

Supporting Information

Placental proteomics provides insights into pathophysiology of pre-eclampsia and predicts possible markers in plasma

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Supporting information: Experimental procedure S1

Label-free analysis MS^E of placental tissue proteome

Nanoscale LC separation was performed with nanoACQUITY system (Waters Corporation) using nanoACQUITY UPLC 10K-2G -V/M Symmetry C₁₈ trapping column (180 µm x 20 mm, 5 µm) and a BEH 130 C₁₈ analytical column (75 µm x 250 mm, 1.7 µm). Peptide digest (2 µl) each sample was injected into trapping, the column was washed with 0.1% solvent A (0.1% formic acid containing water) for 2 min at 10 µl/min flow rate. Sample elution was performed with a gradient of solvent B (0.1% formic acid containing acetonitrile) for 180 min (2 to 5%, 2 min; 5 to 27%, 158 min; 27 to 50%, 13 min; 50 to 90%, 2 min; 90 to 2%, 5 min) at a flow rate of 300 nl/min and column temperature at 40 °C. NanoACQUITY system is coupled to SYNAPT Q-TOF High Definition Mass Spectrometer (Waters Corporation) with NanoLockSpray as the ion source. Lockmass calibrant peptide standard [Glu¹]-fibrinopeptide B (Sigma-Aldrich) was infused into ion source at the flow rate of 300 nl/min and sampled every 60 sec. LC-MS^E data was collected over an m/z range of 50 to 2000, the scan time of 0.7 s, a constant low energy of 4V for MS mode and a step from 10 to 45 V of collision energy during high energy MS^E mode scans.

Each LC-MS^E raw data file was uploaded into TransOmicsTM Informatics for Proteomics software (Waters Corporation), developed in collaboration with Nonlinear Dynamics (Newcastle upon Tyne, UK). Transomics data processing involves peak-modelling algorithm to reduce the raw data file by an order of magnitude and create peak model. The LC runs were then aligned to compensate the variation between runs, using a reference run to generate alignment vectors automatically or manually. The aligned runs were then subjected to peak picking algorithm to generate a single map of peptide ions, which is applied to each sample. TransOmics uses a “quantify and then identify” approach, which promotes identification of low abundance peptides, this is done by the ion abundance algorithm as described by Li et al.

which allows quantification of peptide ions without MS/MS data¹. After quantification, each data was normalized using the spiked peptide ions. The quantified peptides were then subjected to identification using statistical tool filters such as ANOVA, power analysis, and q-values (for FDR). Human reviewed proteome database from Uniprot was downloaded and following search parameters were used: trypsin with two allowed missed cleavage, maximum protein mass 1000kDa, carbamidomethyl C as fixed and oxidation M, deamidation NQ, dehydration ST, as variable modification, automatic peptide and fragment tolerance, less than 4% FDR. ADH was used as internal standard for 'Hi-3' calibration and relative quantification.

Plasma proteome SWATH™ analysis

A total amount 1.5 µg of the sample was loaded onto a reverse phase Eksigent ChromXP trap (200 x 0.5 mm) column and desalted at a flow rate of 2.5 ml per minute for 20 minutes. After desalting, the peptides were separated using an Eksigent C18 column (0.075 x15cm). The peptides were eluted from the column at a flow rate of 300 nl/min using a linear gradient of 5–40% mobile phase B in 75 minutes (mobile phase A was 99.9% water in 0.1% formic acid and mobile phase B was 99.9 % ACN in 0.1% formic acid). The LC eluent was analyzed online using a NanoSpray III Source installed on the Triple-TOF 5600 system. Samples were analyzed using a nebulizing gas of 5; a curtain gas of 25, an ion spray voltage of 2500 V and a heater interface temperature of 130 °C. For ion library generation, typical data-dependent analysis was performed and the mass spectrometer was operated in a manner where a TOF-MS survey scan was performed in the 350–1250 m/z mass range with an accumulation time of 250 milliseconds, from which the 30 most abundant ions were selected for subsequent MS/MS fragmentation with an accumulation time of 70 milliseconds leading to a cycle time of 2.4 seconds. For this experiment, ions were isolated in quadrupole with a unit resolution (0.7 Da) and rolling collision energy with a spread of 5 V. Only the parent ions with a charge state from +2 to +5 was included in the MS/MS fragmentation. The threshold precursor ion intensity was

set as more than 120 cps and was not present on the dynamic exclusion list. Once an ion had been fragmented by MS/MS, its mass and isotopes were excluded for a period of 10 seconds. The MS/MS spectra were acquired in high sensitivity mode.

For SWATH MS-based experiments, the instrument was specifically operated in a “create swath” mode; wherein it was set to allow a quadrupole resolution of 15 Da/mass selections. Using an isolation width of 16 Da (15 Da of optimal ion transmission efficiency and 1 Da for the window overlap), a set of 60 overlapping windows were constructed covering the 350–1250 Da mass range. The collision energy for each window was determined based on the appropriate collision energy set automatically, with a spread of 5 eV. The total duty cycle was 4.3 seconds (the total of 4.2 seconds for stepping through the 60 isolation windows and 0.1 seconds for the optional survey scan). The MS/MS acquisition was performed using a high-sensitivity mode corresponding to a mass resolution of about 15 000, which also enables the extraction of fragment ions with 10–50 ppm accuracy. Three replicate runs were performed to understand the technical variation.

For the ion library generation, all the Wiff files containing MS and MS/MS spectra generated from Triple TOF 5600 (Sciex) were submitted for database searching using the Protein Pilot v5.0 software (Sciex). For the identification of proteins, the paragon algorithm was employed in a “Thorough ID” search mode against the Uniprot-Human reference dataset (20,191 protein). The search parameters allowed for modifications by IAA as cysteine blocking reagent. We applied 1% global protein level False Determination Rate (FDR) for the identification of proteins. For SWATH analysis, the peaks were extracted using the SWATH Acquisition MicroApp in PeakView v. 2.1 (Sciex) software using group files generated from Protein Pilot v 5.0. In parallel, a sciex SWATH library for plasma proteome was uploaded in PeakView 2.1. For both the ion library files, Retention time (RT) calibration was performed separately in PeakView software using high abundant peptides eluted at different RTs across the gradient

and present in both IDA and SWATH run. Group files were extracted using an MS tolerance of 25 ppm and an MS/MS tolerance of 50 ppm, and the following parameters were considered: minimum 2 peptides with 6 transitions, exclude shared peptides, peptide confidence of 99%. These processed. mrkv files from PeakView were then loaded onto MarkerView v. 1.2.1 (Sciex) for further analysis. This export resulted in the generation of three files containing quantitative information about individual ions, the summed intensity of different ions for a particular peptide and the summed intensity of different peptides for a particular protein. The built-in total ion intensity sum plugin was used for the normalization. Proteins having $p < 0.05$ (calculated among technical replicates of every sample) only considered for further deregulation analysis. Fold change > 2 or < 0.5 for up or down-regulated proteins were considered for relative quantitative analysis for all the samples.

1. Li, G. Z.; Vissers, J. P.; Silva, J. C.; Golick, D.; Gorenstein, M. V.; Geromanos, S. J., Database searching and accounting of multiplexed precursor and product ion spectra from the data independent analysis of simple and complex peptide mixtures. *Proteomics* **2009**, 9, 1696-719.

Supporting information: Figure S1

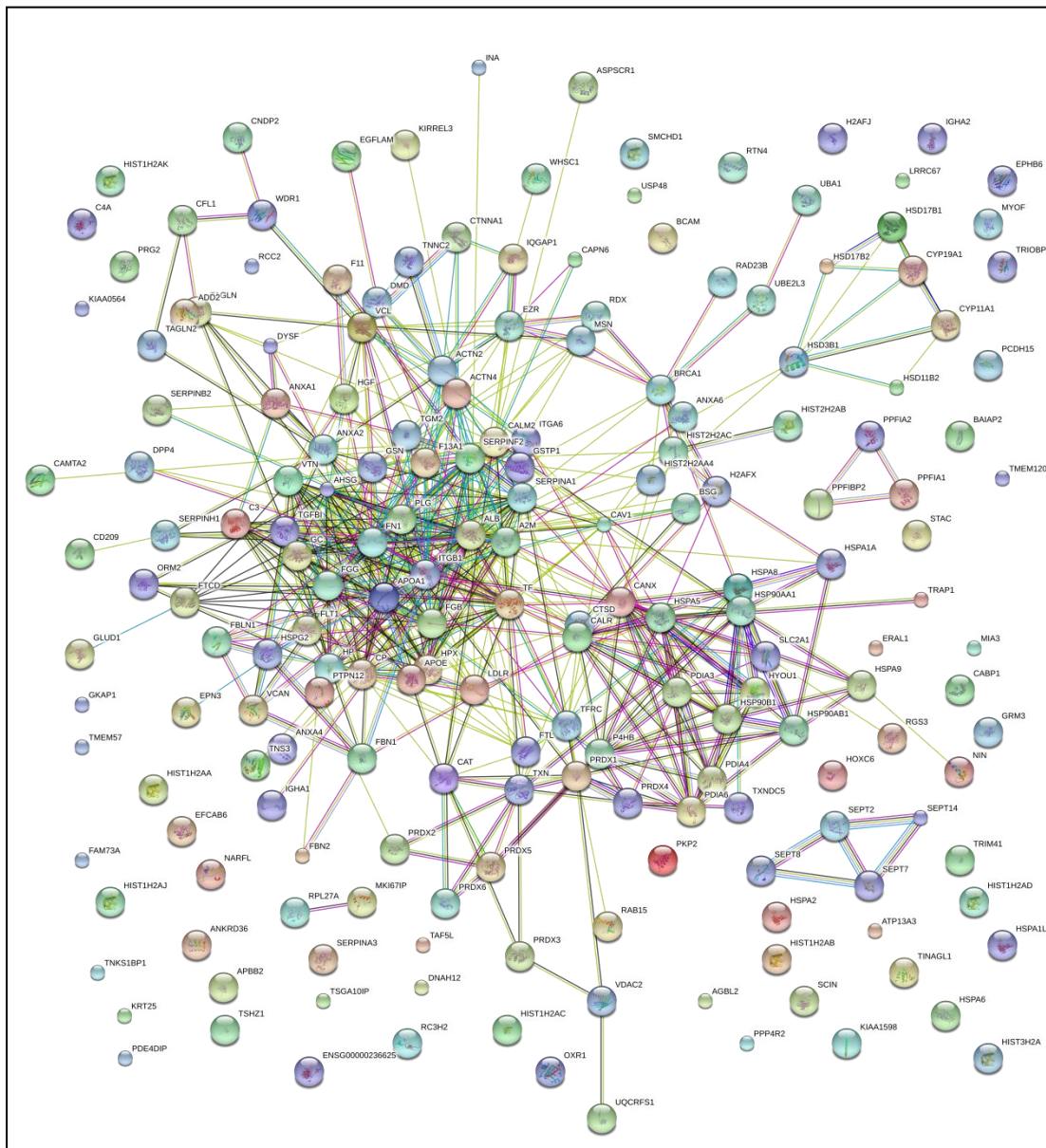
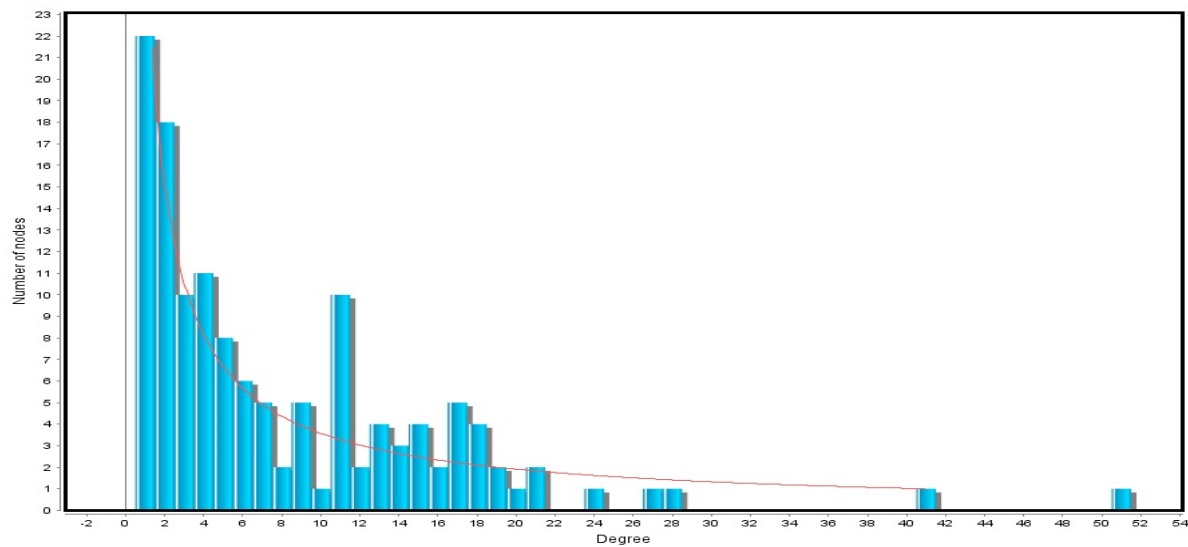


Figure S1- 1: **Protein- protein interaction network predicted by STRING.**

STRING 9.1 database (Search Tool for the Retrieval of Interacting Genes) was used to search for PPIs of 180 differentially expressed proteins (DEGs) (figure S1-1). The PPI obtained from STRING was visualised using Cytoscape software. Network analysis showed 132 nodes and 531 edges, and topological parameter showed that some nodes in the network had a large number of connections (figure S1-2a: degree of nodes), the hub nodes with high degree

included genes such as ALB, FN1, TF, PLG, APOA1, A2M, CANX, CALM2, CALR, FGG, SERPINF2, ITGB1, HPX, TGFBI, SERPINA1, FGB, VTN, APOE, VCL, ACTN2, CP, HSPA5, HP, AHSG, GC, CAV1, ANXA2, ACTN4, HSP90B1, EZR, TXN, GSN, HSP90AA1, P4HB, BRCA1, PDIA3, PRDX1, PDIA6, C3, LDLR, F13A1, CAT, FTL, HSPA8, TFRC (degree >10) (figure S1-2b: hub nodes).

a



b

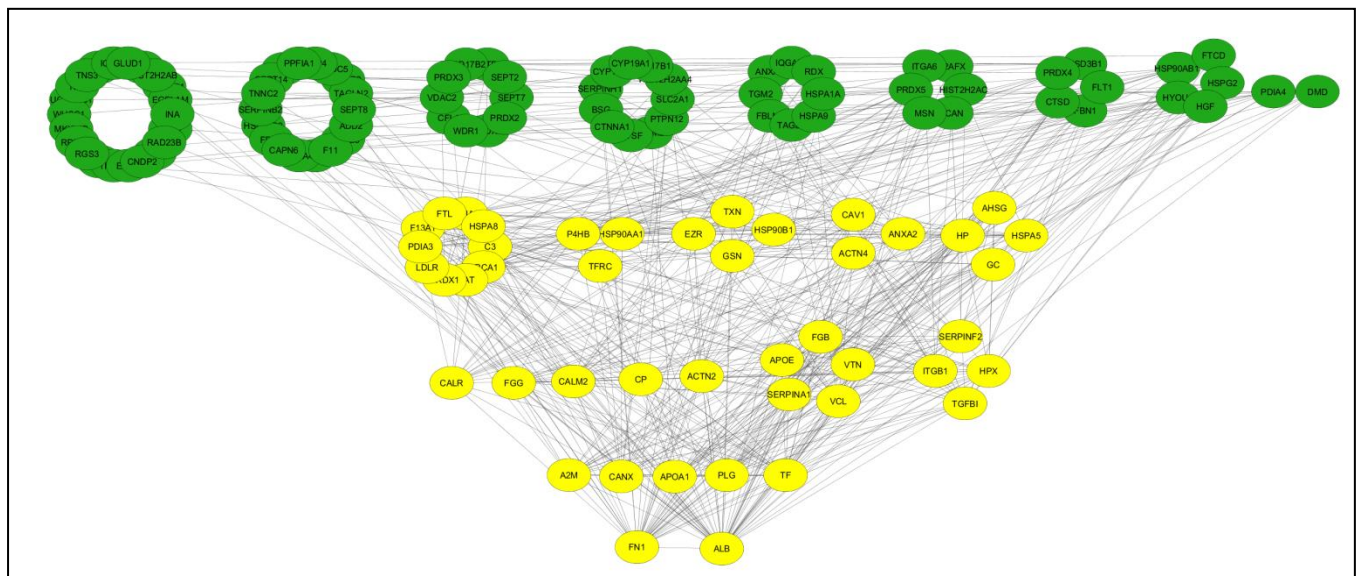
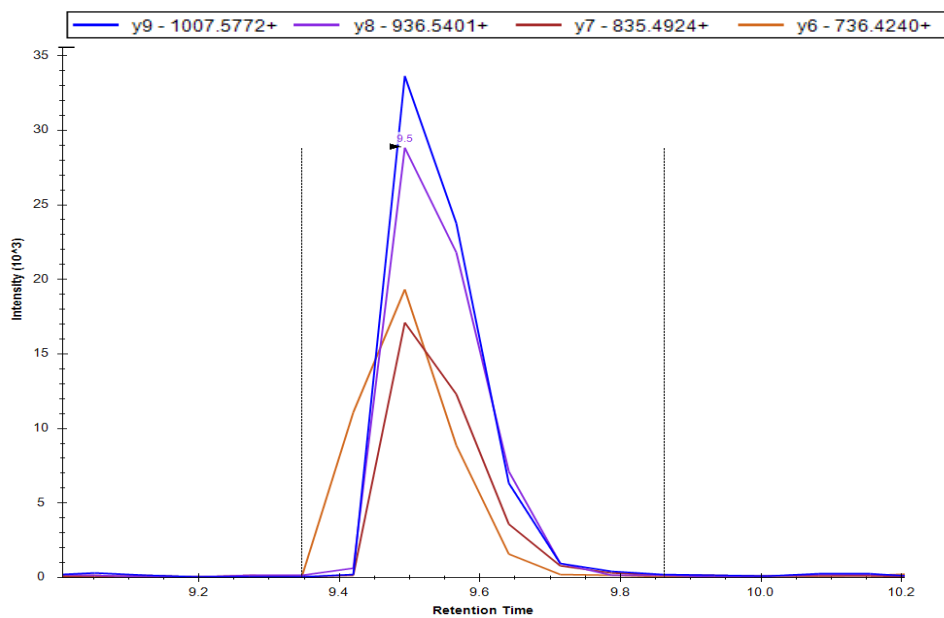
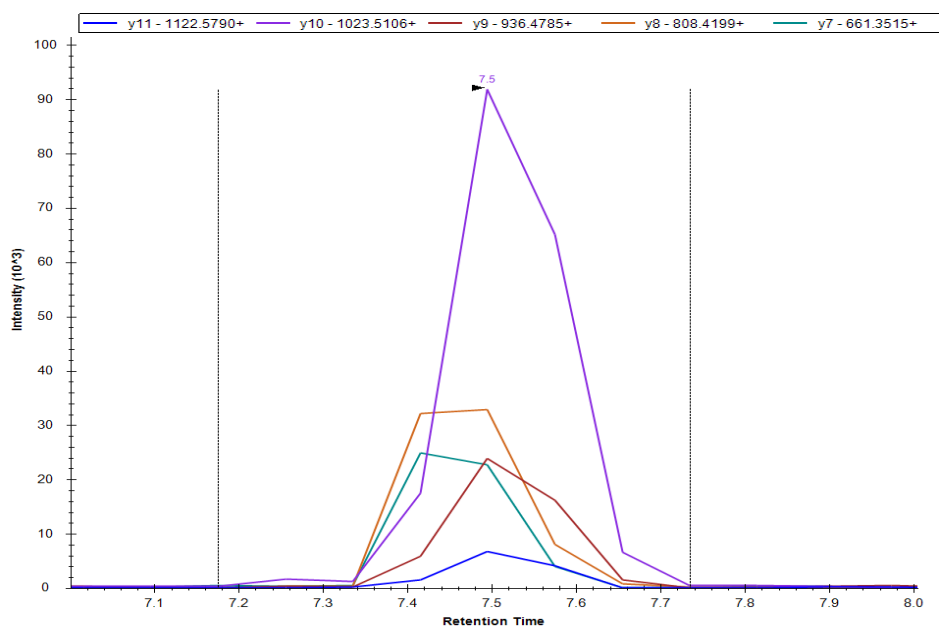
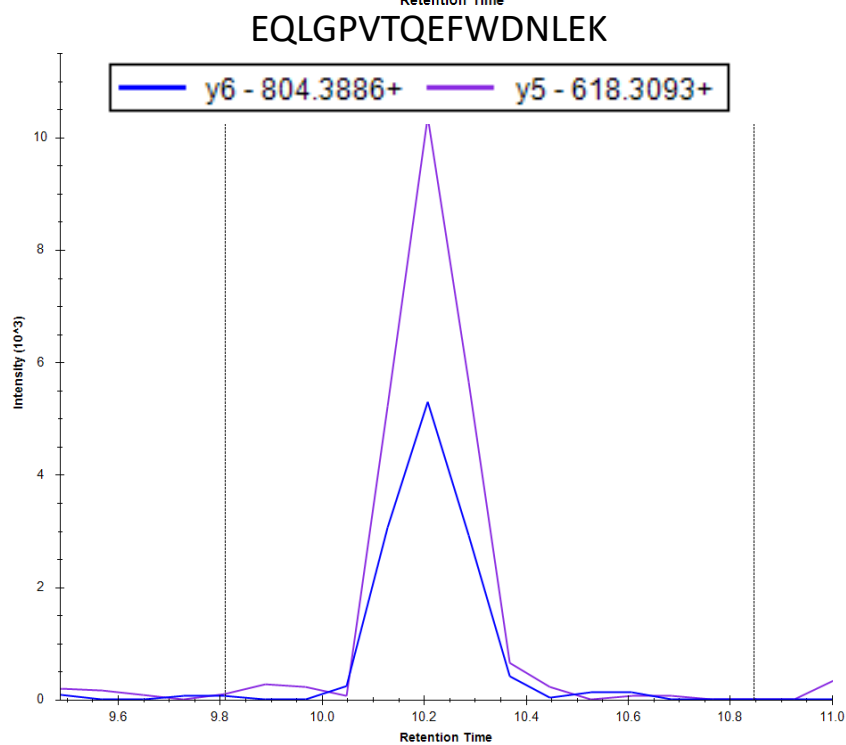
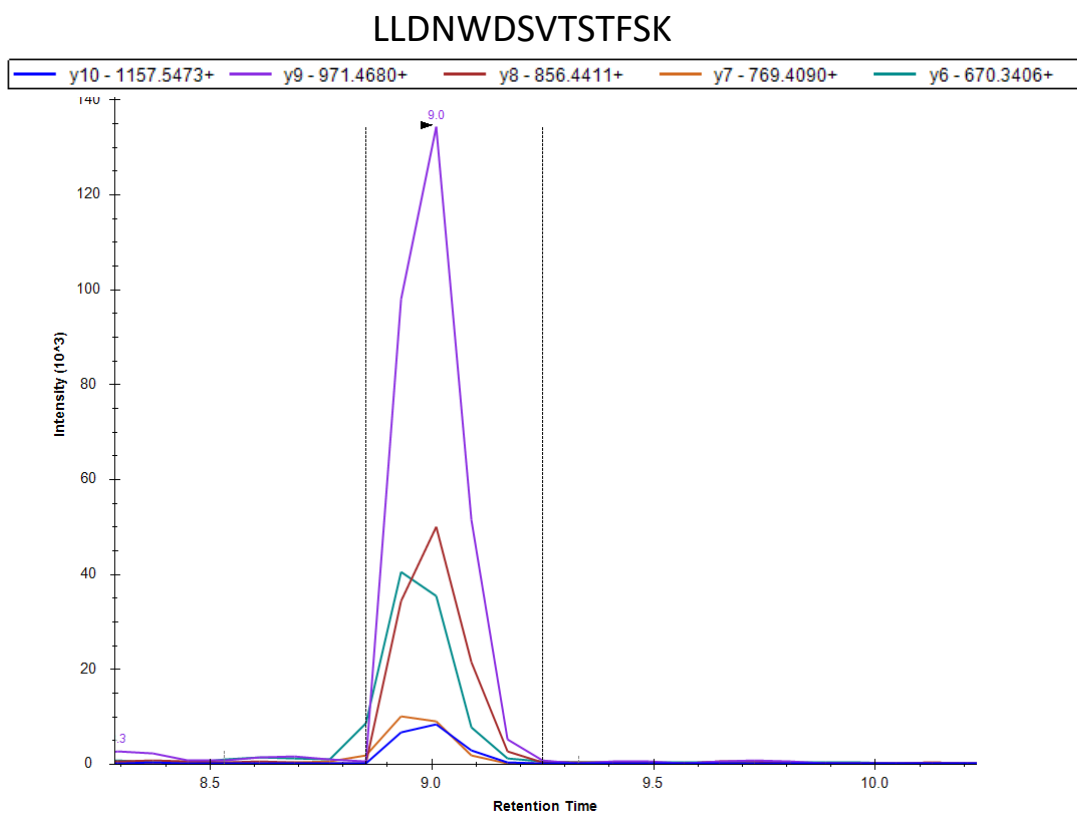
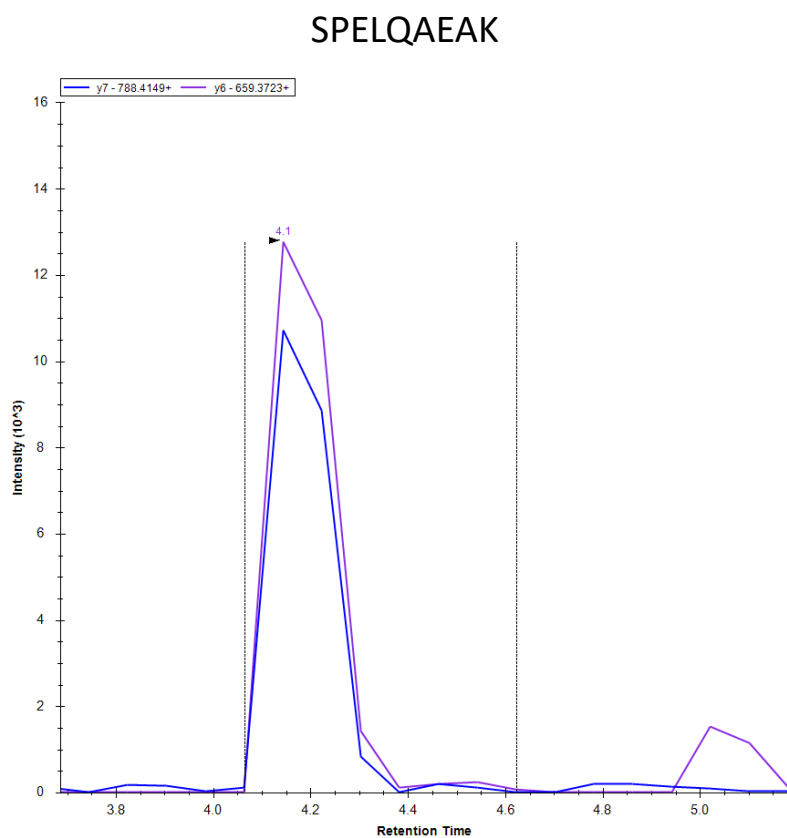
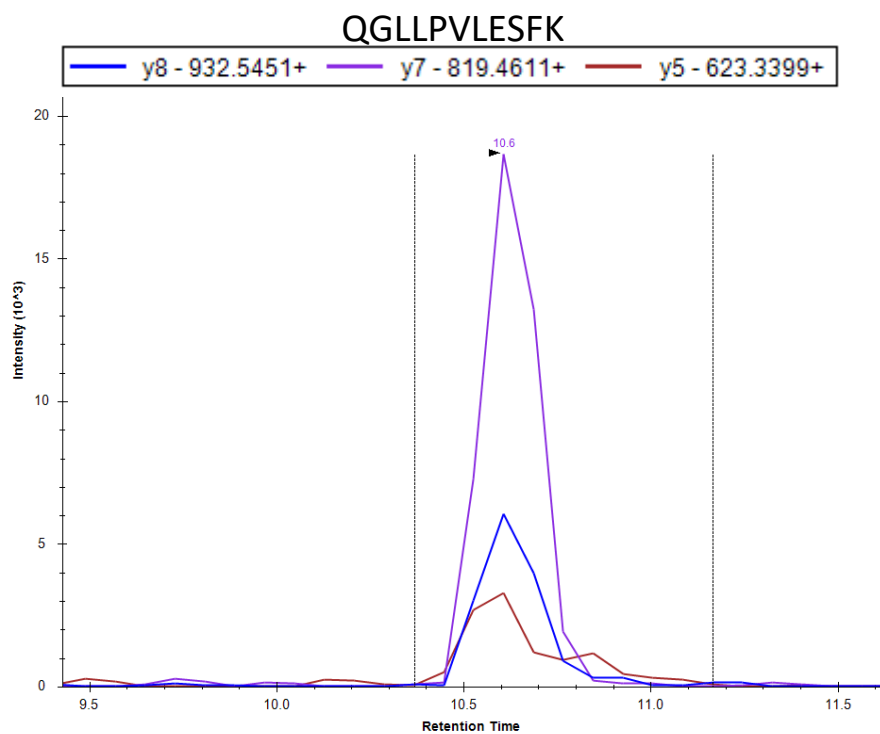


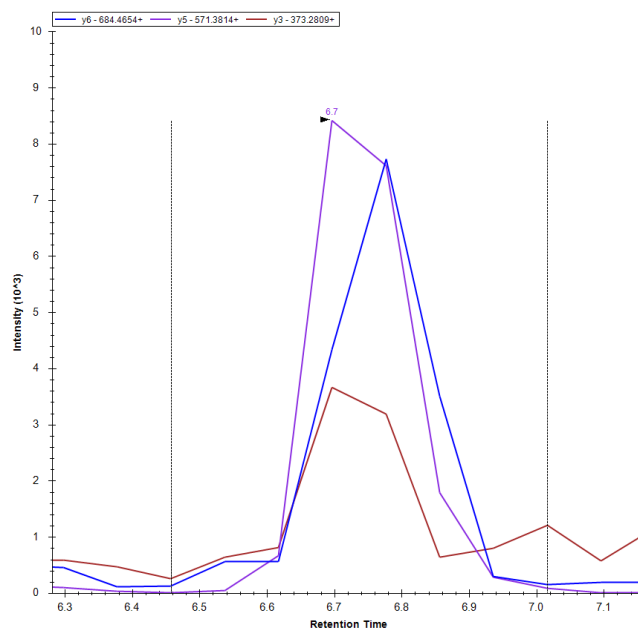
Figure S1-2: a) Degree node, b) Hub node

Supporting information Figure S2: MRM transitions plots for individual peptides**DLATVYVDVLK****DYVSQFEGSALGK**

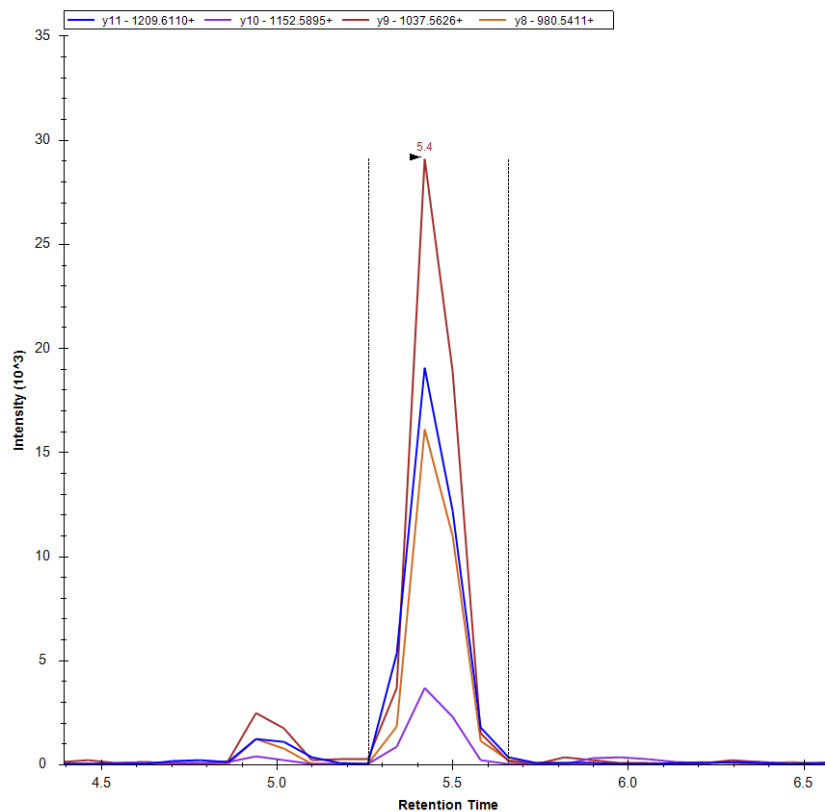




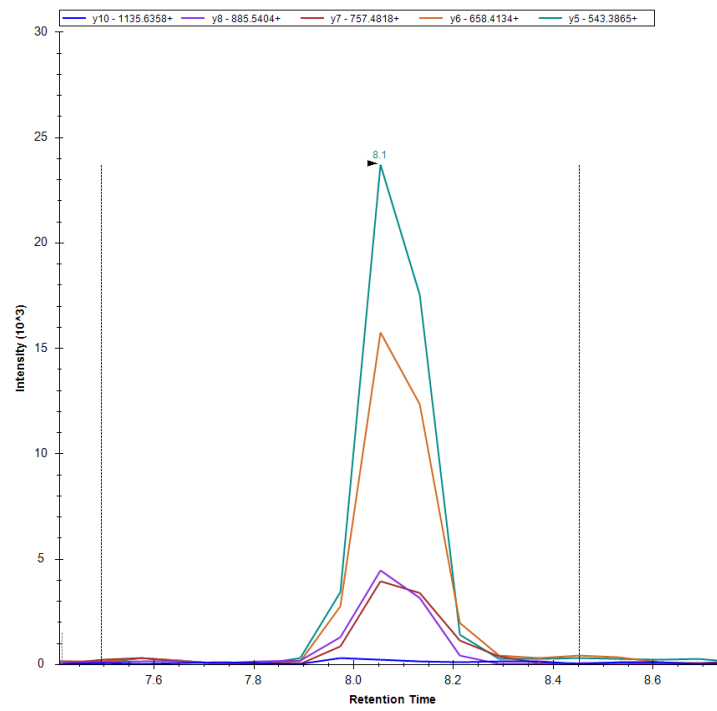
EQLTPLIK



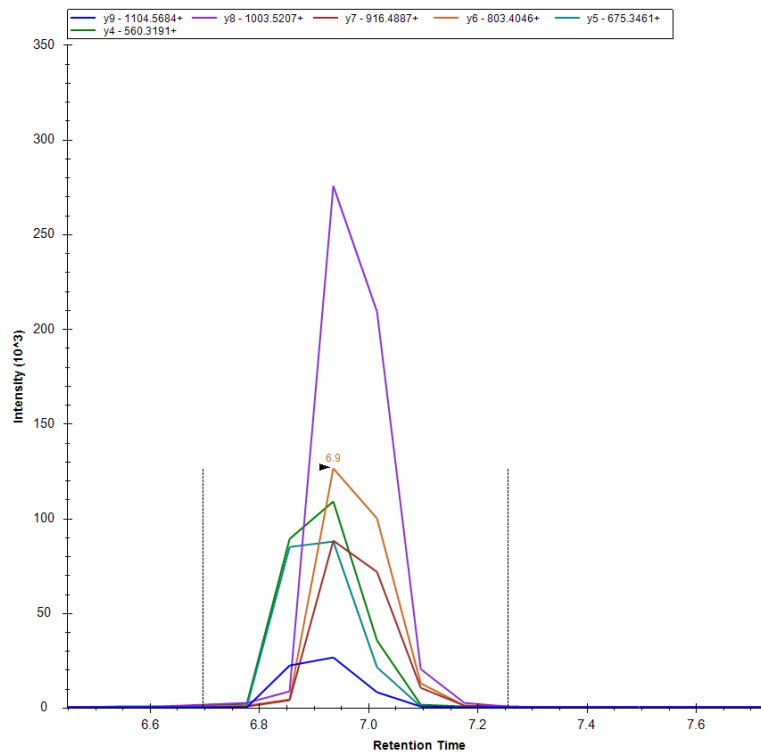
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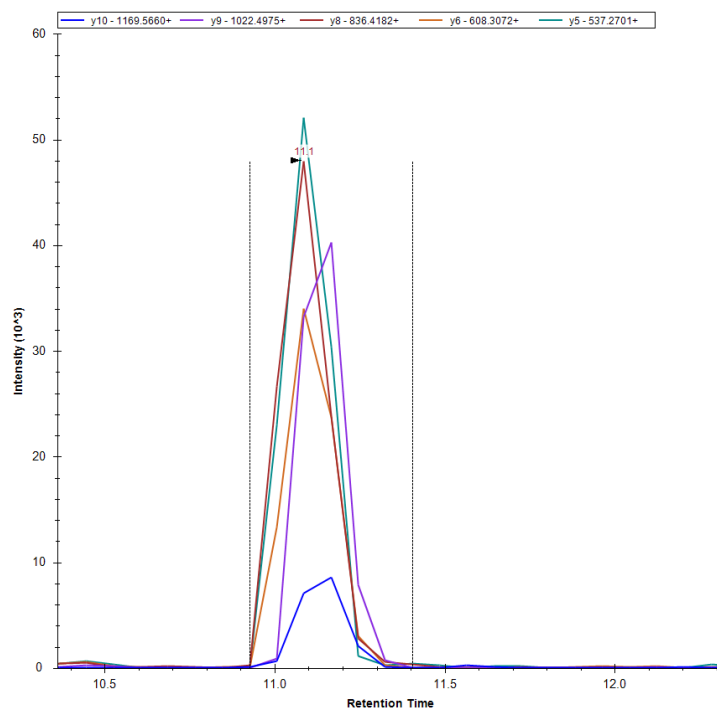
VVLHPNYSQVDIGLIK



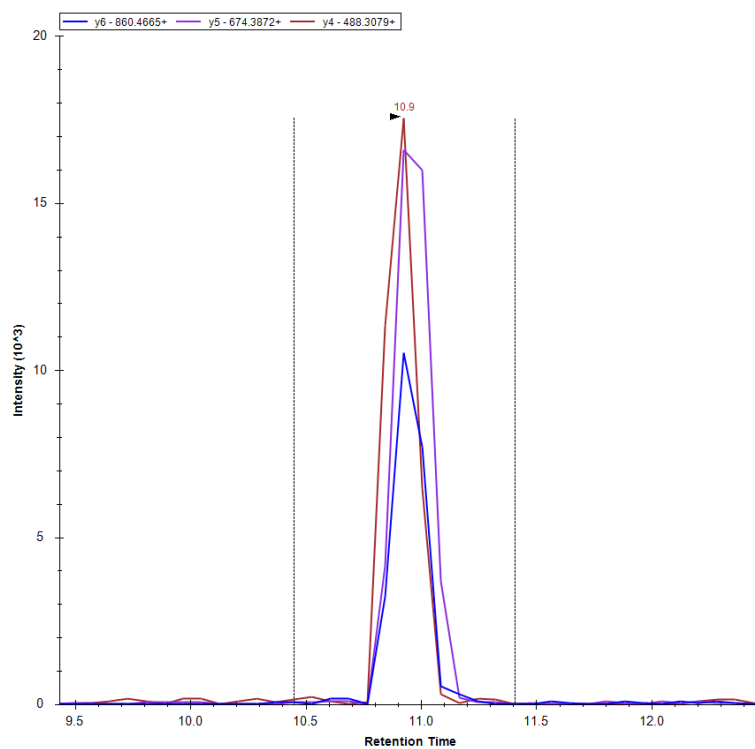
VTSIQDWVQK



EWFWDLATGTMK



LWWLDLK



Supporting information Figure S3 [A] Total ion count of all the MRM runs [B] Graph representing coefficient of variance versus retention time of peptides [C] Test for normality for peptide intensities

Figure S3[A] Total ion count of all the MRM runs

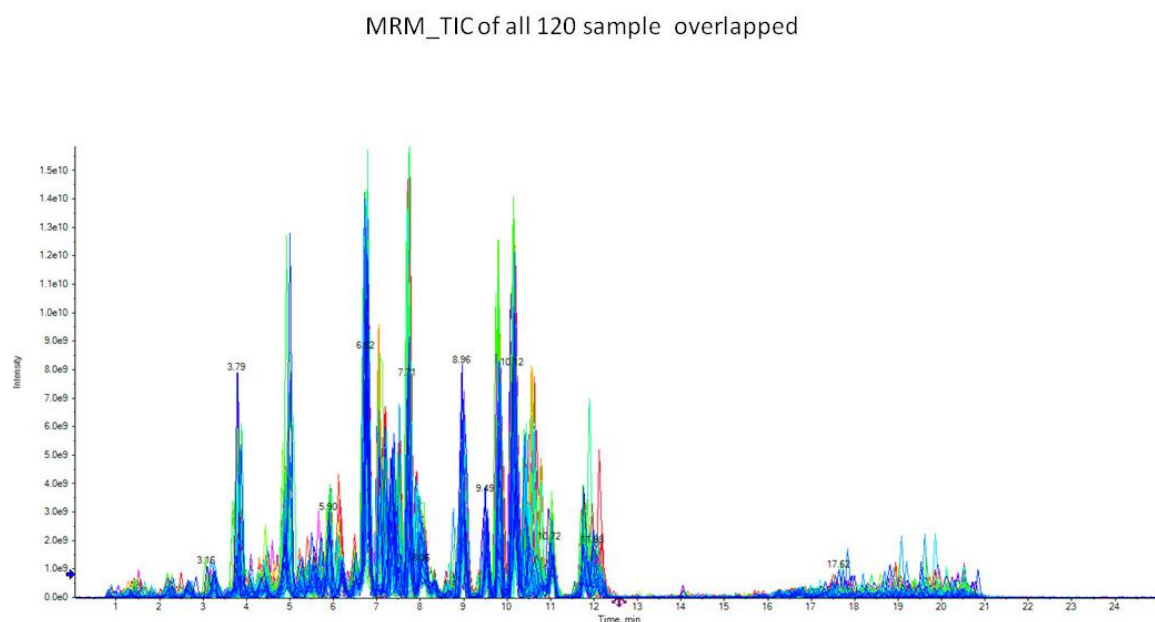
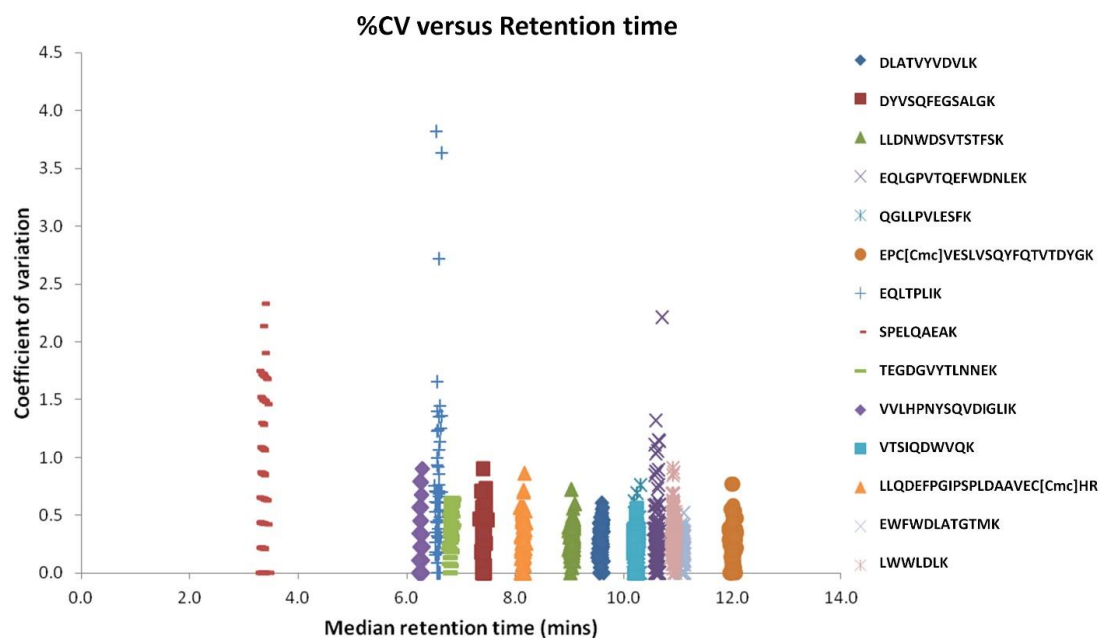


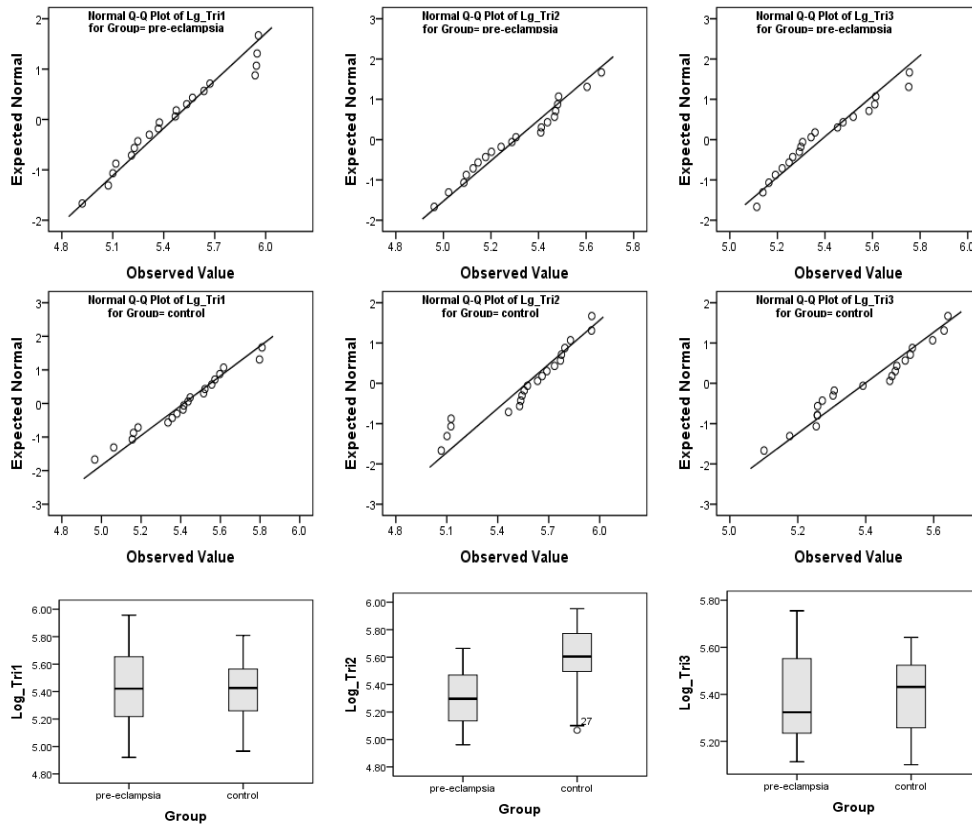
Figure S3 [B] Graph representing coefficient of variance versus retention time of peptides



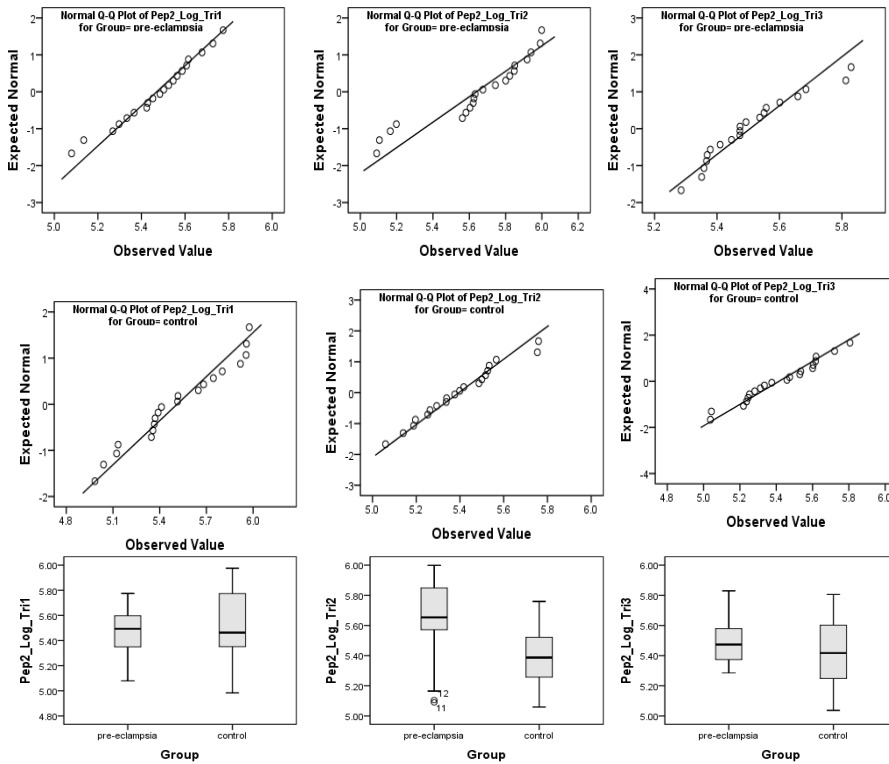
Transitions have consistent retention time with coefficient of variation less than 4%

Figure S3 [C] Test for normality for peptide intensities

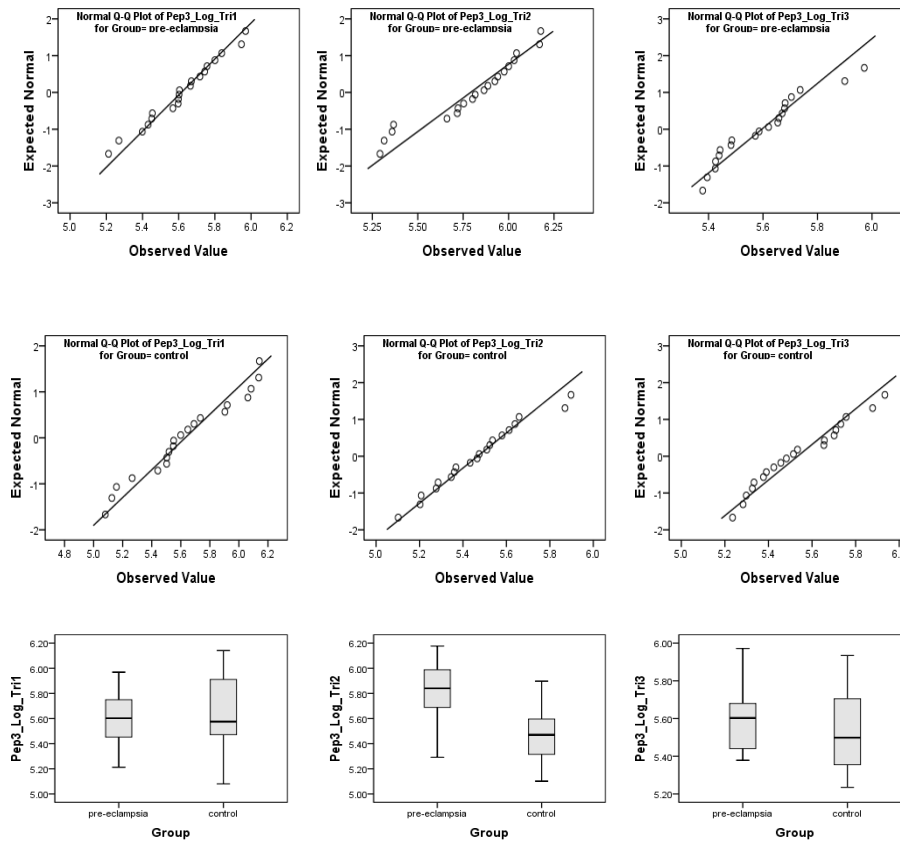
Normal Q-Q plot_ApoA1_DLATVYVDVLK



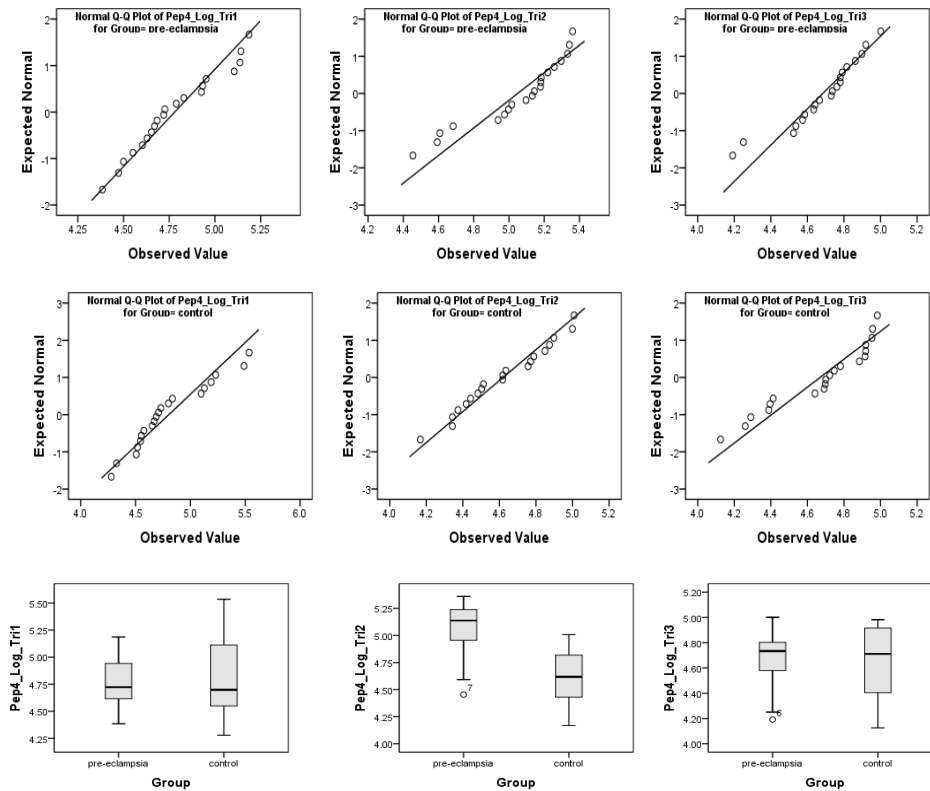
Normal Q-Q plot_ApoA1_DYVSQFEGSALGK



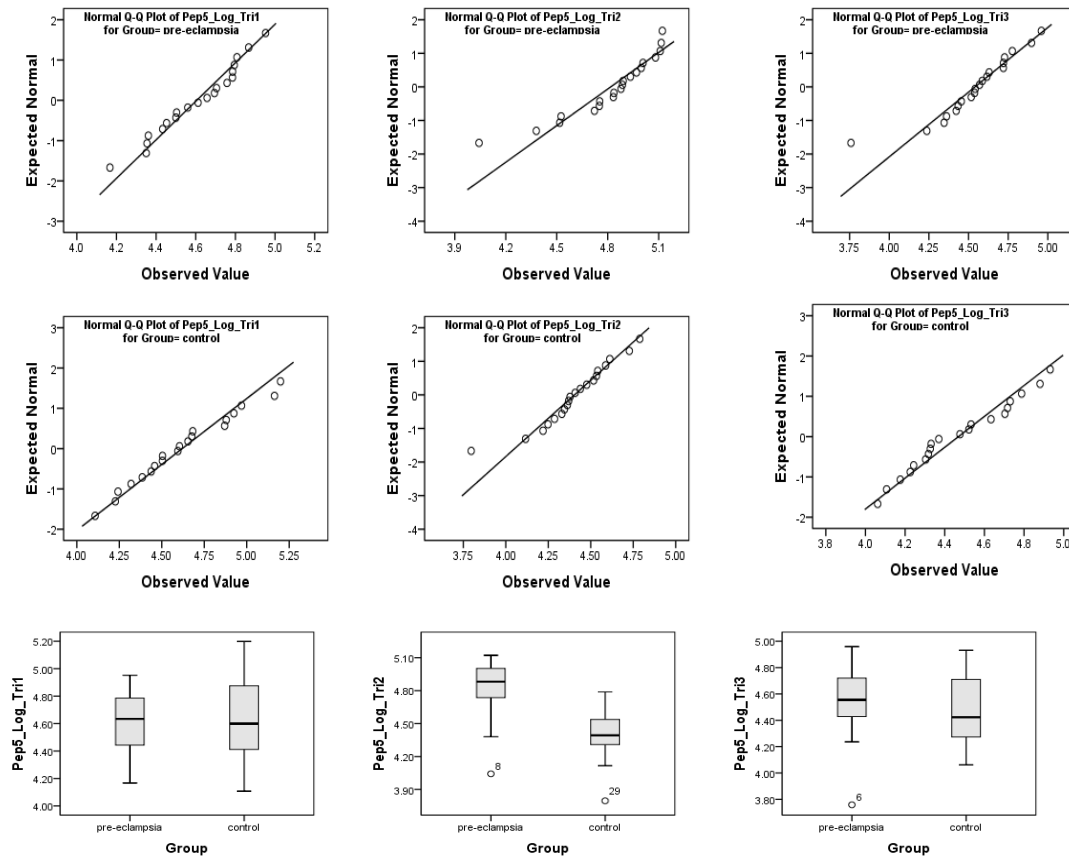
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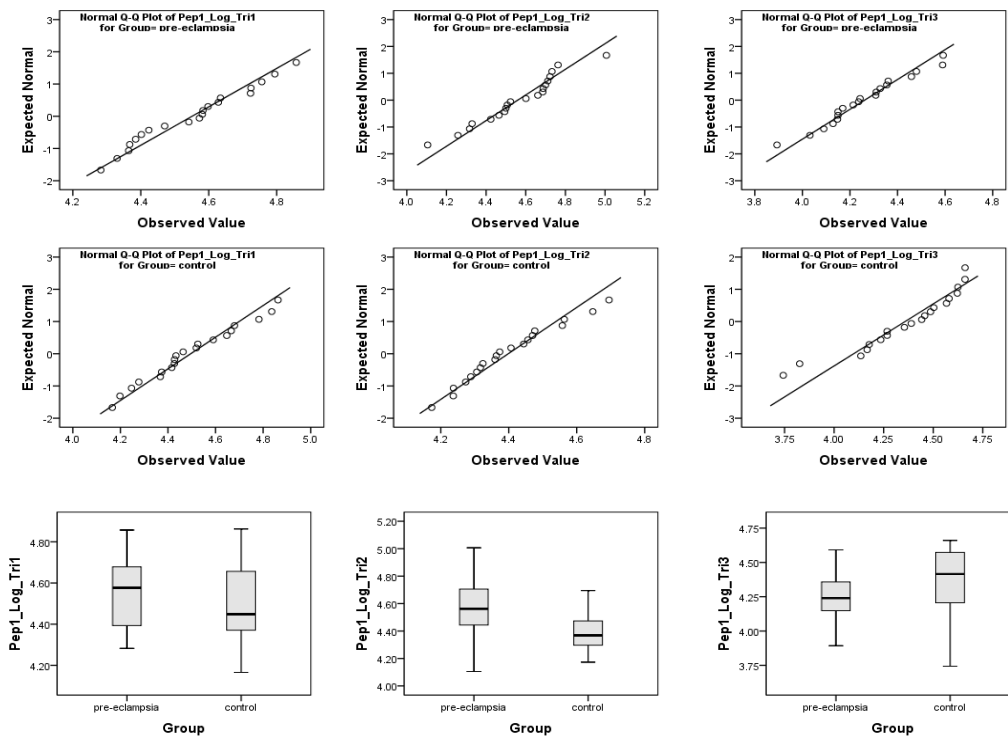
Normal Q-Q plot_ApoA1_EQLGPVTQEFWDNLEK



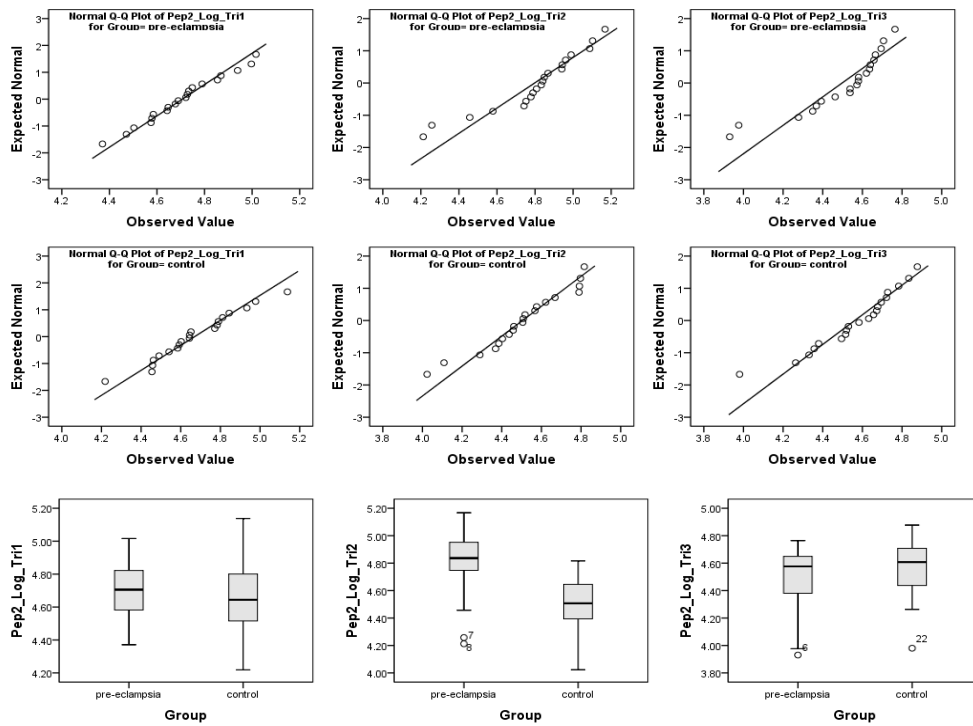
Normal Q-Q plot_ApoA1_QGLLPVLESFK



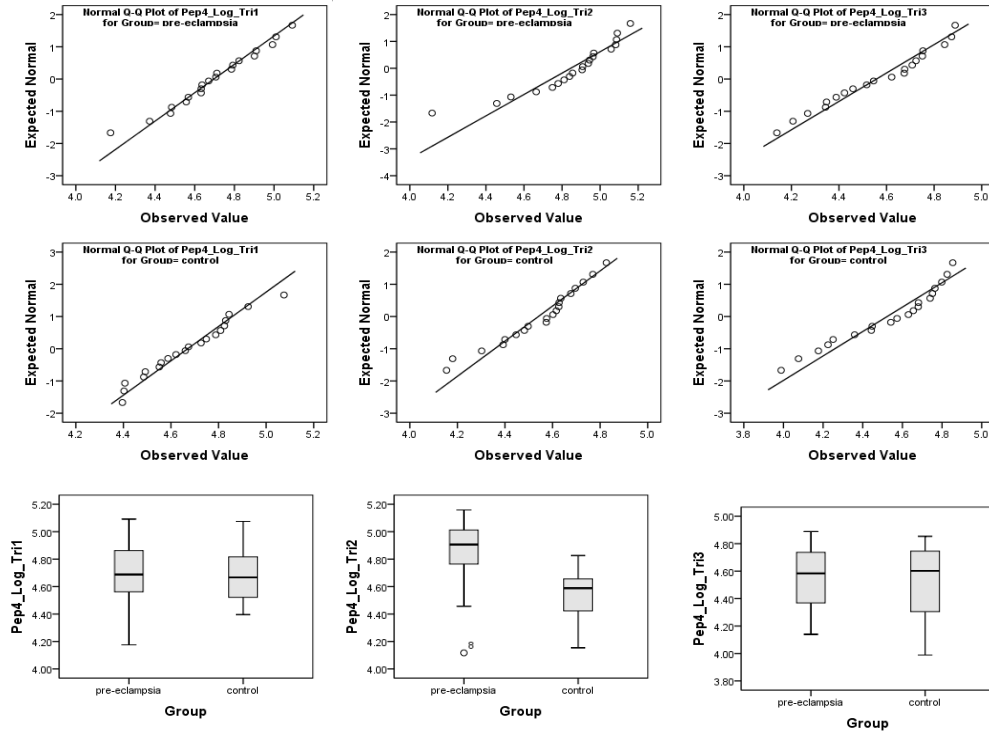
Normal Q-Q plot_ApoA2_EPC[Cmc]VESLVSQYFQTVTDYKG



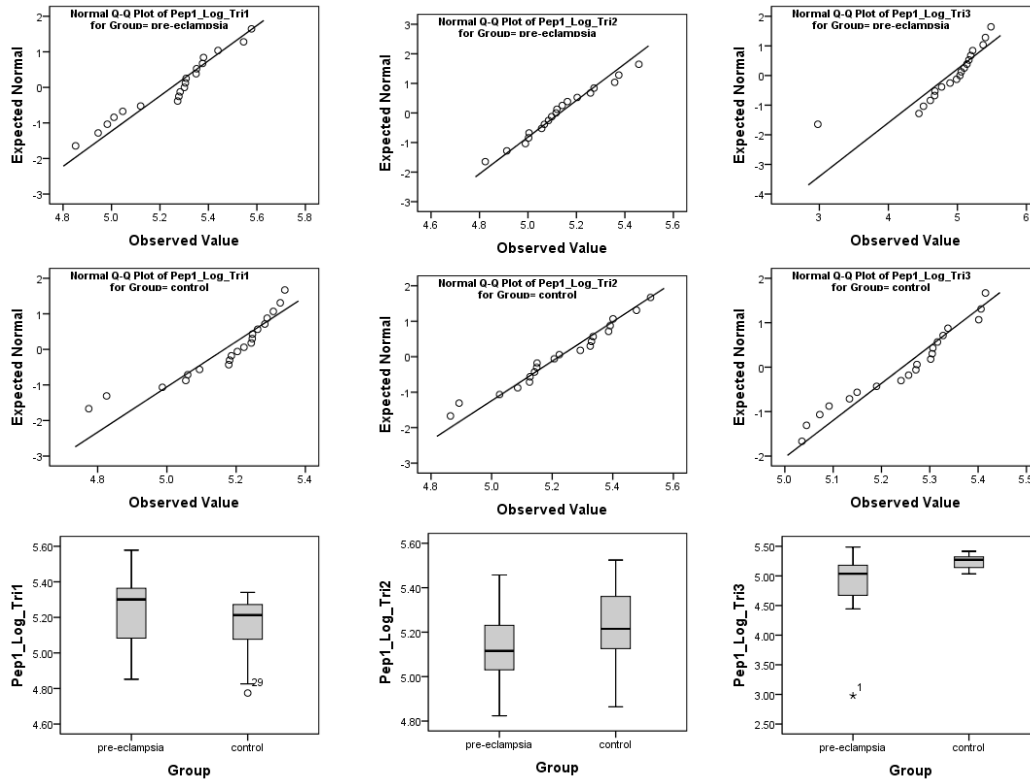
Normal Q-Q plot_ApoA2_EQLTPLIK



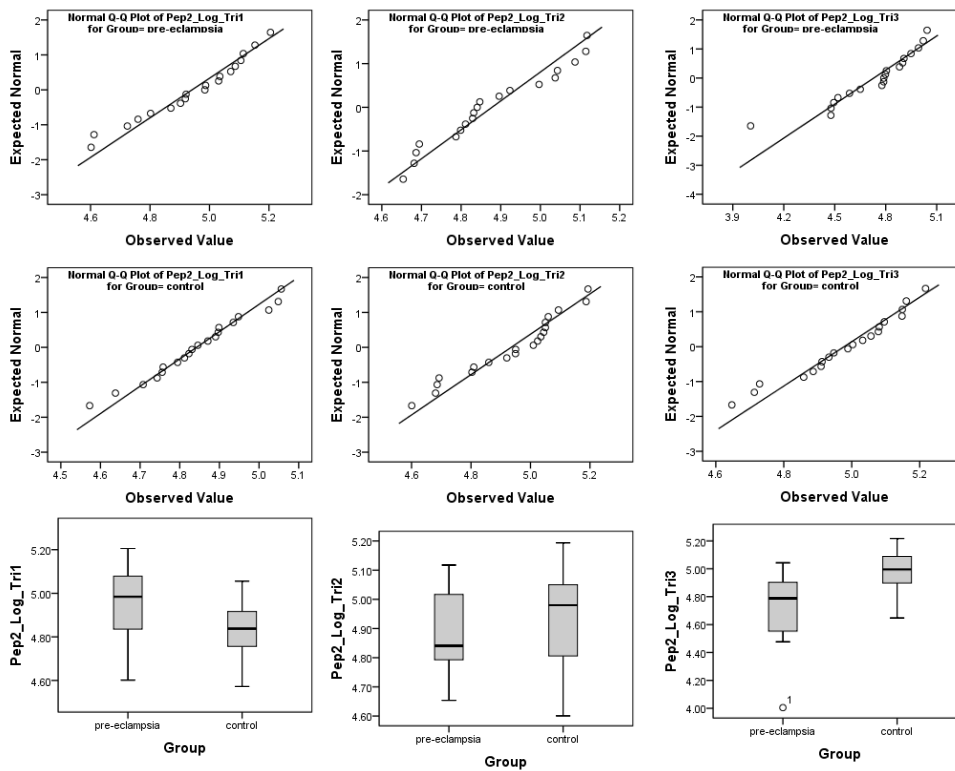
Normal Q-Q plot ApoA2 SPELQAEAK



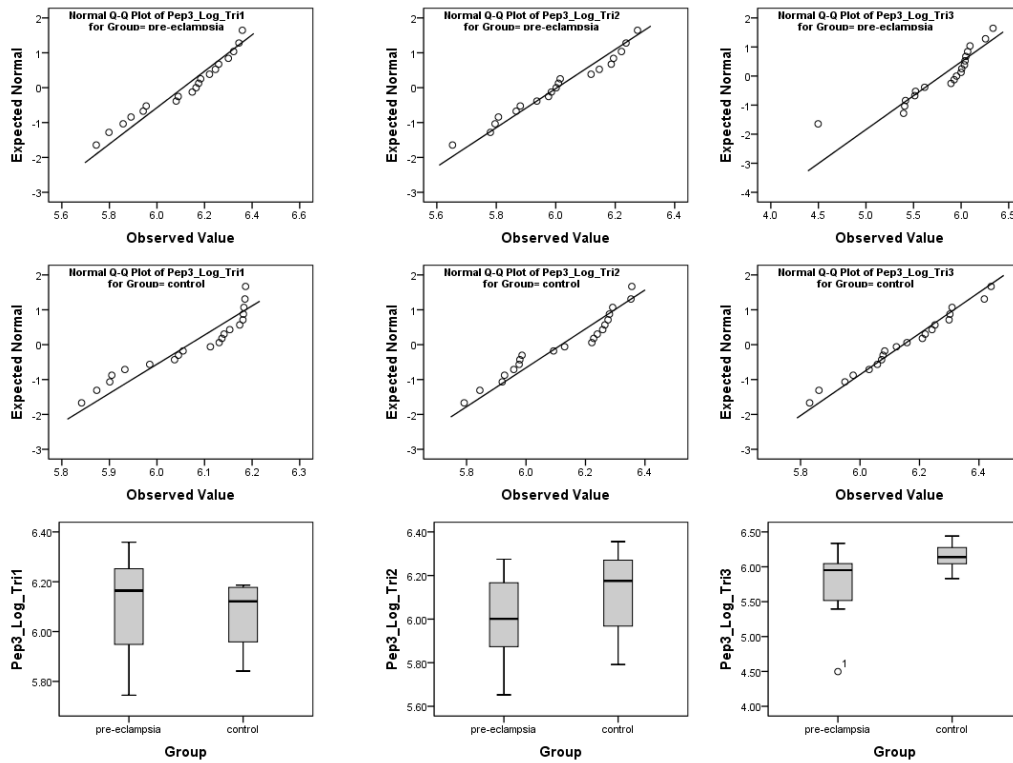
Normal Q-Q plot_Hp_TEGDGVYTLNNEK



Normal Q-Q plot_Hp_VVLHPNYSQVDIGLIK



Normal Q-Q plot_Hp_VTSIQDWVQK



Supporting information; Table S1**List of Differentially expressed proteins****Up-regulated in pre-eclampsia**

Accession	Description	Max fold change	Anova (p value)
P11021	78 kDa glucose-regulated protein OS=Homo sapiens GN=HSPA5 PE=1 SV=2	3.0	0.0072
P08107;P08107-2	Heat shock 70 kDa protein 1A/1B OS=Homo sapiens GN=HSPA1A PE=1 SV=5	2.6	0.0102
P02751;P02751-10;P02751-11;P02751-13;P02751-14;P02751-15;P02751-16;P02751-17;P02751-2;P02751-3;P02751-4;P02751-6;P02751-7;P02751-8;P02751-9	Fibronectin OS=Homo sapiens GN=FN1 PE=1 SV=4	2.5	0.0042
Q12931	Heat shock protein 75 kDa, mitochondrial OS=Homo sapiens GN=TRAP1 PE=1 SV=3	2.1	0.0041
Q68CZ2;Q68CZ2-2;Q68CZ2-3;Q68CZ2-4	Tensin-3 OS=Homo sapiens GN=TNS3 PE=1 SV=2	2.1	0.0064
P02787	Serotransferrin OS=Homo sapiens GN=TF PE=1 SV=3	2.1	0.0166
P02765	Alpha-2-HS-glycoprotein OS=Homo sapiens GN=AHSG PE=1 SV=1	2.0	0.0216
P19652	Alpha-1-acid glycoprotein 2 OS=Homo sapiens GN=ORM2 PE=1 SV=2	2.0	0.0126
Q9NQC3;Q9NQC3-2;Q9NQC3-4;Q9NQC3-5;Q9NQC3-6	Reticulon-4 OS=Homo sapiens GN=RTN4 PE=1 SV=2	2.0	0.0000
P14625	Endoplasmic reticulum chaperone protein OS=Homo sapiens GN=HSP90B1 PE=1 SV=1	2.0	0.0108
P18206;P18206-2;P18206-3	Vinculin OS=Homo sapiens GN=VCL PE=1 SV=4	2.0	0.0000
P11142;P11142-2	Heat shock cognate 71 kDa protein OS=Homo sapiens GN=HSPA8 PE=1 SV=1	1.9	0.0042
P38646	Stress-70 protein, mitochondrial OS=Homo sapiens GN=HSPA9 PE=1 SV=2	1.9	0.0507
O15197;O15197-2;O15197-3	Ephrin type-B receptor 6 OS=Homo sapiens GN=EPHB6 PE=1 SV=4	1.9	0.0038
P54652	Heat shock-related 70 kDa protein 2 OS=Homo sapiens GN=HSPA2 PE=1 SV=1	1.9	0.0323
P45880;P45880-1;P45880-2	Voltage-dependent anion-selective channel protein 2 OS=Homo sapiens GN=VDAC2 PE=1 SV=2	1.9	0.0560
P35556;P35556-2	Fibrillin-2 OS=Homo sapiens GN=FBN2 PE=1 SV=3	1.9	0.0037
Q92599;Q92599-2;Q92599-4	Septin-8 OS=Homo sapiens GN=SEPT8 PE=1 SV=4	1.8	0.0173
P21980;P21980-2;P21980-3	Protein-glutamine gamma-glutamyltransferase 2 OS=Homo sapiens GN=TGM2 PE=1 SV=2	1.8	0.0015
Q5U5Z8	Cytosolic carboxypeptidase 2 OS=Homo sapiens GN=AGBL2 PE=1 SV=2	1.7	0.0256
Q06830	Peroxiredoxin-1 OS=Homo sapiens GN=PRDX1 PE=1 SV=1	1.7	0.0374
P27824	Calnexin OS=Homo sapiens GN=CANX PE=1 SV=2	1.7	0.0272
O75923;O75923-10;O75923-11;O75923-12;O75923-13;O75923-14;O75923-15;O75923-2;O75923-3;O75923-4;O75923-5;O75923-6;O75923-7;O75923-8;O75923-9	Dysferlin OS=Homo sapiens GN=DYSF PE=1 SV=1	1.7	0.0036

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P46940	Ras GTPase-activating-like protein IQGAP1 OS=Homo sapiens GN=IQGAP1 PE=1 SV=1	1.7	0.0033
P98160	Basement membrane-specific heparan sulfate proteoglycan core protein OS=Homo sapiens GN=HSPG2 PE=1 SV=4	1.7	0.0007
P02647	Apolipoprotein A-I OS=Homo sapiens GN=APOA1 PE=1 SV=1	1.7	0.0053
P17066	Heat shock 70 kDa protein 6 OS=Homo sapiens GN=HSPA6 PE=1 SV=2	1.7	0.0123
P30048;P30048-2	Thioredoxin-dependent peroxide reductase, mitochondrial OS=Homo sapiens GN=PRDX3 PE=1 SV=3	1.7	0.0435
Q8ND30;Q8ND30-2	Liprin-beta-2 OS=Homo sapiens GN=PPFIBP2 PE=1 SV=3	1.6	0.0106
P08133;P08133-2	Annexin A6 OS=Homo sapiens GN=ANXA6 PE=1 SV=3	1.6	0.0039
P50895	Basal cell adhesion molecule OS=Homo sapiens GN=BCAM PE=1 SV=2	1.6	0.0223
Q15582	Transforming growth factor-beta-induced protein ig-h3 OS=Homo sapiens GN=TGFBI PE=1 SV=1	1.6	0.0083
P34931	Heat shock 70 kDa protein 1-like OS=Homo sapiens GN=HSPA1L PE=1 SV=2	1.6	0.0063
Q96QU1;Q96QU1-6	Protocadherin-15 OS=Homo sapiens GN=PCDH15 PE=1 SV=2	1.5	0.0138
P06396;P06396-3	Gelsolin OS=Homo sapiens GN=GSN PE=1 SV=1	1.5	0.0087
P27487	Dipeptidyl peptidase 4 OS=Homo sapiens GN=DPP4 PE=1 SV=2	1.5	0.0318
Q13162	Peroxiredoxin-4 OS=Homo sapiens GN=PRDX4 PE=1 SV=1	1.5	0.0350
P00367	Glutamate dehydrogenase 1, mitochondrial OS=Homo sapiens GN=GLUD1 PE=1 SV=2	1.5	0.0176
P35241;P35241-4;P35241-5	Radixin OS=Homo sapiens GN=RDY PE=1 SV=1	1.5	0.0026
P07900-2;P07900;Q14568	Isoform 2 of Heat shock protein HSP 90-alpha OS=Homo sapiens GN=HSP90AA1	1.5	0.0281
P02649	Apolipoprotein E OS=Homo sapiens GN=APOE PE=1 SV=1	1.5	0.0306
P09211	Glutathione S-transferase P OS=Homo sapiens GN=GSTP1 PE=1 SV=2	1.5	0.0435
P00747	Plasminogen OS=Homo sapiens GN=PLG PE=1 SV=2	1.5	0.0041
Q6ZSZ6-2;Q6ZSZ6	Isoform 2 of Teashirt homolog 1 OS=Homo sapiens GN=TSHZ1	1.5	0.0131
Q5THR3;Q5THR3-2;Q5THR3-3;Q5THR3-4;Q5THR3-5	EF-hand calcium-binding domain-containing protein 6 OS=Homo sapiens GN=EFCAB6 PE=1 SV=1	1.4	0.0046
Q8WV44-2	Isoform 2 of E3 ubiquitin-protein ligase TRIM41 OS=Homo sapiens GN=TRIM41	1.4	0.0062
P17948;P17948-5;P17948-6;P17948-7;P17948-8	Vascular endothelial growth factor receptor 1 OS=Homo sapiens GN=FLT1 PE=1 SV=2	1.4	0.0087
A6QL64;A6QL64-4;A6QL64-5	Ankyrin repeat domain-containing protein 36A OS=Homo sapiens GN=ANKRD36 PE=2 SV=3	1.4	0.0007
P35221;P35221-2;P35221-3	Catenin alpha-1 OS=Homo sapiens GN=CTNNA1 PE=1 SV=1	1.4	0.0004
P30101	Protein disulfide-isomerase A3 OS=Homo sapiens GN=PDIA3 PE=1 SV=4	1.4	0.0017
P08238	Heat shock protein HSP 90-beta OS=Homo sapiens GN=HSP90AB1 PE=1 SV=4	1.4	0.0448
Q16181;Q16181-2;Q92599-3	Septin-7 OS=Homo sapiens GN=SEPT7 PE=1 SV=2	1.4	0.0025
Q8N4C6-5;Q8N4C6;Q8N4C6-10;Q8N4C6-11;Q8N4C6-	Isoform 5 of Ninein OS=Homo sapiens GN=NIN	1.4	0.0234

2;Q8N4C6-4;Q8N4C6-6;Q8N4C6-7;Q8N4C6-9			
P32119;P32119-2	Peroxiredoxin-2 OS=Homo sapiens GN=PRDX2 PE=1 SV=5	1.4	0.0107
P27797	Calreticulin OS=Homo sapiens GN=CALR PE=1 SV=1	1.4	0.0245
P02786	Transferrin receptor protein 1 OS=Homo sapiens GN=TFRC PE=1 SV=2	1.4	0.0381
A3KMH1;A3KMH1-2;A3KMH1-3	von Willebrand factor A domain-containing protein 8 OS=Homo sapiens GN=VWA8 PE=1 SV=2	1.3	0.0350
Q9BZE9-3;Q9BZE9;Q9BZE9-2	Isoform 3 of Tether containing UBX domain for GLUT4 OS=Homo sapiens GN=ASPSCR1	1.3	0.0507
Q9Y6Q1	Calpain-6 OS=Homo sapiens GN=CAPN6 PE=1 SV=2	1.3	0.0034
Q8N5G2;Q8N5G2-2;Q8N5G2-3	Macoilin OS=Homo sapiens GN=TMEM57 PE=1 SV=1	1.3	0.0275
Q99469	SH3 and cysteine-rich domain-containing protein OS=Homo sapiens GN=STAC PE=1 SV=1	1.3	0.0207
P30041	Peroxiredoxin-6 OS=Homo sapiens GN=PRDX6 PE=1 SV=3	1.3	0.0227
Q9Y4L1	Hypoxia up-regulated protein 1 OS=Homo sapiens GN=HYOU1 PE=1 SV=1	1.3	0.0186
Q9H6Q4;Q9H6Q4-3	Cytosolic Fe-S cluster assembly factor NARFL OS=Homo sapiens GN=NARFL PE=1 SV=1	1.3	0.0442
Q05209	Tyrosine-protein phosphatase non-receptor type 12 OS=Homo sapiens GN=PTPN12 PE=1 SV=3	1.3	0.0344
Q7Z4L9	Protein phosphatase 1 regulatory subunit 42 OS=Homo sapiens GN=PPP1R42 PE=2 SV=2	1.3	0.0522
P16104	Histone H2AX OS=Homo sapiens GN=H2AFX PE=1 SV=2	1.3	0.0026
Q16777;P04908;P0C0S8;P20671;Q6F113;Q7L7L0;Q8IUE6;Q93077;Q96KK5;Q96QV6;Q99878;Q9BTM1;Q9BTM1-2	Histone H2A type 2-C OS=Homo sapiens GN=HIST2H2AC PE=1 SV=4	1.3	0.0026
P54727;P54727-2	UV excision repair protein RAD23 homolog B OS=Homo sapiens GN=RAD23B PE=1 SV=1	1.3	0.0237
P04040	Catalase OS=Homo sapiens GN=CAT PE=1 SV=3	1.3	0.0062
Q9UQB8;Q9UQB8-2;Q9UQB8-3;Q9UQB8-4;Q9UQB8-5;Q9UQB8-6	Brain-specific angiogenesis inhibitor 1-associated protein 2 OS=Homo sapiens GN=BAIAP2 PE=1 SV=1	1.3	0.0363

Down-regulated in pre-eclampsia (negative sign indicates downregulation)

Accession	Description	Max fold change	Anova (p value)
P01009;P01009-2;P01009-3	Alpha-1-antitrypsin OS=Homo sapiens GN=SERPINA1 PE=1 SV=3	0.35	0.0424
Q9NY27	Serine/threonine-protein phosphatase 4 regulatory subunit 2 OS=Homo sapiens GN=PPP4R2 PE=1 SV=3	0.43	0.0014
Q16352	Alpha-internexin OS=Homo sapiens GN=INA PE=1 SV=2	0.47	0.0017
P01011	Alpha-1-antichymotrypsin OS=Homo sapiens GN=SERPINA3 PE=1 SV=2	0.47	0.0347
O43707;O43707-2;O43707-3	Alpha-actinin-4 OS=Homo sapiens GN=ACTN4 PE=1 SV=2	0.52	0.0011
Q5VU43;Q5VU43-10;Q5VU43-11;Q5VU43-4;Q5VU43-6;Q5VU43-7	Myomegalin OS=Homo sapiens GN=PDE4DIP PE=1 SV=1	0.55	0.0023
P35613-2;P35613;P35613-3;P35613-4	Isoform 2 of Basigin OS=Homo sapiens GN=BSG	0.55	0.0117

Q8NAN2;Q8NAN2-2	Protein FAM73A OS=Homo sapiens GN=FAM73A PE=2 SV=1	0.55	0.0090
P35612;P35612-2;P35612-3;P35612-4;P35612-5;P35612-6;P35612-7	Beta-adducin OS=Homo sapiens GN=ADD2 PE=1 SV=3	0.55	0.0000
P02790	Hemopexin OS=Homo sapiens GN=HPX PE=1 SV=2	0.58	0.0013
Q96KP4;Q96KP4-2	Cytosolic non-specific dipeptidase OS=Homo sapiens GN=CNDP2 PE=1 SV=2	0.58	0.0323
P23528	Cofilin-1 OS=Homo sapiens GN=CFL1 PE=1 SV=3	0.58	0.0074
P05556;P05556-2;P05556-3;P05556-4	Integrin beta-1 OS=Homo sapiens GN=ITGB1 PE=1 SV=2	0.58	0.0044
Q9H7F0;Q9H7F0-2	Probable cation-transporting ATPase 13A3 OS=Homo sapiens GN=ATP13A3 PE=1 SV=4	0.58	0.0242
Q08043	Alpha-actinin-3 OS=Homo sapiens GN=ACTN3 PE=1 SV=2	0.58	0.0004
P01024	Complement C3 OS=Homo sapiens GN=C3 PE=1 SV=2	0.58	0.0003
P26038	Moesin OS=Homo sapiens GN=MSN PE=1 SV=3	0.58	0.0000
Q13136;Q13136-2	Liprin-alpha-1 OS=Homo sapiens GN=PPFIA1 PE=1 SV=1	0.58	0.0092
O96028;O96028-3;O96028-5	Histone-lysine N-methyltransferase NSD2 OS=Homo sapiens GN=WHSC1 PE=1 SV=1	0.62	0.0061
Q6ZU15	Septin-14 OS=Homo sapiens GN=SEPT14 PE=1 SV=2	0.62	0.0002
P01023	Alpha-2-macroglobulin OS=Homo sapiens GN=A2M PE=1 SV=3	0.62	0.0061
Q8IZU9;Q8IZU9-2	Kin of IRRE-like protein 3 OS=Homo sapiens GN=KIRREL3 PE=1 SV=1	0.62	0.0450
P00738	Haptoglobin OS=Homo sapiens GN=HP PE=1 SV=1	0.62	0.0470
P68036-2;P68036;P68036-3	Isoform 2 of Ubiquitin-conjugating enzyme E2 L3 OS=Homo sapiens GN=UBE2L3	0.62	0.0380
O75529;O75529-2	TAF5-like RNA polymerase II p300/CBP-associated factor-associated factor 65 kDa subunit 5L OS=Homo sapiens GN=TAF5L PE=1 SV=1	0.62	0.0282
P13727	Bone marrow proteoglycan OS=Homo sapiens GN=PRG2 PE=1 SV=2	0.66	0.0095
P14210;P14210-2;P14210-3;P14210-4;P14210-5;P14210-6	Hepatocyte growth factor OS=Homo sapiens GN=HGF PE=1 SV=2	0.66	0.0004
Q8NBS9;Q8NBS9-2	Thioredoxin domain-containing protein 5 OS=Homo sapiens GN=TXNDC5 PE=1 SV=2	0.66	0.0235
Q5VSY0;Q5VSY0-2	G kinase-anchoring protein 1 OS=Homo sapiens GN=GKAP1 PE=1 SV=2	0.66	0.0035
A0MZ66-6;A0MZ66;A0MZ66-2;A0MZ66-3;A0MZ66-4;A0MZ66-5;A0MZ66-7;A0MZ66-8	Isoform 6 of Shootin-1 OS=Homo sapiens GN=KIAA1598	0.66	0.0192
O95954;O95954-2;O95954-3;O95954-4	Formimidoyltransferase-cyclodeaminase OS=Homo sapiens GN=FTCD PE=1 SV=2	0.66	0.0046
P37802	Transgelin-2 OS=Homo sapiens GN=TAGLN2 PE=1 SV=3	0.66	0.0058
P03951-2;P03951	Isoform 2 of Coagulation factor XI OS=Homo sapiens GN=F11	0.66	0.0039
Q9BXJ8-2;Q9BXJ8	Isoform 2 of Transmembrane protein 120A OS=Homo sapiens GN=TMEM120A	0.66	0.0059
Q9H201;Q9H201-2	Epsin-3 OS=Homo sapiens GN=EPN3 PE=2 SV=1	0.66	0.0164
P35609	Alpha-actinin-2 OS=Homo sapiens GN=ACTN2 PE=1 SV=1	0.66	0.0134
P02679-2;P02679	Isoform Gamma-A of Fibrinogen gamma chain OS=Homo sapiens GN=FGG	0.66	0.0013

O75334;O75334-2;O75334-3;O75334-4;O75334-5;O75334-6;O75334-7;O75334-8	Liprin-alpha-2 OS=Homo sapiens GN=PPFIA2 PE=1 SV=2	0.66	0.0190
Q9C0C2;Q9C0C2-2	182 kDa tankyrase-1-binding protein OS=Homo sapiens GN=TNKS1BP1 PE=1 SV=4	0.66	0.0006
P35555	Fibrillin-1 OS=Homo sapiens GN=FBN1 PE=1 SV=3	0.66	0.0040
P07339	Cathepsin D OS=Homo sapiens GN=CTSD PE=1 SV=1	0.66	0.0436
P47985;P0C7P4	Cytochrome b-c1 complex subunit Rieske, mitochondrial OS=Homo sapiens GN=UQCRFS1 PE=1 SV=2	0.66	0.0007
P09525	Annexin A4 OS=Homo sapiens GN=ANXA4 PE=1 SV=4	0.66	0.0029
P16284;P16284-2;P16284-3;P16284-4;P16284-5;P16284-6	Platelet endothelial cell adhesion molecule OS=Homo sapiens GN=PECAM1 PE=1 SV=1	0.66	0.0004
P23142;P23142-2;P23142-3	Fibulin-1 OS=Homo sapiens GN=FBLN1 PE=1 SV=4	0.66	0.0103
Q63HQ2;Q63HQ2-2;Q63HQ2-4	Pikachurin OS=Homo sapiens GN=EGFLAM PE=1 SV=2	0.66	0.0022
P05108;P05108-2	Cholesterol side-chain cleavage enzyme, mitochondrial OS=Homo sapiens GN=CYP11A1 PE=1 SV=2	0.66	0.0255
O75616-2;O75616	Isoform HERA-B of GTPase Era, mitochondrial OS=Homo sapiens GN=ERAL1	0.71	0.0140
Q15019;Q15019-2	Septin-2 OS=Homo sapiens GN=SEPT2 PE=1 SV=1	0.71	0.0062
P23229;P23229-2;P23229-3;P23229-4;P23229-5;P23229-6;P23229-7;P23229-9	Integrin alpha-6 OS=Homo sapiens GN=ITGA6 PE=1 SV=5	0.71	0.0011
P14060	3 beta-hydroxysteroid dehydrogenase/Delta 5-->4-isomerase type 1 OS=Homo sapiens GN=HSD3B1 PE=1 SV=2	0.71	0.0005
Q9GZM7;Q9GZM7-3	Tubulointerstitial nephritis antigen-like OS=Homo sapiens GN=TINAGL1 PE=1 SV=1	0.71	0.0105
P04004	Vitronectin OS=Homo sapiens GN=VTN PE=1 SV=1	0.71	0.0001
P07237	Protein disulfide-isomerase OS=Homo sapiens GN=P4HB PE=1 SV=3	0.71	0.0132
O94983-2;O94983;O94983-3;O94983-4;O94983-5;O94983-6	Isoform 2 of Calmodulin-binding transcription activator 2 OS=Homo sapiens GN=CAMTA2	0.71	0.0000
Q14832	Metabotropic glutamate receptor 3 OS=Homo sapiens GN=GRM3 PE=1 SV=2	0.71	0.0046
Q8N573-5;Q8N573;Q8N573-3;Q8N573-8	Isoform 5 of Oxidation resistance protein 1 OS=Homo sapiens GN=OXR1	0.71	0.0271
P50454	Serpin H1 OS=Homo sapiens GN=SERPINH1 PE=1 SV=2	0.71	0.0026
P08697;P08697-2	Alpha-2-antiplasmin OS=Homo sapiens GN=SERPINF2 PE=1 SV=3	0.71	0.0021
P13667	Protein disulfide-isomerase A4 OS=Homo sapiens GN=PDIA4 PE=1 SV=2	0.71	0.0142
P04083	Annexin A1 OS=Homo sapiens GN=ANXA1 PE=1 SV=2	0.71	0.0001
P49796-6;P49796;P49796-1;P49796-2;P49796-4;P49796-7;P49796-8	Isoform 6 of Regulator of G-protein signaling 3 OS=Homo sapiens GN=RGS3	0.71	0.0014
P02675	Fibrinogen beta chain OS=Homo sapiens GN=FGB PE=1 SV=2	0.71	0.0040
P01130;P01130-3;P01130-4;P01130-5;P01130-6	Low-density lipoprotein receptor OS=Homo sapiens GN=LDLR PE=1 SV=1	0.71	0.0224
P11532;P11532-10;P11532-2;P11532-3;P11532-4;P11532-5;P11532-6;P11532-7;P11532-8;P11532-9	Dystrophin OS=Homo sapiens GN=DMD PE=1 SV=3	0.71	0.0008
P15311	Ezrin OS=Homo sapiens GN=EZR PE=1 SV=4	0.71	0.0054

Q6ZR08-3	Isoform 3 of Dynein heavy chain 12, axonemal OS=Homo sapiens GN=DNAH12	0.71	0.0171
P11166	Solute carrier family 2, facilitated glucose transporter member 1 OS=Homo sapiens GN=SLC2A1 PE=1 SV=2	0.71	0.0022
Q99959;Q99959-2	Plakophilin-2 OS=Homo sapiens GN=PKP2 PE=1 SV=2	0.71	0.0069
P46776	60S ribosomal protein L27a OS=Homo sapiens GN=RPL27A PE=1 SV=2	0.71	0.0001
P13611;P13611-2;P13611-3;P13611-4;P13611-5	Versican core protein OS=Homo sapiens GN=VCAN PE=1 SV=3	0.71	0.0143
P02768;P02768-2	Serum albumin OS=Homo sapiens GN=ALB PE=1 SV=2	0.71	0.0001
O75083	WD repeat-containing protein 1 OS=Homo sapiens GN=WDR1 PE=1 SV=4	0.71	0.0045
Q9H2D6;Q9H2D6-2;Q9H2D6-3;Q9H2D6-4;Q9H2D6-5;Q9H2D6-7	TRIO and F-actin-binding protein OS=Homo sapiens GN=TRIOBP PE=1 SV=3	0.71	0.0435
P00488	Coagulation factor XIII A chain OS=Homo sapiens GN=F13A1 PE=1 SV=4	0.71	0.0010
P02774;P02774-2;P02774-3	Vitamin D-binding protein OS=Homo sapiens GN=GC PE=1 SV=1	0.71	0.0059
P14061	Estradiol 17-beta-dehydrogenase 1 OS=Homo sapiens GN=HSD17B1 PE=1 SV=3	0.71	0.0014
P09630;P09630-2	Homeobox protein Hox-C6 OS=Homo sapiens GN=HOXC6 PE=2 SV=3	0.71	0.0084
P07355;P07355-2	Annexin A2 OS=Homo sapiens GN=ANXA2 PE=1 SV=2	0.71	0.0024
P11511	Aromatase OS=Homo sapiens GN=CYP19A1 PE=1 SV=3	0.71	0.0020
Q5JRA6;Q5JRA6-2;Q5JRA6-3	Melanoma inhibitory activity protein 3 OS=Homo sapiens GN=MIA3 PE=1 SV=1	0.71	0.0005
P0C0L5;P0C0L4;P0C0L4-2	Complement C4-B OS=Homo sapiens GN=C4B PE=1 SV=2	0.71	0.0065
Q9P258	Protein RCC2 OS=Homo sapiens GN=RCC2 PE=1 SV=2	0.71	0.0039
P22314	Ubiquitin-like modifier-activating enzyme 1 OS=Homo sapiens GN=UBA1 PE=1 SV=3	0.71	0.0035
P05120	Plasminogen activator inhibitor 2 OS=Homo sapiens GN=SERPINB2 PE=1 SV=2	0.71	0.0003
Q9NZU7;Q9NZU7-1;Q9NZU7-2;Q9NZU7-3	Calcium-binding protein 1 OS=Homo sapiens GN=CABP1 PE=1 SV=5	0.71	0.0375
P37059	Estradiol 17-beta-dehydrogenase 2 OS=Homo sapiens GN=HSD17B2 PE=1 SV=1	0.71	0.0167
Q03135;Q03135-2	Caveolin-1 OS=Homo sapiens GN=CAV1 PE=1 SV=4	0.71	0.0452
Q9GZM7-2	Isoform 2 of Tubulointerstitial nephritis antigen-like OS=Homo sapiens GN=TINAGL1	0.71	0.0023
P10599-2;P10599	Isoform 2 of Thioredoxin OS=Homo sapiens GN=TXN	0.76	0.0125
Q92870;Q92870-2;Q92870-4	Amyloid beta A4 precursor protein-binding family B member 2 OS=Homo sapiens GN=APBB2 PE=1 SV=3	0.76	0.0394
Q9Y6U3;Q9Y6U3-2;Q9Y6U3-3	Adseverin OS=Homo sapiens GN=SCIN PE=1 SV=4	0.76	0.0075
Q9NZM1;Q9NZM1-2;Q9NZM1-3;Q9NZM1-5;Q9NZM1-6;Q9NZM1-7;Q9NZM1-8	Myoferlin OS=Homo sapiens GN=MYOF PE=1 SV=1	0.76	0.0005
Q01995	Transgelin OS=Homo sapiens GN=TAGLN PE=1 SV=4	0.76	0.0043
P80365	Corticosteroid 11-beta-dehydrogenase isozyme 2 OS=Homo sapiens GN=HSD11B2 PE=1 SV=2	0.76	0.0098
Q86UV5;Q86UV5-2;Q86UV5-3;Q86UV5-5;Q86UV5-6	Ubiquitin carboxyl-terminal hydrolase 48 OS=Homo sapiens GN=USP48 PE=1 SV=1	0.76	0.0191

Supporting information

Q9NNX6-6;Q9NNX6;Q9NNX6-10;Q9NNX6-11;Q9NNX6-2;Q9NNX6-5;Q9NNX6-7	Isoform 6 of CD209 antigen OS=Homo sapiens GN=CD209	0.76	0.0212
Q9HBD1;Q9HBD1-4;Q9HBD1-6	Roquin-2 OS=Homo sapiens GN=RC3H2 PE=1 SV=2	0.76	0.0546
P30044;P30044-2;P30044-3	Peroxiredoxin-5, mitochondrial OS=Homo sapiens GN=PRDX5 PE=1 SV=4	0.76	0.0192
P38398;P38398-4	Breast cancer type 1 susceptibility protein OS=Homo sapiens GN=BRCA1 PE=1 SV=2	0.76	0.0165
P02792	Ferritin light chain OS=Homo sapiens GN=FTL PE=1 SV=2	0.76	0.0331
P62158;P02585	Calmodulin OS=Homo sapiens GN=CALM1 PE=1 SV=2	0.76	0.0075
Q3SY00;Q3SY00-2	Testis-specific protein 10-interacting protein OS=Homo sapiens GN=TSGA10IP PE=2 SV=1	0.76	0.0092
Q15084-2;Q15084	Isoform 2 of Protein disulfide-isomerase A6 OS=Homo sapiens GN=PDIA6	0.76	0.0001
Q9BYG3	MKI67 FHA domain-interacting nucleolar phosphoprotein OS=Homo sapiens GN=NIFK PE=1 SV=1	0.76	0.0303
P01876;P01877	Ig alpha-1 chain C region OS=Homo sapiens GN=IGHA1 PE=1 SV=2	0.76	0.0301
P59190-2	Isoform 2 of Ras-related protein Rab-15 OS=Homo sapiens GN=RAB15	0.76	0.0111
P00450	Ceruloplasmin OS=Homo sapiens GN=CP PE=1 SV=1	0.76	0.0081
Q7Z3Z0	Keratin, type I cytoskeletal 25 OS=Homo sapiens GN=KRT25 PE=1 SV=1	0.76	0.0303

Supporting information: Table S4**List of proteins with secreted GO term from DAVID analysis**

List of 41 protein with secreted GO term from DAVID analysis

Up regulated

Sr.No	Protein Name	Uniprot Accession
1	Ephrin type-B receptor 6; EPH receptor B6	O15197
2	von Willebrand factor A domain-containing protein 8	A3KMH1
3	alpha-2-HS-glycoprotein	P02765
4	apolipoprotein A-I	P02647
5	calreticulin	P27797
6	dipeptidyl-peptidase 4	P27487
7	fibrillin 2	P35556
8	Gelsolin	P06396
9	heparan sulfate proteoglycan 2	P98160
10	apolipoprotein E	P02649
11	orosomucoid 2	P19652
12	peroxiredoxin 4	Q13162
13	Plasminogen	P00747
14	transferrin	P02787
15	transferrin receptor 1	P02786
16	vegfr-1	P17948
17	Annexin A6	P08133

Down regulated

Sr.No	Protein Name	Uniprot Accession
1	EGF-like, fibronectin type III and laminin G domains	Q63HQ2
2	alpha-2-macroglobulin	P01023
3	annexin A2	P07355
4	ceruloplasmin	P00450
5	coagulation factor XI	P03951
6	coagulation factor XIII, A1 polypeptide	P00488
7	complement component 4B	P0C0L5
8	fibrillin 1	P35555
9	fibrinogen beta chain	P02675
10	fibrinogen gamma chain	P02679
11	fibrinogen alpha chain	P02671
12	fibulin 1	P23142
13	vitamin D binding protein	P02774
14	haptoglobin	P00738
15	hemopexin	P02790
16	Plasminogen activator inhibitor 2	P05120
17	proteoglycan 2	P13727
18	Alpha-1-antitrypsin	P01009
19	Alpha-1-antichymotrypsin	P01011
20	alpha-2 antiplasmin	P08697
21	Complement C3	P01024
22	tubulointerstitial nephritis antigen-like 1	Q9GZM7
23	Vitronectin	P04004
24	versican	P13611