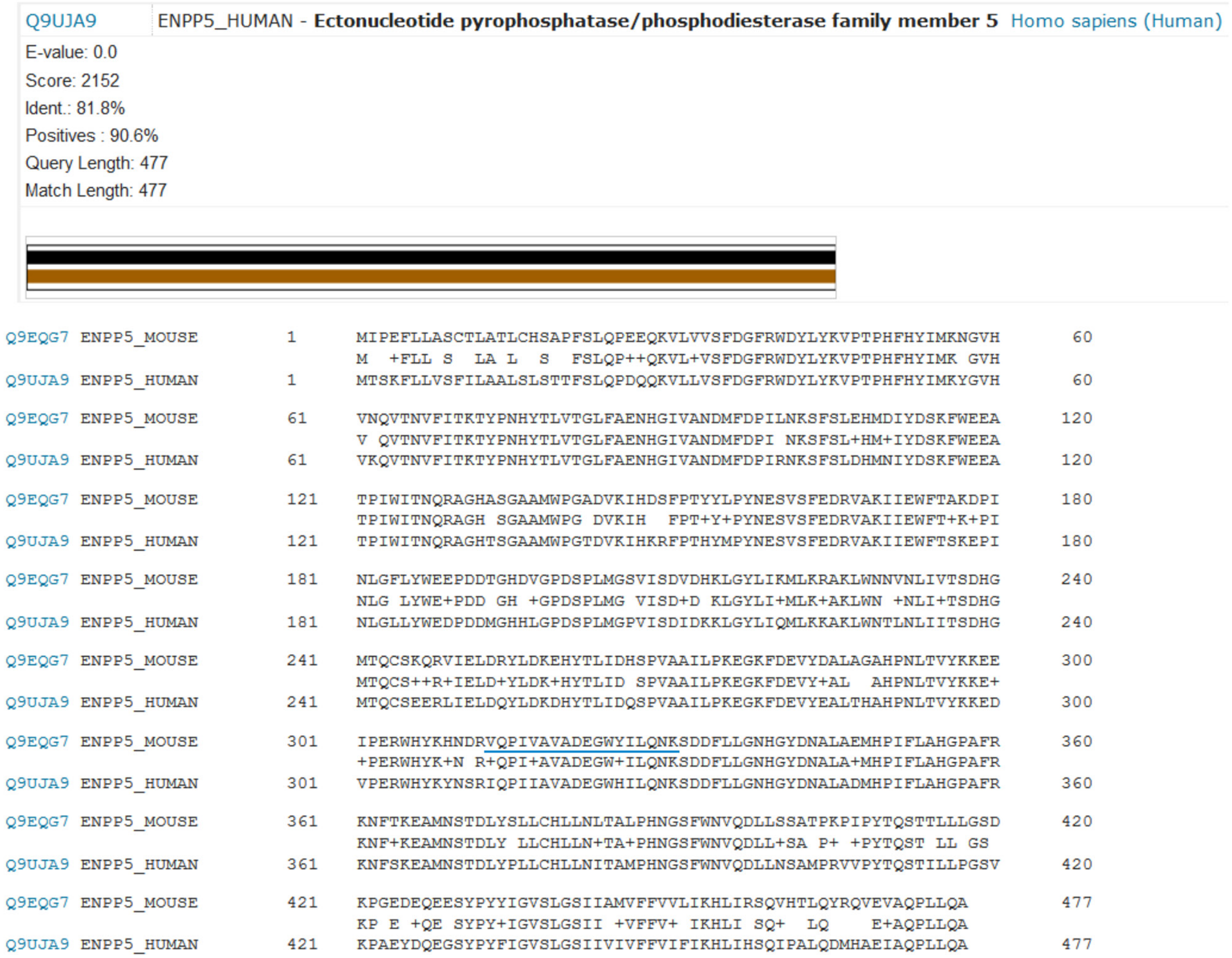
Supplementary Figure 32: IPA® analysis reveals humoral immune response as one of top N-glycosylated protein networks activated in tumor stroma. Molecule selected for orthogonal cross-validation (i.e., NPP-5) is marked with red asterisk.



Sequence: VQPIVAVADEGWYILQNK, N17-Asn->Asp (0.98402 Da)
Charge: +3, Monoisotopic m/z: 682.03052 Da (+0.65 mmu/+0.95 ppm), MH+: 2044.07700 Da, RT: 77.46 min,
Identified with: Sequest HT (v1.3); XCorr:4.19, Ions matched by search engine: 0/0
Fragment match tolerance used for search: 0.6 Da

Extracted from: D:\JB\Tissues\Mouse_JB\Mouse_A549_Tumors\01_TTJB_Elite_MouseA549_MEMgly092515_A02.raw #10258 RT: 77.46
ITMS, CID@35.00, z=+3, Mono m/z=682.03052 Da, MH+=2044.07700 Da, Match Tol.=0.6 Da

Supplementary Figure 33: MS^2 spectrum depicting unambiguous identification of the mouse-derived NPP-5 ortholog (i.e., Enpp5 gene product) in tumor stroma by detection of protein- and species-specific N-glycopeptide.



Q9UJA9 ENPP5_HUMAN - **Ectonucleotide pyrophosphatase/phosphodiesterase family member 5** Homo sapiens (Human)

E-value: 0.0
Score: 2152
Ident.: 81.8%
Positives : 90.6%
Query Length: 477
Match Length: 477

```
Q9EQG7 ENPP5_MOUSE       1        MIPEFLLASCTLATLCHSAPFSLQPEEQKVLVVSFDGFRWDYLYKVPTPHFHYIMKNGVH     60
                                  M  +FLL S  LA L  S  FSLQP++QKVL+VSFDGFRWDYLYKVPTPHFHYIMK GVH
Q9UJA9 ENPP5_HUMAN       1        MTSKFLLVSFILAALSLSTTFSLQPDQQKVLLVSFDGFRWDYLYKVPTPHFHYIMKYGVH     60

Q9EQG7 ENPP5_MOUSE       61       VNQVTNVFITKTYPNHYTLVTGLFAENHGIVANDMFDPILNKSFSLEHMDIYDSKFWEEA     120
                                  V QVTNVFITKTYPNHYTLVTGLFAENHGIVANDMFDPI NKSFSL+HM+IYDSKFWEEA
Q9UJA9 ENPP5_HUMAN       61       VKQVTNVFITKTYPNHYTLVTGLFAENHGIVANDMFDPIRNKSFSLDHMNIYDSKFWEEA     120

Q9EQG7 ENPP5_MOUSE       121      TPIWITNQRAGHASGAAMWPGADVKIHDSFPTYYLPYNESVSFEDRVAKIIEWFTAKDPI     180
                                  TPIWITNQRAGH SGAAMWPG DVKIH  FPT+Y+PYNESVSFEDRVAKIIEWFT+K+PI
Q9UJA9 ENPP5_HUMAN       121      TPIWITNQRAGHTSGAAMWPGTDVKIHKRFPTHYMPYNESVSFEDRVAKIIEWFTSKEPI     180

Q9EQG7 ENPP5_MOUSE       181      NLGFLYWEEPDDTGHDVGPDSPLMGSVISDVDHKLGYLIKMLKRAKLWNNVNLIVTSDHG     240
                                  NLG LYWE+PDD GH +GPDSPLMG VISD+D KLGYLI+MLK+AKLWN +NLI+TSDHG
Q9UJA9 ENPP5_HUMAN       181      NLGLLYWEDPDDMGHHLGPDSPLMGPVISDIDKKLGYLIQMLKKAKLWNTLNLIITSDHG     240

Q9EQG7 ENPP5_MOUSE       241      MTQCSKQRVIELDRYLDKEHYTLIDHSPVAAILPKEGKFDEVYDALAGAHPNLTVYKKEE     300
                                  MTQCS++R+IELD+YLDK+HYTLID SPVAAILPKEGKFDEVY+AL  AHPNLTVYKKE+
Q9UJA9 ENPP5_HUMAN       241      MTQCSEERLIELDQYLDKDHYTLIDQSPVAAILPKEGKFDEVYEALTHAHPNLTVYKKED     300

Q9EQG7 ENPP5_MOUSE       301      IPERWHYKHNDRVQPIVAVADEGWYILQNKSDDFLLGNHGYDNALAEMHPIFLAHGPAFR     360
                                  +PERWHYK+N R+QPI+AVADEGW+ILQNKSDDFLLGNHGYDNALA+MHPIFLAHGPAFR
Q9UJA9 ENPP5_HUMAN       301      VPERWHYKYNSRIQPIIAVADEGWHILQNKSDDFLLGNHGYDNALADMHPIFLAHGPAFR     360

Q9EQG7 ENPP5_MOUSE       361      KNFTKEAMNSTDLYSLLCHLLNLTALPHNGSFWNVQDLLSSATPKPIPYTQSTTLLLGSD     420
                                  KNF+KEAMNSTDLY LLCHLLN+TA+PHNGSFWNVQDLL+SA P+ +PYTQST LL GS
Q9UJA9 ENPP5_HUMAN       361      KNFSKEAMNSTDLYPLLCHLLNITAMPHNGSFWNVQDLLNSAMPRVVPYTQSTILLPGSV     420

Q9EQG7 ENPP5_MOUSE       421      KPGEDEQEESYPYYIGVSLGSIIAMVFFVVLIKHLIRSQVHTLQYRQVEVAQPLLQA        477
                                  KP E +QE SYPY+IGVSLGSII +VFFV+ IKHLI SQ+  LQ    E+AQPLLQA
Q9UJA9 ENPP5_HUMAN       421      KPAEYDQEGSYPYFIGVSLGSIIVIVFFVIFIKHLIHSQIPALQDMHAEIAQPLLQA        477
```

Supplementary Figure 34: BLAST analysis confirming protein and species specificity of identified N-glycopeptides from stromal mouse-derived NPP-5, Enpp5 gene product. Underlined in blue are protein and species-specific peptides identified solely in tumor stroma.

Supplementary Table 1: (A) N-glycosylated human peptides identified in A549 murine tumor xenograft using TCSG. (B) N-glycosylated mouse peptides identified in A549 murine tumor xenograft using TCSG.

See Supplementary File 1

Supplementary Table 2: (A) N-glycosylated peptides identified in normal mouse lung tissue using TCSG. (B) N-glycosylated peptides identified in 2D-grown A549 human lung-adenocarcinoma cell line using TCSG.

See Supplementary File 2

Supplementary Table 3: (A) All human N-glycopeptides identified in tumor xenograft and 2D-grown A549 cells using TCSG. (B) All mouse N-glycopeptides identified in tumor xenograft and normal mouse lungs using TCSG.

See Supplementary File 3

Supplementary Table 4: (A) N-glycosylated mouse proteins identified in tumor xenograft using TCSG. (B) N-glycosylated human proteins identified in tumor xenograft using TCSG.

See Supplementary File 4

Supplementary Table 5: (A) All mouse N-glycoproteins identified in tumor xenograft and normal mouse lungs obtained from healthy mice using TCSG. (B) All human N-glycoproteins identified in tumor xenograft and 2D-grown A549 cells using TCSG.

See Supplementary File 5

Supplementary Table 6: (A) Mouse-stromal N-glycoproteins identified in tumor xenograft by at least a single species-specific N-glycosylated peptide. (B) Human-parenchymal N-glycoproteins identified in tumor xenograft by at least a single species-specific N-glycosylated peptide.

See Supplementary File 6

Supplementary Table 7: (A) N-glycoproteins identified in normal mouse lungs by at least a single species-specific N-glycosylated peptide. (B) N-glycoproteins identified in 2D-grown A549 human lung-denocarcinoma cell line by at least a single species-specific N-glycosylated peptide.

See Supplementary File 7

Supplementary Table 8: (A) Stroma-uniqe mouse proteins revealed by subtractive TCSG analysis. (B) Parenchyma-uniqe human proteins revealed by subtractive TCSG analysis.

See Supplementary File 8

Supplementary Table 9: (A) Stroma-specific protein panel uncovered by compartive subtractive TCSG analysis. (B) Parenchyma-specific protein panel uncovered by compartive subtractive TCSG analysis. (C) A549-specific protein panel uncovered by compartive subtractive TCSG analysis. (C) Normal lung-specific protein panel uncovered by compartive subtractive TCSG analysis.

See Supplementary File 9

Supplementary Table 10: Diferently regulated orthologues detected in both tumor parenchyma and tumor stroma by at least one species-specific N-glycosylated peptide.

See Supplementary File 10

Supplementary Table 11: (A) Top parenchymal functional protein networks in terms of statistical significance and number of detected interacting protein species. (B) Top stromal functional protein networks in terms of statistical significance and number of detected interacting protein species.

See Supplementary File 11