Distinct Degradome Signature in Triple Negative Breast Cancer Revealed by Large-Scale Comparative Peptidomic Analysis

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Breast Cancer in the U.S.

Estimated New Cases*

Males			Females		
Prostate	233,000	27%	Breast	232,670	29%
Lung & bronchus	116,000	14%	Lung & bronchus	108,210	13%
Colorectum	71,830	8%	Colorectum	65,000	8%
Urinary bladder	56,390	7%	Uterine corpus	52,630	6%
Melanoma of the skin	43,890	5%	Thyroid	47,790	6%
Kidney & renal pelvis	39,140	5%	Non-Hodgkin lymphoma	32,530	4%
Non-Hodgkin lymphoma	38,270	4%	Melanoma of the skin	32,210	4%
Oral cavity & pharynx	30,220	4%	Kidney & renal pelvis	24,780	3%
Leukemia	30,100	4%	Pancreas	22,890	3%
Liver & intrahepatic bile duct	24,600	3%	Leukemia	22,280	3%
All Sites	**855,220**	**100%**	**All Sites**	**810,320**	**100%**

Estimated Deaths

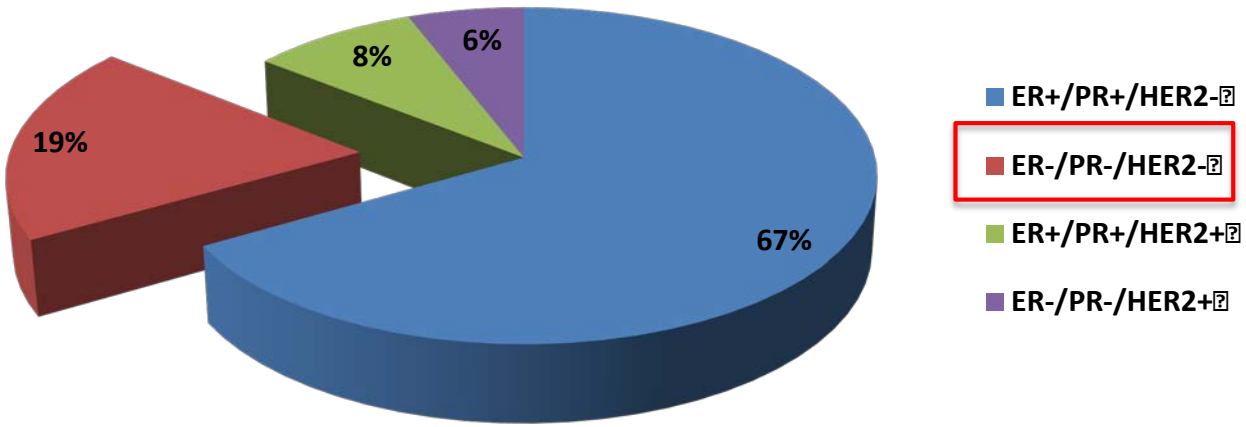
Males			Females		
Lung & bronchus	86,930	28%	Lung & bronchus	72,330	26%
Prostate	29,480	10%	Breast	40,000	15%
Colorectum	26,270	8%	Colorectum	24,040	9%
Pancreas	20,170	7%	Pancreas	19,420	7%
Liver & intrahepatic bile duct	15,870	5%	Ovary	14,270	5%
Leukemia	14,040	5%	Leukemia	10,050	4%
Esophagus	12,450	4%	Uterine corpus	8,590	3%
Urinary bladder	11,170	4%	Non-Hodgkin lymphoma	8,520	3%
Non-Hodgkin lymphoma	10,470	3%	Liver & intrahepatic bile duct	7,130	3%
Kidney & renal pelvis	8,900	3%	Brain & other nervous system	6,230	2%
All Sites	**310,010**	**100%**	**All Sites**	**275,710**	**100%**

FIGURE 1. Ten Leading Cancer Types for the Estimated New Cancer Cases and Deaths by Sex, United States, 2014.
*Estimates are rounded to the nearest 10 and exclude basal cell and squamous cell skin cancers and in situ carcinoma except urinary bladder.

Siegel et al. CA CANCER J CLIN 2014;64:9–29

Triple Negative Breast Cancer

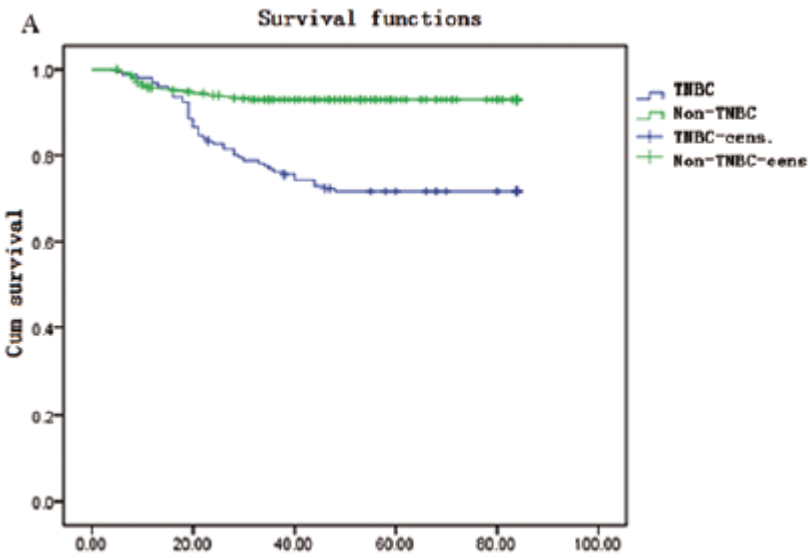
Estrogen receptor (ER)
Progesterone receptor (PR)
Human epidermal growth factor receptor 2 (HER2)



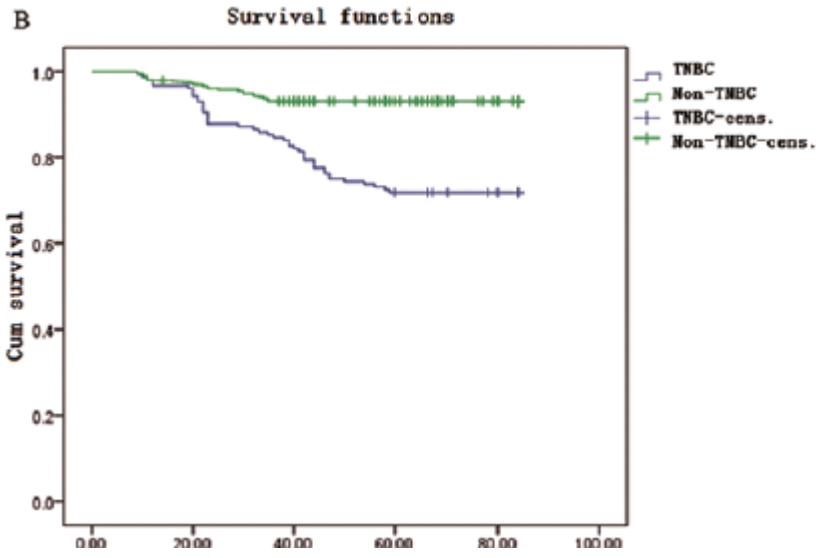
Triple Negative Breast Cancer

Lund et al. Cancer. 2010; 116(11):2549-2559.

TNBC is associated with poor survival



Disease free survival

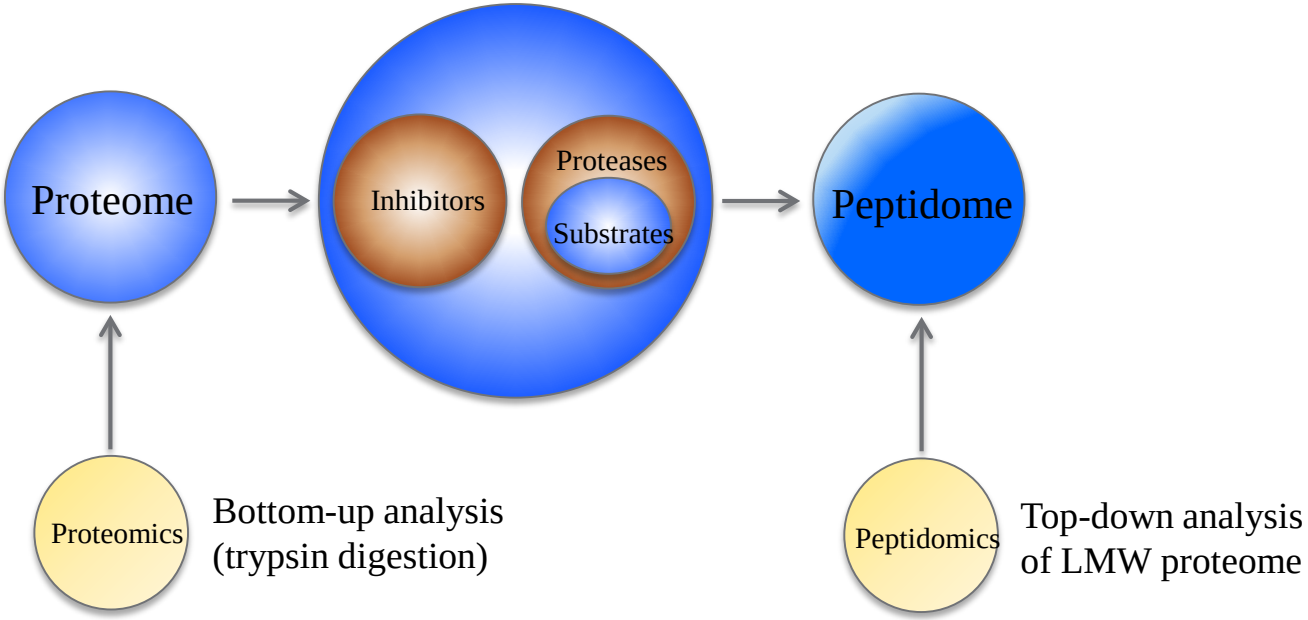


Overall survival

Yuan et al. MOLECULAR AND CLINICAL ONCOLOGY 2: 245-251, 2014

Need effective biomarkers for TNBC!

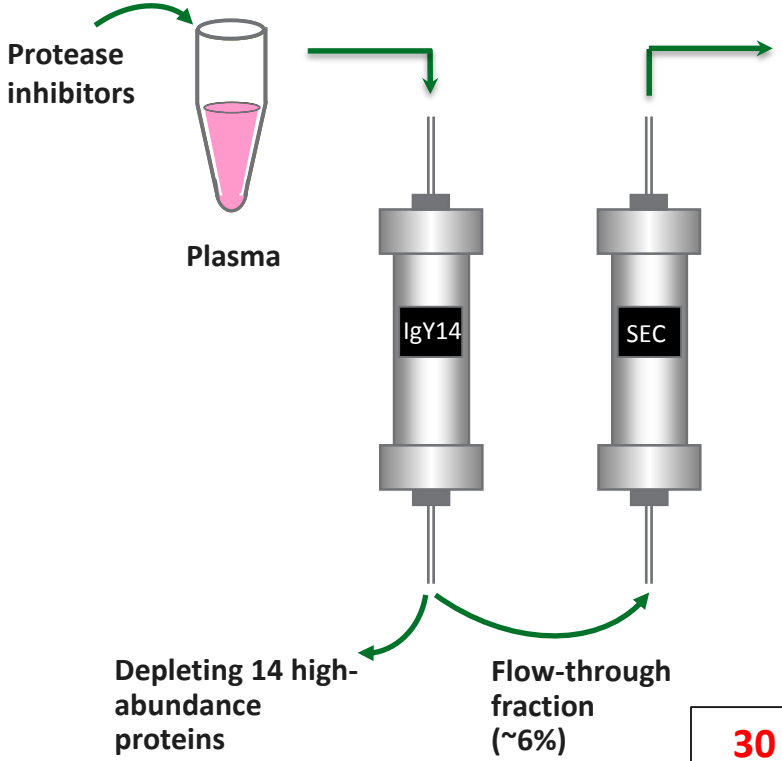
Peptidomics: promising, but challenging

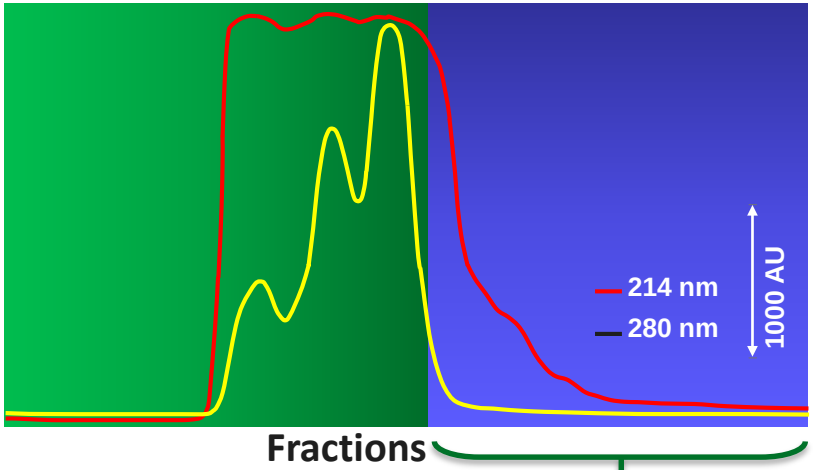


- ✓ Proteolysis affects protein abundances, function and activities
- ✓ Elevated expression/activities in cancer (e.g., OE of MMPs in BrCa: tumor growth)
- ✓ Provides a complementary (or otherwise unseen) view of the proteome

- ▪ Challenges in sample preparation and informatics analysis

Peptidome isolation from blood plasma





Peptidome sample

albumin, IgG, 1-antitrypsin, IgA, IgM, transferrin, Haptoglobin, 1-acid glycoprotein, 2-macroglobulin, apolipoprotein A-I, apolipoprotein A-II, fibrinogen, C3, LDL

30 TNBC patients vs. 30 healthy controls

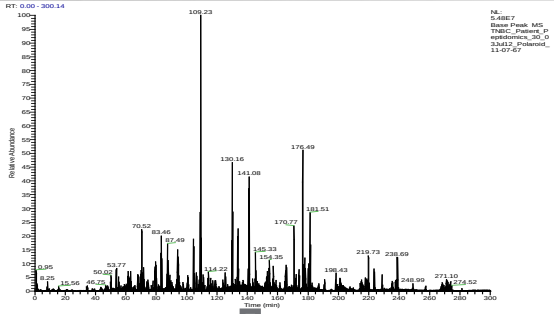
Age, menopausal status and race matched

Sample concentrated by ~330 fold [i.e., 1/(6% × 5%)]

Shen et al., J Proteome Res. 2010, 9(5):2339-46
Shen et al., PLoS One. 2010, 5(10):e13133

Data analysis workflow

Data Acquisition



Database Searching

MSGF+, Sequest, Ms-Align+

MTDB Construction

MT_Human_TNBC_Peptidomics

Label-free Quantification

Informed Quantification

Data Interpretation

Bioinformatics Analysis

LC-MS/MS conditions:
3 µm Jupiter C18
110 cm x 75 µm i.d. column
300-min gradient
flow rate 200 nL/min

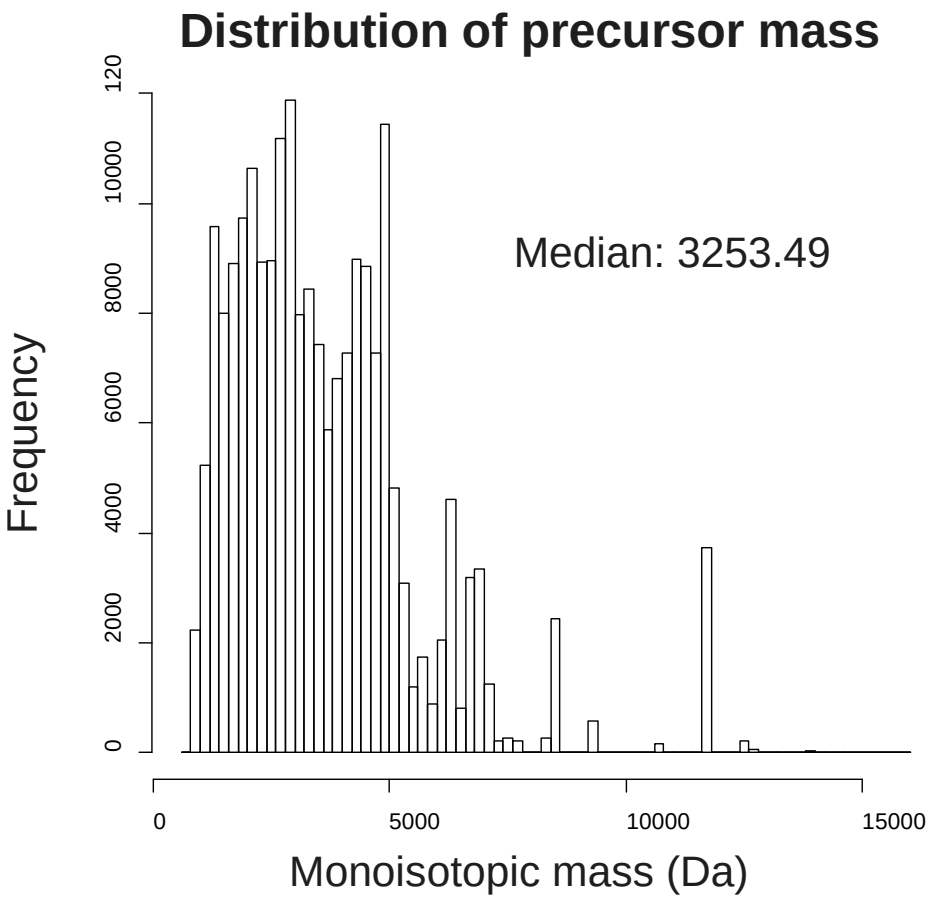
LTQ-Orbitrap Velos
Full MS @ 60K resolution
top 10 CID MS/MS @ 15K resolution

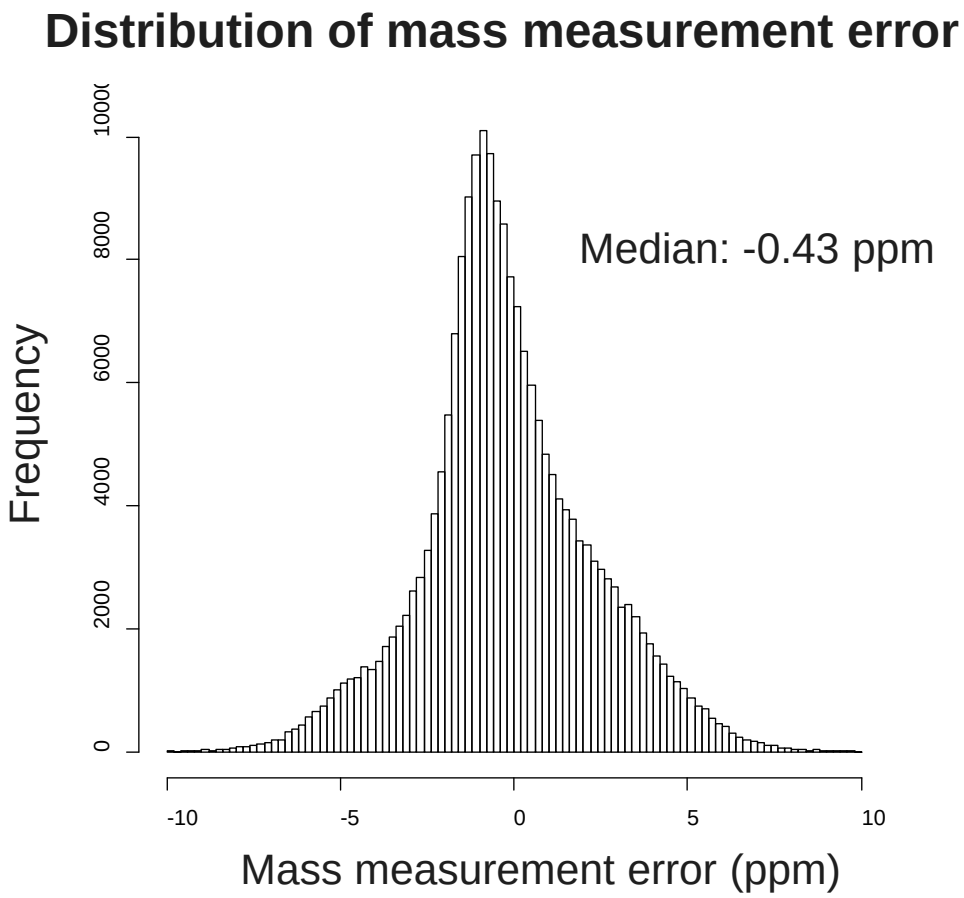
Peptidomics Poster 169: Zhe et al.

Oral talk: Fujimoto et al.

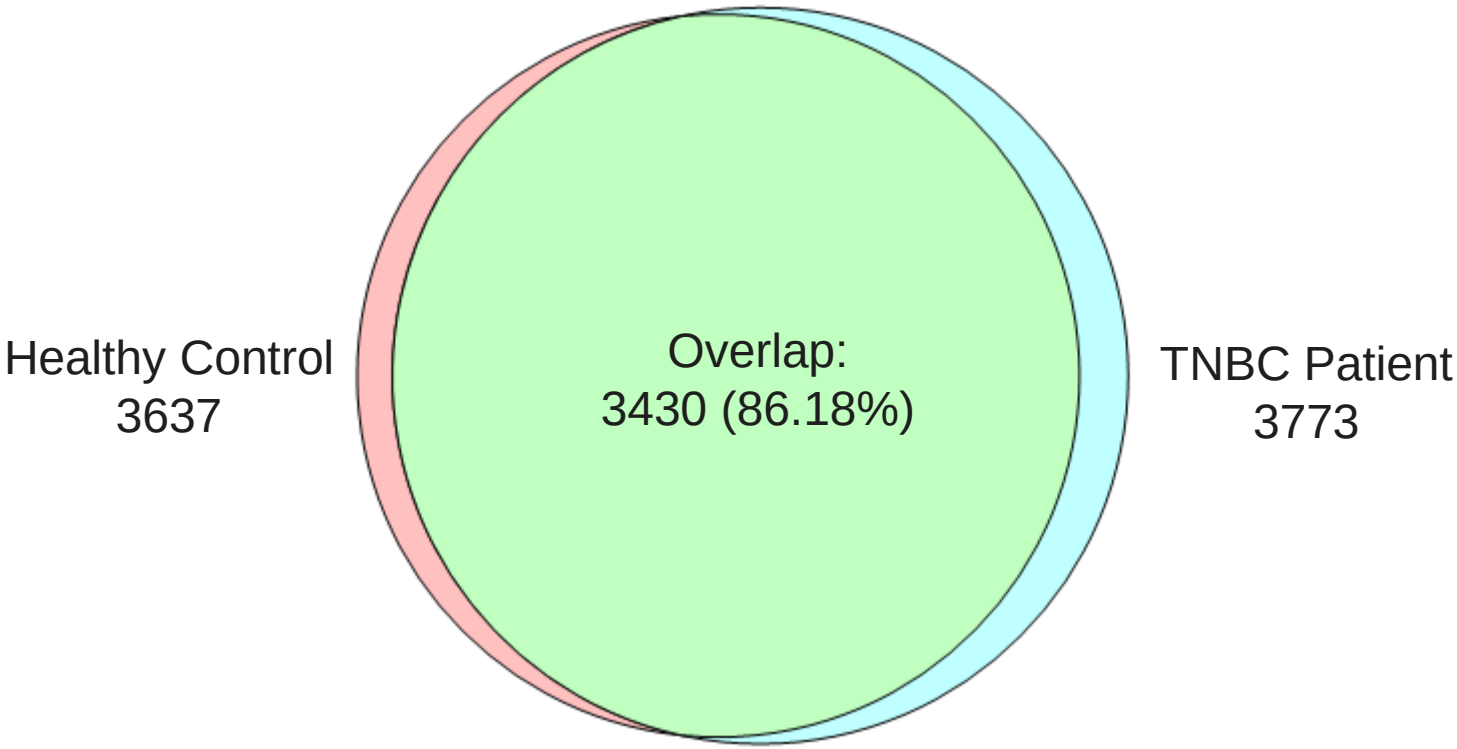
3980 unique peptides
436 protein groups

Distribution of precursor mass and mass measurement error



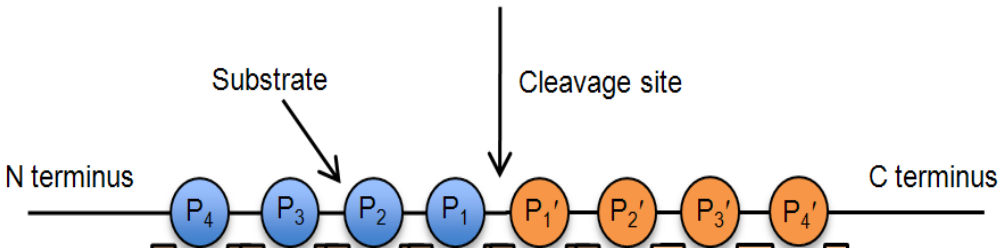


Comparison of peptide IDs in the two groups



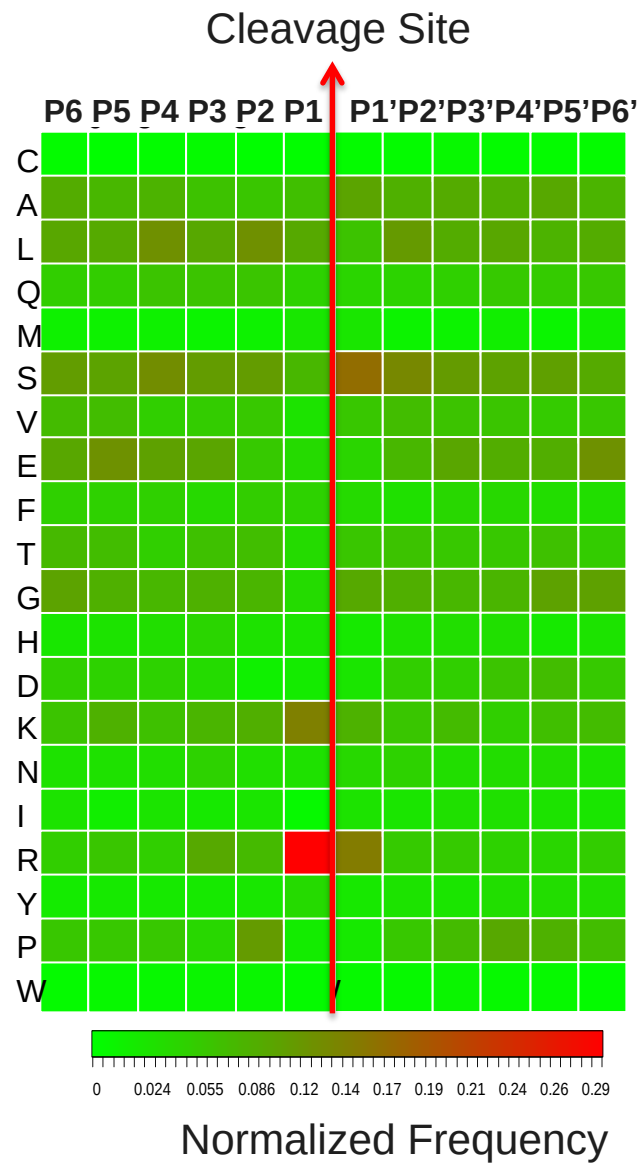
Total: 3980 unique peptide IDs

Trypsin like protease cleavage specificity in all identified peptides



N-Term: with or without the first methionine
C-Term

	N-Term	Internal	C-Term
Number of matches	98	4411	183



Serine related protease cleavage specificity in TNBC group

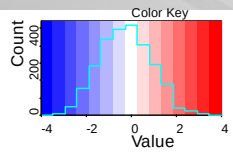
ADAM19
XPNPEP3

Background (3980)

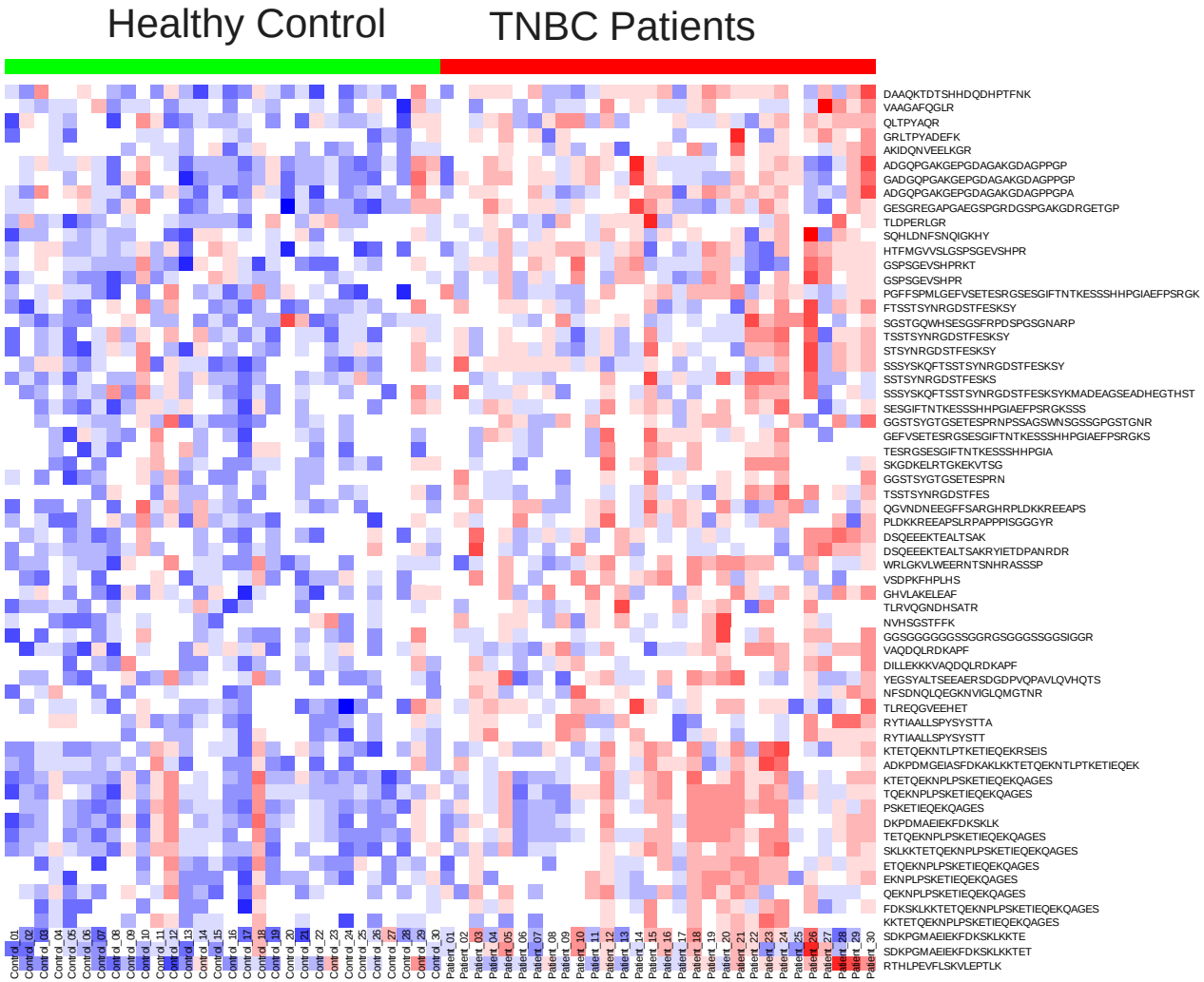
1.5 fold P/C >30 samples (497)

2 fold P/C >30 samples (212)

Significantly increased peptidome level in TNBC samples



Heatmap



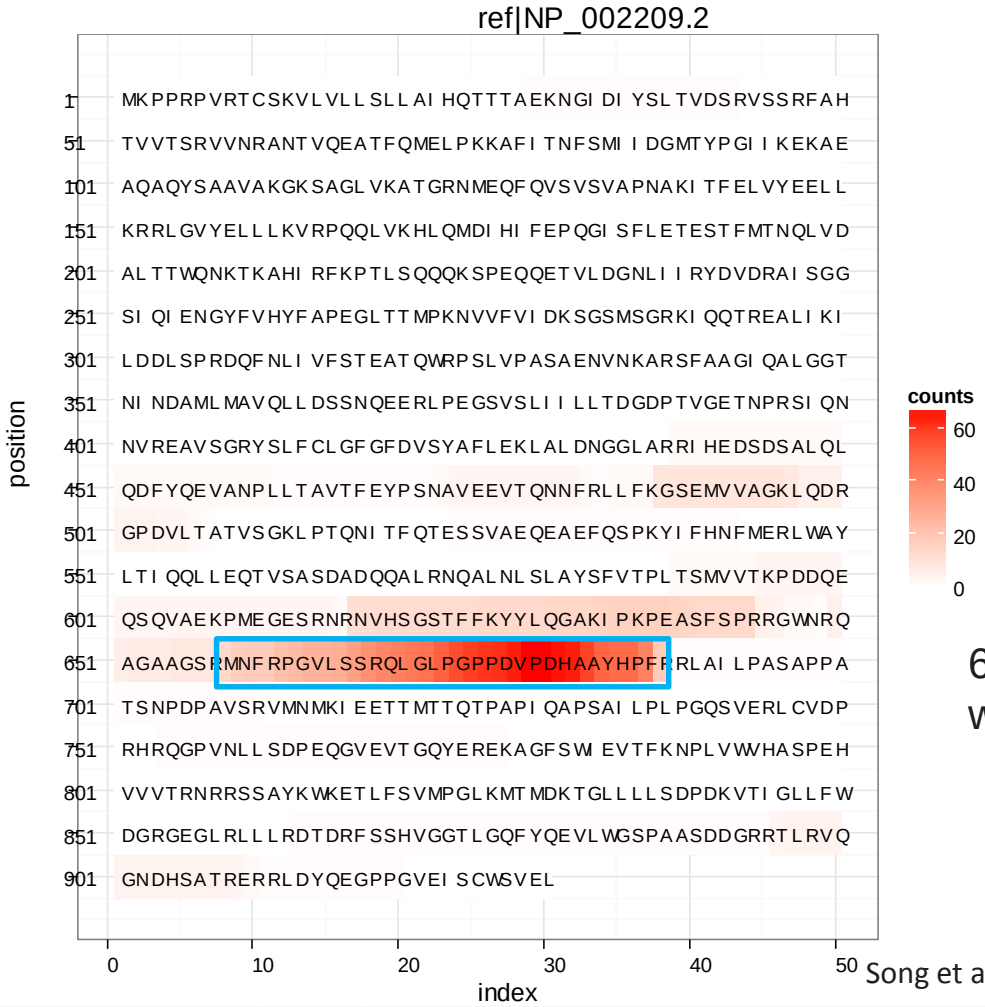
T-test
62 significant peptides
($p < 0.01$)

Protein name	Protein description	Peptide
A2GL_HUMAN	Leucine-rich alpha-2-glycoprotein	1
APOA4_HUMAN	Apolipoprotein A-IV;	3
CO1A1_HUMAN	Collagen alpha-1(I)	4
CO3_HUMAN	Complement C3	1
FA5_HUMAN	Coagulation factor V	1
FIBA_HUMAN	Fibrinogen alpha chain	15
FIBB_HUMAN	Fibrinogen beta chain	2
GELS_HUMAN	Gelsolin	2
GLR_HUMAN	Glucagon receptor	1
IBP3_HUMAN	Insulin-like growth factor-binding protein 3	1
IGF2_HUMAN	Insulin-like growth factor II	1
ITIH4_HUMAN	Inter-alpha-trypsin inhibitor heavy chain H4	2
FETUA_HUMAN	Alpha-2-HS-glycoprotein	3
A1AT_HUMAN	Alpha-1-antitrypsin	1
K2C1_HUMAN	Keratin, type II cytoskeletal 1	1
MA1A1_HUMAN	Mannosyl-oligosaccharide 1,2-alpha-mannosidase IA;	2
SDPR_HUMAN	Serum deprivation-response protein	1
TAGL2_HUMAN	Transgelin-2	1
TLN1_HUMAN	Talin-1	1
TTHY_HUMAN	Transthyretin	2
TYB10_HUMAN	Thymosin beta-10	2
TYB4_HUMAN	Thymosin beta-4	11
TYB4Y_HUMAN	Thymosin beta-4, Y-chromosomal	2
VTDB_HUMAN	Vitamin D-binding protein	1

Protein name	Protein description	Peptide
A2GL_HUMAN	Leucine-rich alpha-2-glycoprotein	1
APOA4_HUMAN	Apolipoprotein A-IV;	3
CO1A1_HUMAN	Collagen alpha-1(I)	4
CO3_HUMAN	Complement C3	1
FA5_HUMAN	Coagulation factor V	1
FIBA_HUMAN	Fibrinogen alpha chain	15
FIBB_HUMAN	Fibrinogen beta chain	2
GELS_HUMAN	Gelsolin	2
GLR_HUMAN	Glucagon receptor	1
IBP3_HUMAN	Insulin-like growth factor-binding protein 3	1
IGF2_HUMAN	Insulin-like growth factor II	1
ITIH4_HUMAN	Inter-alpha-trypsin inhibitor heavy chain H4	2
FETUA_HUMAN	Alpha-2-HS-glycoprotein	3
A1AT_HUMAN	Alpha-1-antitrypsin	1
K2C1_HUMAN	Keratin, type II cytoskeletal 1	1
MA1A1_HUMAN	Mannosyl-oligosaccharide 1,2-alpha-mannosidase IA;	2
SDPR_HUMAN	Serum deprivation-response protein	1
TAGL2_HUMAN	Transgelin-2	1
TLN1_HUMAN	Talin-1	1
TTHY_HUMAN	Transthyretin	2
TYB10_HUMAN	Thymosin beta-10	2
TYB4_HUMAN	Thymosin beta-4	11
TYB4Y_HUMAN	Thymosin beta-4, Y-chromosomal	2
VTDB_HUMAN	Vitamin D-binding protein	1

Highly proteolytically active region in ITIH4

119 unique peptides identified for ITIH4

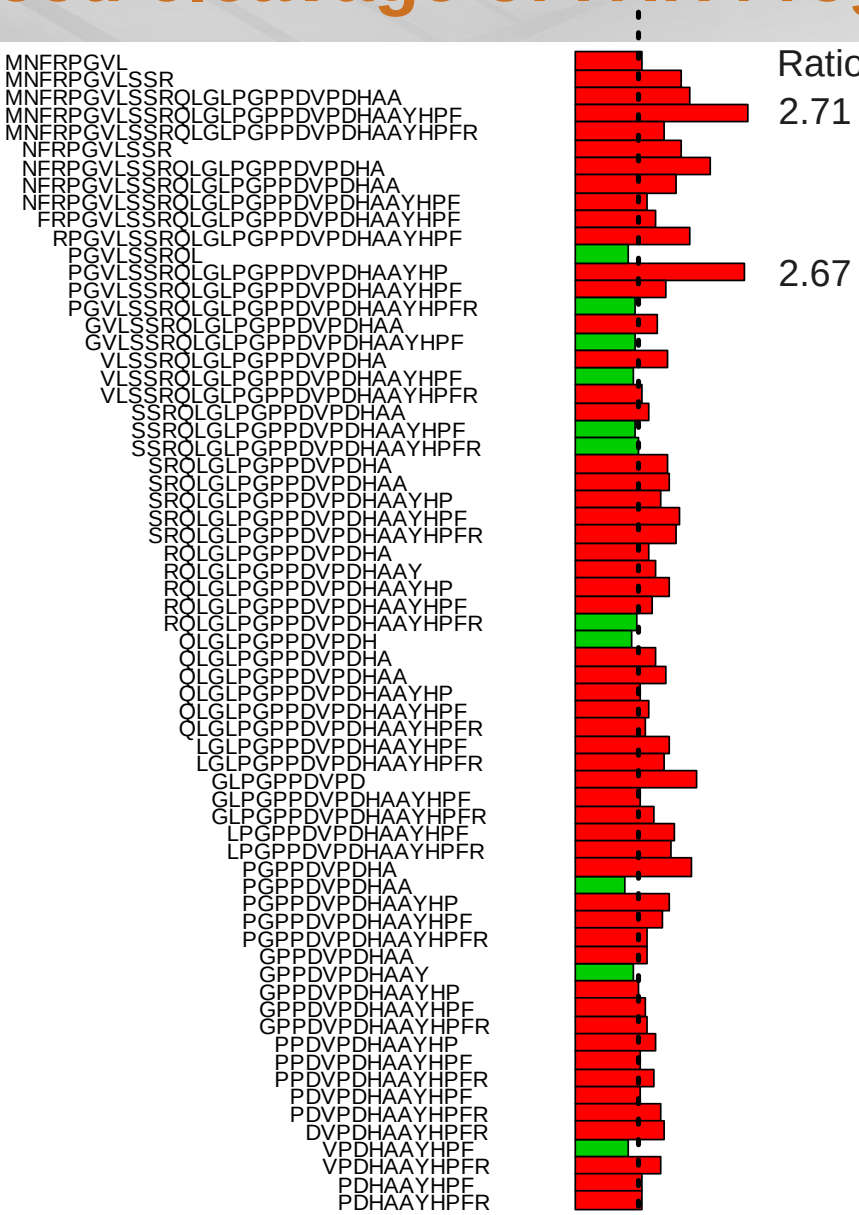


66 Unique peptides within the blue box

Song et al. Clinical Chemistry 52, No. 6, 2006

Region	658 – 688	31	Proline-rich (PRR) potential bioactive peptide

Enhanced cleavage of ITIH4 region in TNBC patients



p value: 8.382e-05 (Fisher)

Dashed line: Patient/Control Ratio=1

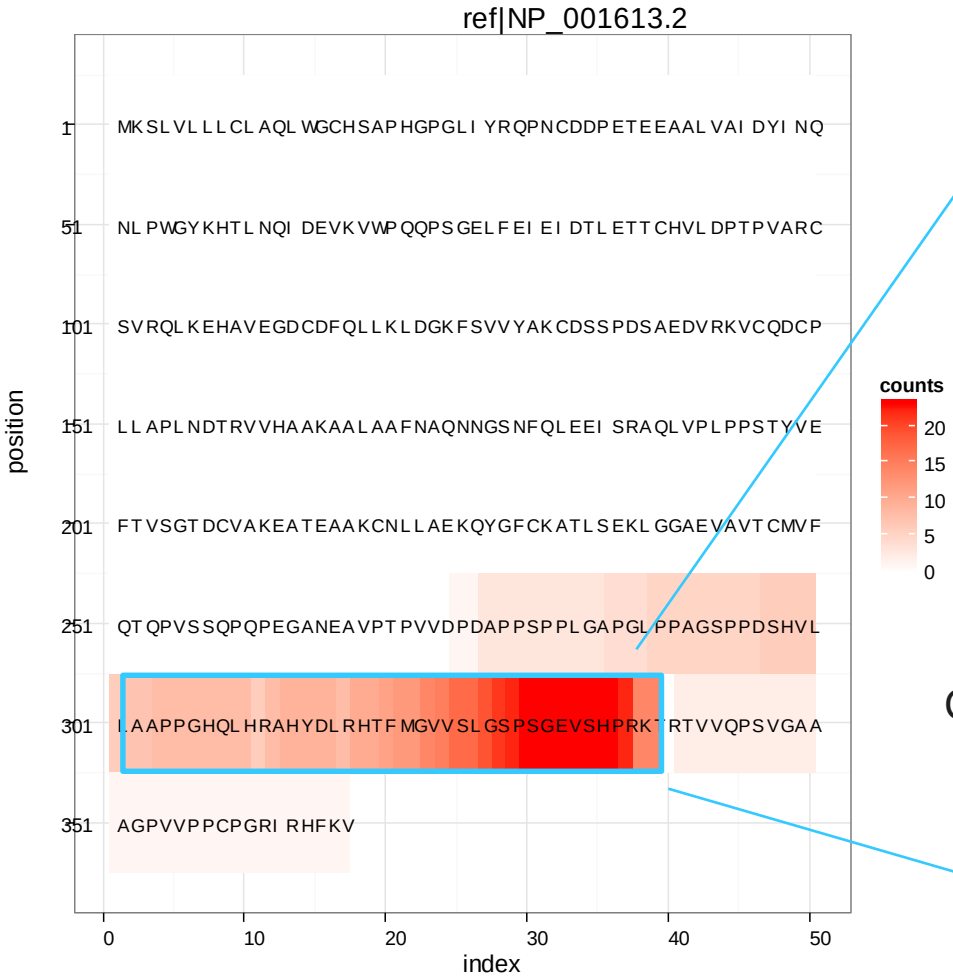
Red: >1
Green: <1

C-term:

R	**19**
F	**20**
A	**14**

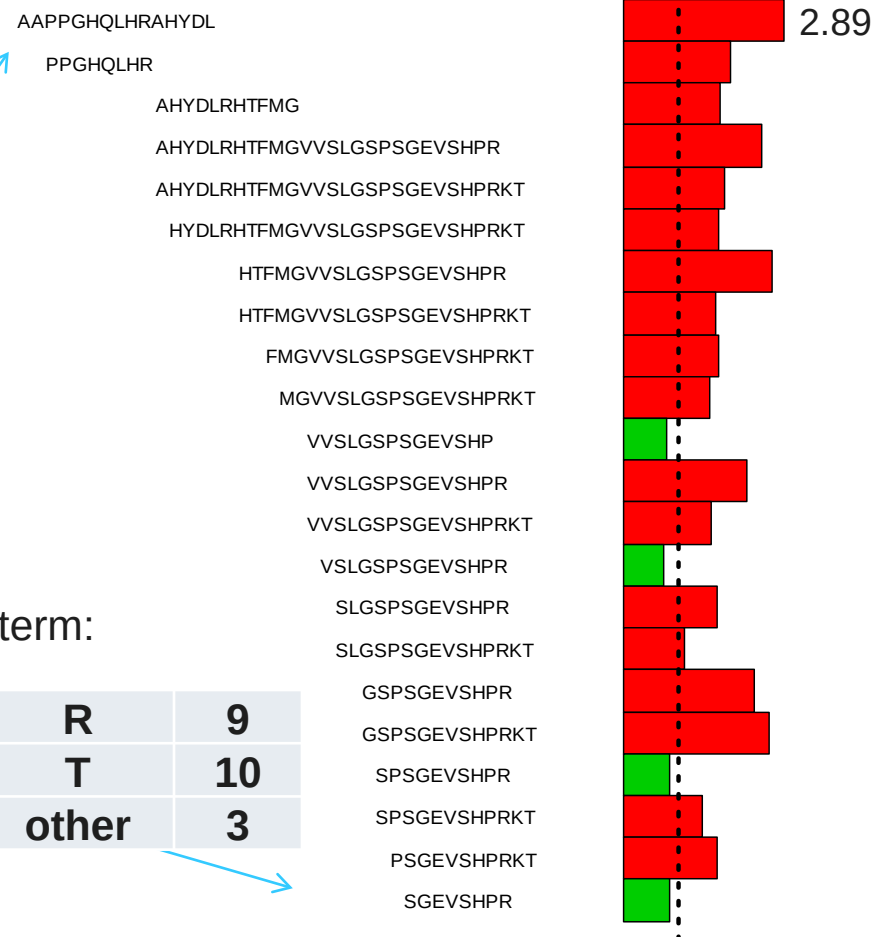
Highly proteolytically active region in AHSG

30 unique peptides identified for AHSG



22 peptides within blue box

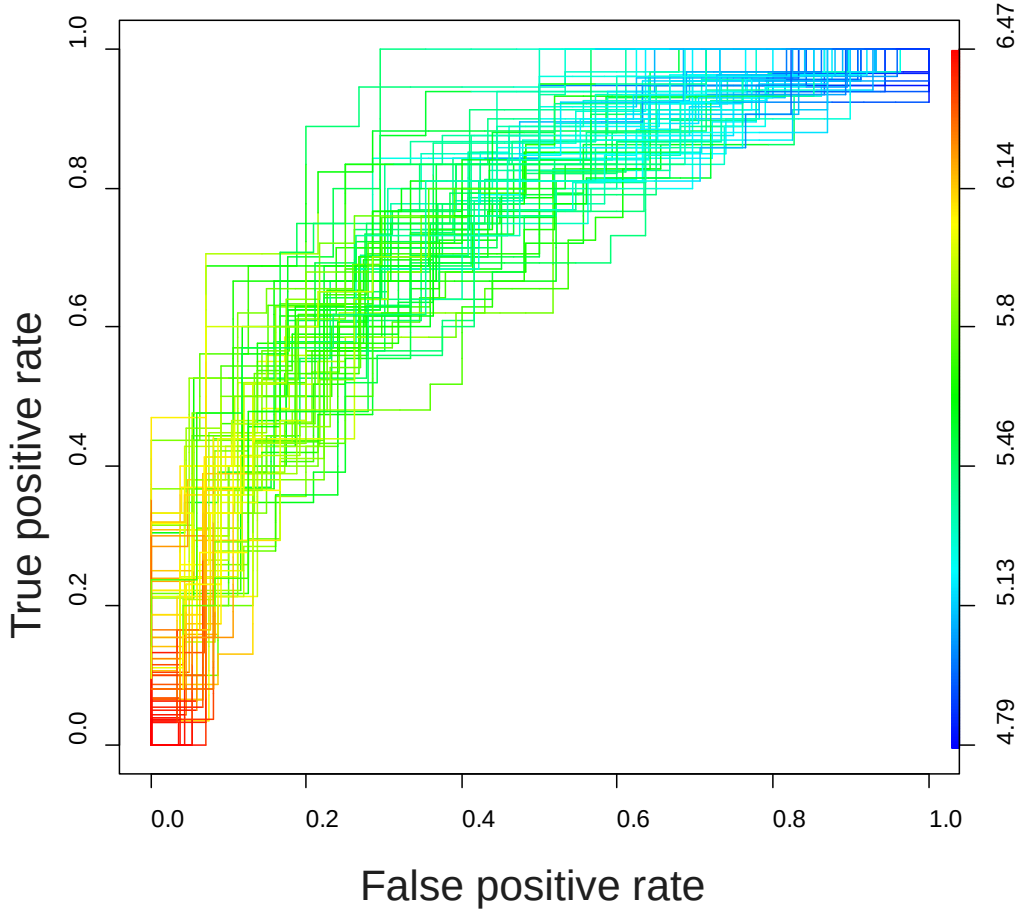
Patient/Control Ratio
Red: >1
Green: <1

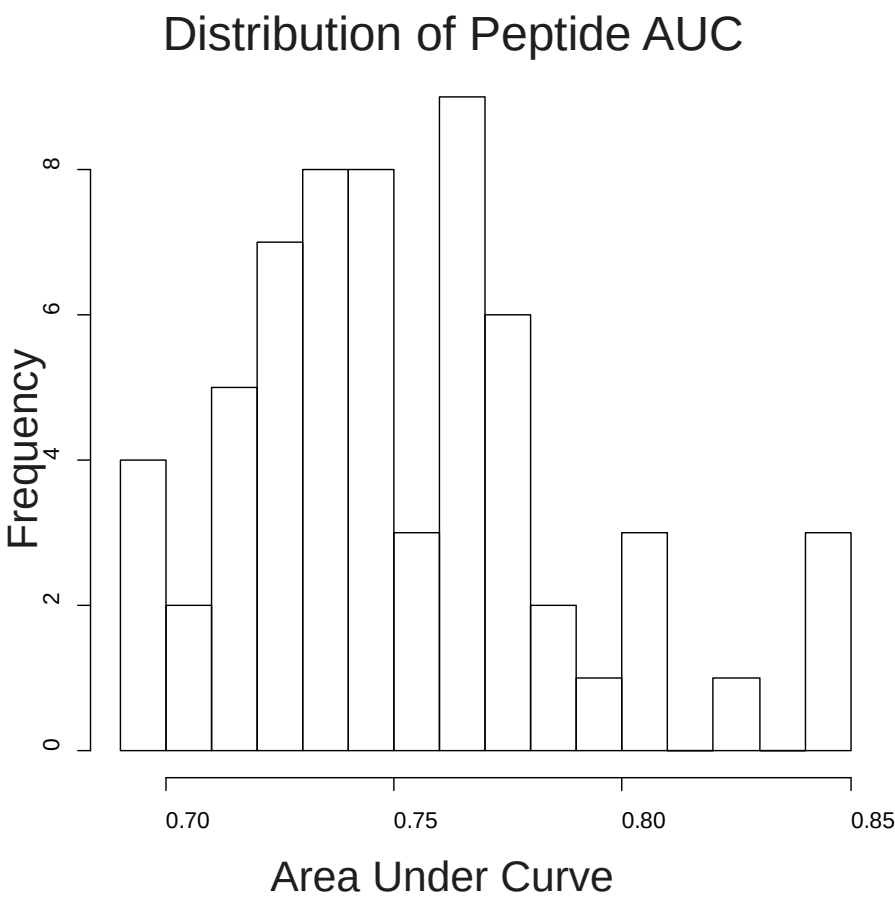


C-term:

R	**9**
T	**10**
other	**3**

ROC curve for all 62 significant peptides

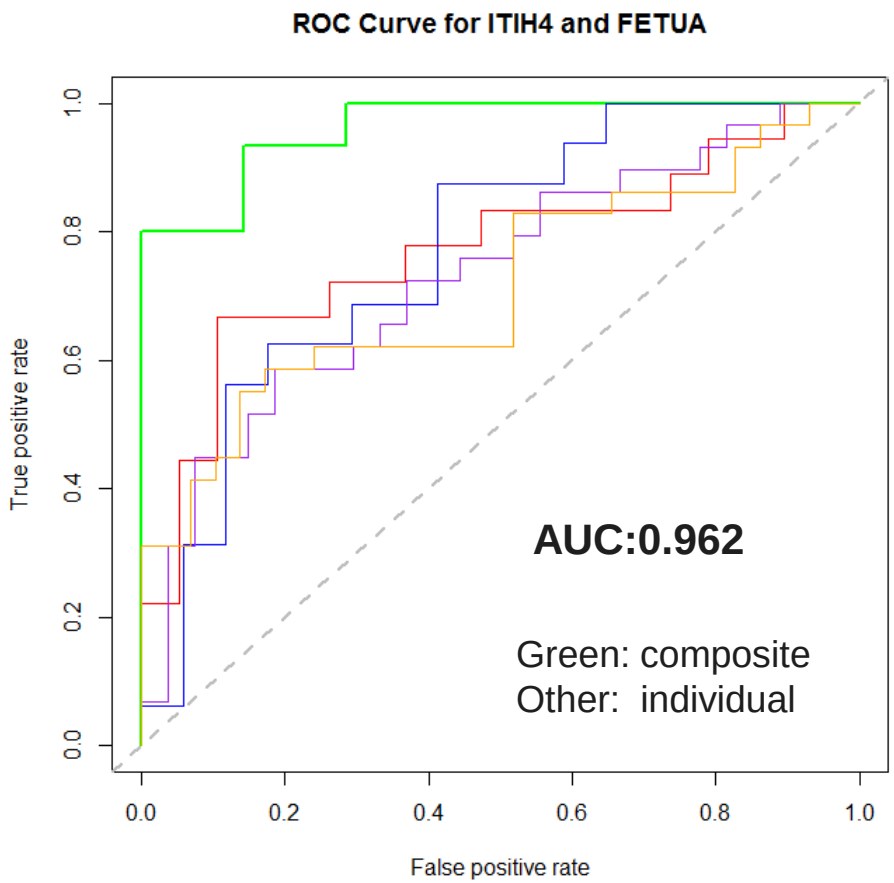




Most peptides showed moderate AUC value.

ROC curve consisting of ITIH4 and AHSG

	AHSG_1	AHSG_2	ITIH4_1	ITIH4_2
AUC	0.769	0.772	0.731	0.712



29 samples
(14 Control, 15 TNBC)

Peptidome signature peptide of ITIH4 and AHSG pair can be a potential cooperative biomarkers for TNBC

Summary and perspectives

- Enhanced peptidome/degradome activity observed in the cancer group
- 62 significantly-changing peptides separate TNBC group and control group
- Peptidome signature of peptide pairs from ITIH4-AHSG are a potential indicator for TNBC
- Peptidomic biomarker discovery in plasma is interesting but still challenging
- Future work may focus on tumor peptidomics to identify cancer specific biomarker and follow up in plasma and serum

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  NYU Langone Medical Center

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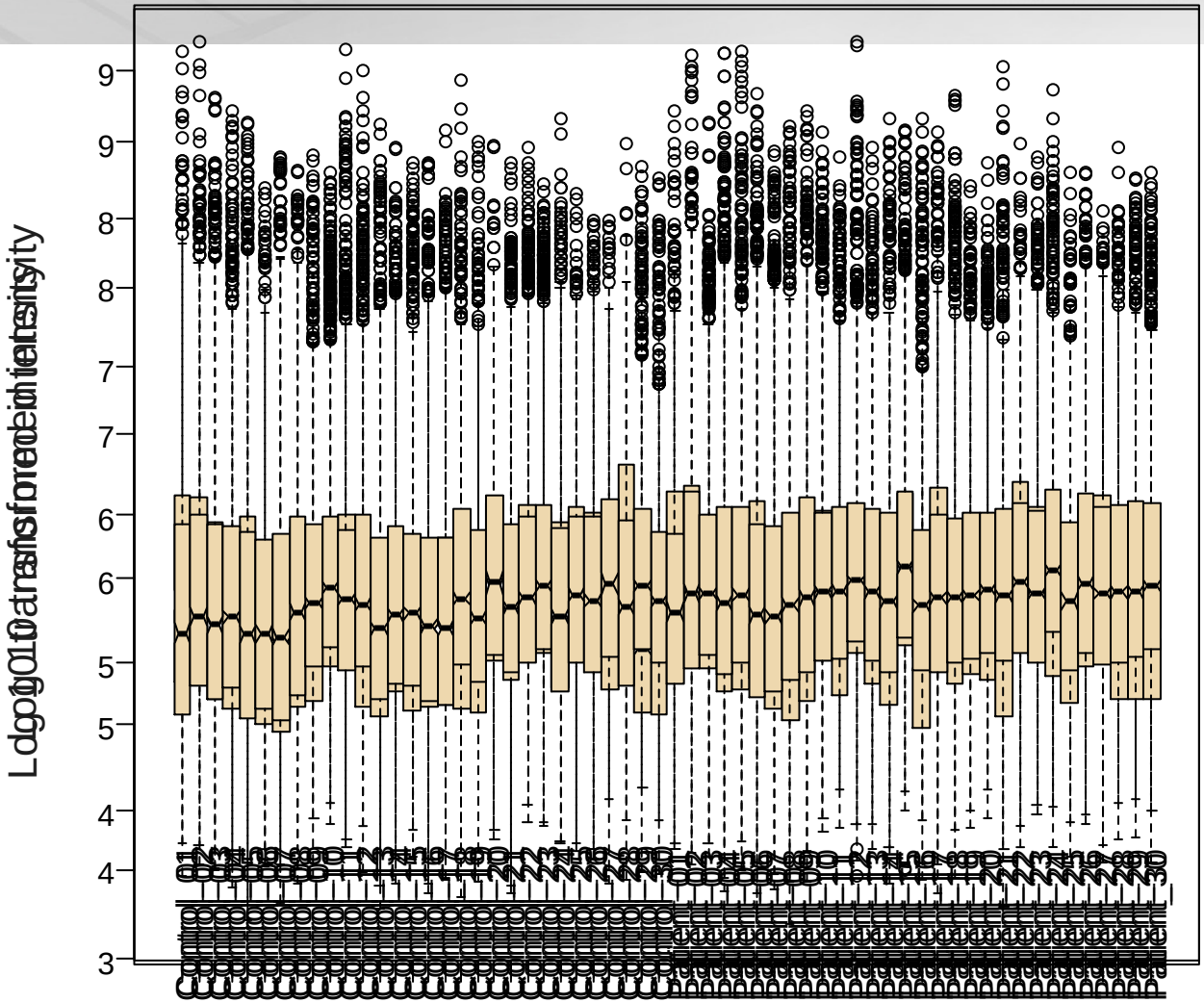
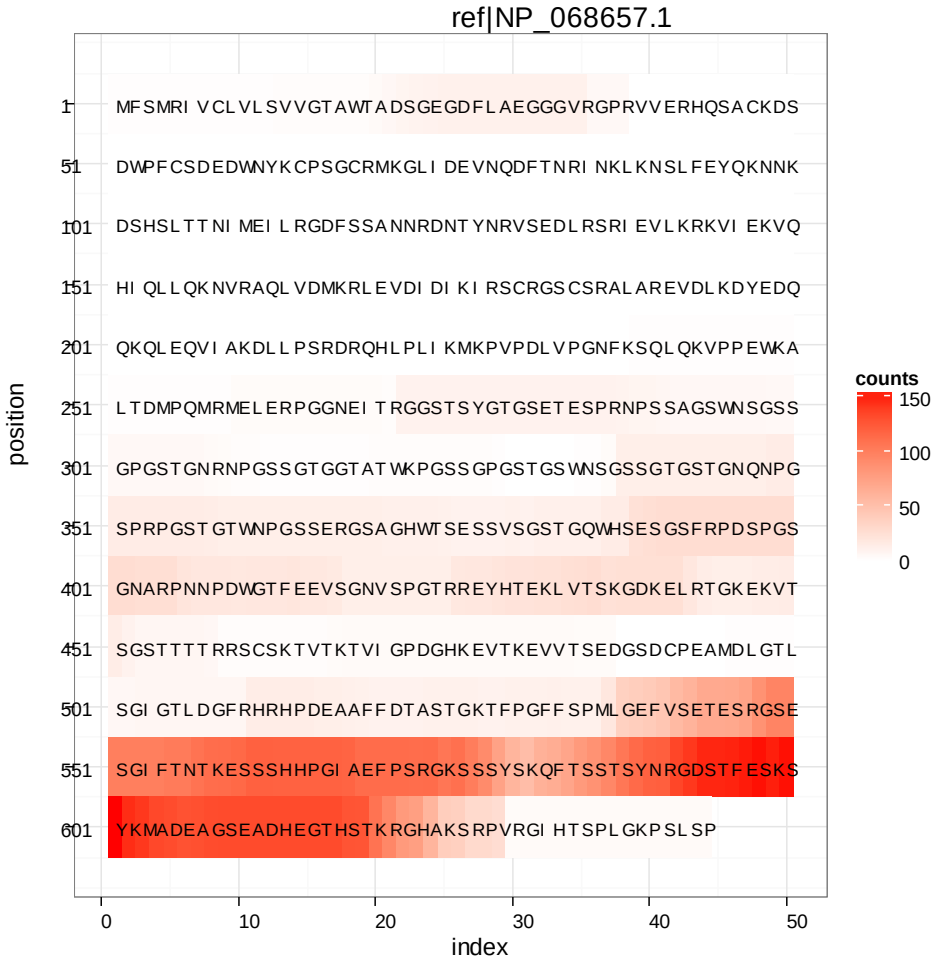
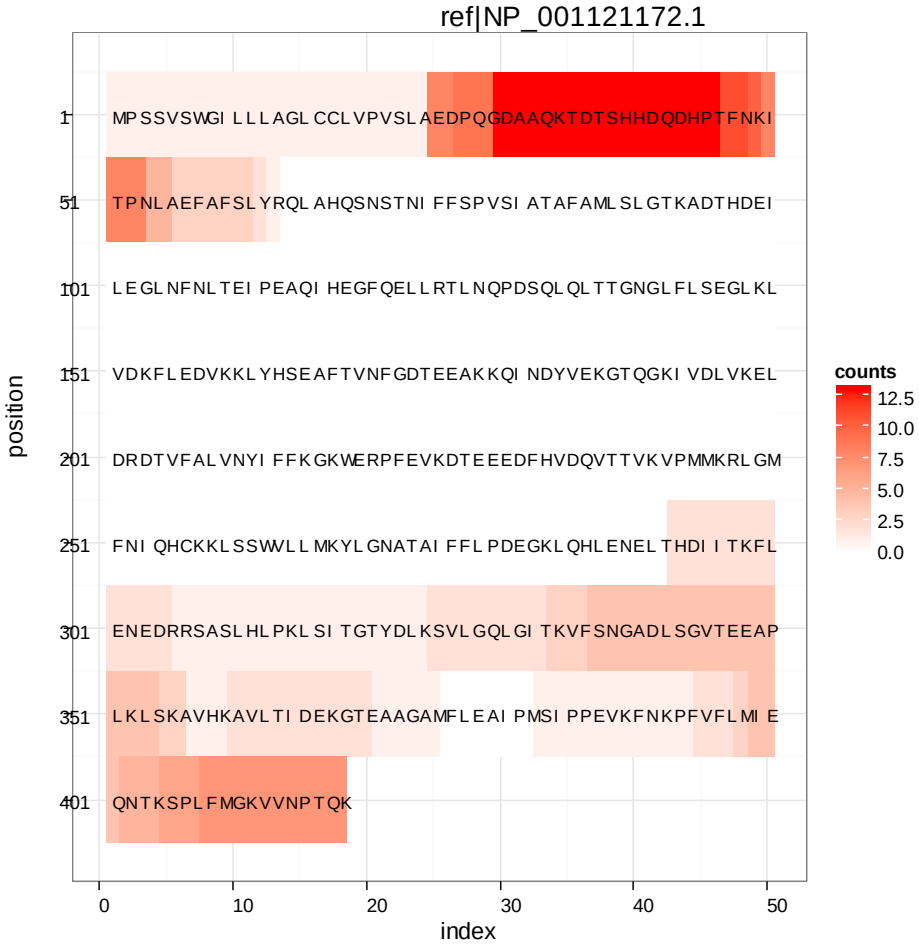
  NIH Biomedical Technology
  Research Resource (BTRR)
  P41GM103493

Normalization

1. Peptides identified in >=10 samples are retained
2. p-value based median normalization
(p value > 0.75 as the background)



Fibrinogen alpha chain



Alpha-1-antitrypsin