Supplementary Data:

Supplementary_Methods

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Supplementary Methods

Automated, solid-phase peptide extraction. Serum peptide profiling is done using a technology platform that was developed in our laboratory for simultaneous measurement of large numbers of serum polypeptides (1). It uses magnetic bead-based, solid-phase extraction of predominantly small peptides followed by a MALDI-TOF MS read-out. The system is intrinsically more sensitive than any surface capture on chips as spherical particles have larger combined surface areas than small-diameter spots. When combined with high-resolution MS, hundreds of peptides are detected in a single droplet of serum. In the current version, peptides are captured and concentrated using SiMAG-C8/K superparamagnetic, silica-based particles (≤1 micron diameter; 80% iron oxide; non-porous), bearing C8 reversed-phase (RP) ligands (Chemicell). All analyses were performed in a 96-well format, using the same batch of C8 magnetic particles, in 0.2-mL polypropylene tubes (8x12-tube 'Temp Plate II'; USA Scientific). The protocol is based on a detailed investigation of serum handling, RP ligand and eluant selection (1), and is automated using a 'Genesis Freedom 100' (Tecan) liquid handling workstation for throughput and reproducibility. The system was programmed either directly via its standard software or, when individual wells needed to be accessed independently, indirectly through its work-lister capability. This system automates all of the liquid-handling steps, including magnetic separation via a robotic manipulating arm, mixing of eluates with MALDI matrix and deposition onto the Bruker 384-spot MALDI target plates. A computer randomization program is used to position case and control samples for both solid-phase extraction and mass spectrometry.

Mass spectrometry. Peptide profiles were analyzed with an Autoflex MALDI-TOF mass spectrometer (Bruker) equipped with a 337 nm nitrogen laser, a gridless ion source, delayed-extraction (DE) electronics, a high-resolution timed ion selector (TIS), and a 2 GHz digitizer. Separate spectra were obtained for two restricted mass-to-charge (*m/z*) ranges, corresponding to polypeptides with molecular mass of 0.7-4 kDa ("≤4kD") and 4-15 kDa ("≥4kD") (assuming $z = 1$), under specifically optimized instrument settings. Each spectrum was the result of 400 laser shots, per *m/z* segment per sample, delivered in four sets of 100 shots (at 50-Hz frequency) to each of four different locations on the surface of the spot. A weekly performance test with commercial human reference serum (# S-7023, lot 034K8937; Sigma) was done, and the effective laser energy delivered to the target was adjusted when necessary. The entire irradiation program was automated using the instrument's 'AutoXecute' function. Spectra were acquired in linear mode geometry under 20 kV (18.6 kV during DE) of ion accelerating and -1.3 kV multiplier potentials, and with gating of mass ions ≤ 400 *m/z* (≤4kD segment) or ≤ 3,000 *m/z* (≥4kD segment). DE was maintained for 80 (≤4kD) or 50 nanoseconds (≥4kD) to give appropriate time-lag focusing after each laser shot. Peptide samples were always mixed with two volumes of premade a-cyano-4-hydroxycinnamic acid (ACCA) matrix solution (Agilent), deposited onto the stainless steel target surface, in every other column of the 384-spot layout, and allowed to dry at room temperature. Thirty fmoles (per peptide) and 500 fmoles (per protein) of commercially available calibration standards (Bruker # 206195 (<4kD) and # 206355 (>4kD)) were also mixed with ACCA matrix and separately deposited onto the target

plates, adjacent to each spotted serum sample (one sample / one standard), in the alternating columns. All spectra were acquired within less than 1-2 hours after completion of robotic sample processing, as we have noticed an adverse effect of increasing times between crystallization and mass spectral acquisition. The AutoFlex MALDI-TOF has a probe at the output of the laser, before the attenuator. We verified the accuracy of this monitoring device and then calibrated the settings of the attenuator (displayed on the computer screen as an arbitrary scale of 100 - 0 %) by measuring transmitted energy at varying %. This allowed us to generate a calibration curve to convert before-to-after attenuation laser energy. The optimal laser setting that had been empirically determined was then measured to yield 16-μJ energy per pulse, post-attenuation. Laser output energy is measured and documented on a weekly basis and adjustments are made accordingly to compensate for fading laser energy over time.

Assigning peptide sequences. A set of peptides previously selected on the basis of statistical differences in ion intensity between cancers and control groups was analyzed by MALDI-TOF/TOF tandem mass spectrometry, using an UltraFlex TOF/TOF instrument (Bruker) operated in 'LIFT' mode. The mono-isotopic masses were first assigned by one-dimensional reflectron-TOF MS, in the presence of three peptide calibrants (6 fmoles each; calculated monoisotopic masses of 2,108.155 Da, 1,307.762 Da and 969.575 Da in the protonated form), as described (2). Spectra were obtained by averaging multiple signals; laser irradiance and number of acquisitions (typically 100-150) were operator adjusted to yield maximal peak deflections derived from the digitizer in real time. Mono-isotopic masses were assigned for all selected and other prominent

peaks after visual inspection, and the low- and high-end internal standards were used for recalibration. The pass/fail criterion for recalibration is a correct assignment of an *m/z* value for the 'middle' calibrant with a mass accuracy equal or better than 12 ppm. Alternatively, a QSTAR XL Hybrid quadrupole (Q) time-of-flight mass spectrometer (Applied Biosystems/MDS Sciex), equipped with an o-MALDI ion source, was used for both duplicate and additional tandem-MS analyses. By selecting precursor ions of interest in 'Q1' (operated in the mass-filter mode), mass measurements of fragment ions could be obtained in the TOF detector following collision-induced dissociation (CID) in 'Q2'. Typically, a mass window of 3 Da was selected in order to transmit the entire isotopic envelope of the precursor ion species. Collision energy was operator adjusted to yield maximum number and intensities of the fragment ions.

Fragment ion spectra resulting from TOF/TOF and Q/TOF analyses (300-1,000 acquisitions averaged per spectrum) were taken to search a "non-redundant" human database ('NCBInr'; release data: 05-20-2005; 134,668 entries; National Center for Biotechnology Information) using the MASCOT MS/MS ion search program, version 2.0.04 for Windows (Matrix Science Ltd.), with the following search parameters: mono-isotopic precursor mass tolerance of 35 ppm, fragment mass tolerance of 0.5 Da, and without a specified protease cleavage site. Mascot 'mowse' scores greater than 35 were considered significant. Any identification thus obtained was verified independently by two different people by comparing the computer-generated fragment ion series of the predicted peptide with the experimental MS/MS data. Some sequence assignments had below-threshold scores but could nonetheless be unequivocally assigned as the

precursor ion mass and selected fragment ion masses (higher-intensity b" or y" ions resulting from preferential CID of Xaa-Pro and Asp/Glu-Xaa peptide bonds (3)) matched a particular peptide, representing a rung in one of the serum peptide sequence ladders (see Figure 5 and Supplementary Table 3).

Signal processing. *Setup*: Once acquired, all data are stored with a naming convention that allows each sample to be associated with its calibrant. The spectra are first converted from binary format to ASCII files containing two columns of data (x: m/z; y: intensity) by a custom written macro in FlexAnalysis (Bruker). For the lower mass range (700-4,000 Da), about 48,000 x,y-points were generated while for the upper mass range (4-15 kDa), there were about 77,000 points. Further data processing was done in MATLAB with a custom script called 'Qcealign' using only the ASCII versions of the raw spectra. 'Qcealign' used the 'Qpeaks' program (Spectrum Square Associates) for smoothing, baseline subtraction and peak labeling (4). The singletwidth parameter required by 'Qpeaks' was set to -400 for the lower mass range and -200 for the upper mass range, thereby specifying the resolution, $(m/z)/\Delta(m/z)$, for processing. Peak information was used automatically by 'Qpeaks' in setting the parameters for smoothing, baseline-subtraction, and binning. The noise statistics were assumed 'Normal'.

Processing: Following parameter selection, a setup file is created. 'Qcealign' then queries the setup file to obtain a list of all the directories for processing. During a single processing run, all data files in all listed directories are aligned with each other. For each directory, singletwidth information is provided in the setup file, along with parameters controlling calibration, peak labeling sensitivity, alignment, etc. The files

containing the polypeptide standards are calibrated first. The centroid positions of peaks in these calibration files are obtained from the peak table created by 'Qpeaks', compared to the known polypeptide peak positions, and a quadratic calibration equation for correcting the measured masses in each calibration file is created. The calibration equations are saved to disk for use in calibrating the mass axes of the sample files. Next, 'Qcealign' creates a reference file to which all sample spectra will later be aligned. The first data file is loaded and calibrated by applying the curve calculated from its associated calibrant spectrum. This file's x-axis (*m/z*) becomes the x-axis (and thus the calibration) used in the reference file. 'Qcealign' then loads all other sample files, calibrates them, and adds their intensities to the reference file's intensity. After all samples have been added, the reference spectrum becomes the average of all the sample files. The reference is processed with 'Qpeaks' to find a baseline, which is subtracted, and is then normalized to unit size by dividing each intensity value by the Total Ion Count (TIC). Once normalized, a scaling factor is added by multiplying each intensity value by a user-selected number (e.g., 10^7). This scaling factor is constant within a data set and is used to convert the normalized spectrum to a "user friendly" scale, where most peak heights are greater than one. Next, 'Qcealign' processes each sample file with 'Qpeaks' to create a peak table, smoothed curve and a baseline. This spectrum is then taken for alignment.

Alignment: A custom alignment algorithm, 'Entropycal', aligns sample data files to a reference file using a minimum entropy algorithm by taking unsmoothed ('raw'), baseline-corrected data (4). Taking raw spectra for alignment facilitates the use of all

statistical information in the data; processed data contains less information. The alignment is performed in two steps: 'Entropycal' and binning. 'Entropycal' slides each data file by 'n' data points to the right or left along the x-axis of the reference file. At each relative position n, the Shannon entropy of the sum of the two files is computed. The optimal alignment occurs at the shift that produces the minimum Shannon entropy. Once aligned, the smoothed spectrum, which is produced as a byproduct of the 'Qpeaks' processing, is then updated to reflect the aligned m/z values and saved to disk after baseline-subtraction, normalization and scaling. The previously generated peak table is updated to reflect the baseline-subtraction, normalization, scaling and alignment. The peak lists are then binned by using the resolution of the peaks: all peaks in rows within Δ(m/z) of the strongest peak at a given value of m/z are binned together, and a spreadsheet is created for further statistical analysis.

MATLAB software tools. Three software modules, developed in MATLAB, were used for visualization and signal processing of the spectra (4). (I) *Signal Processing & Preview (SPP)*, a graphical viewer for spectra in ASCII format, allows plotting raw and processed spectra side-by-side to review the outcome of signal processing. Furthermore, parameters of 'Qpeaks' (the signal processing software) can be adjusted. (II) *Mass Spectra Viewer (MSV)*, a visual interface for processed spectral data, plots spectra as X-Y curves (mass vs. magnitude) for examining the signatures of several groups of samples. MSV supports regular browsing functions such as scroll, zoom, highlighting, etc. (III) *HeatMap (HM)* displays spectra as a 2D heat map images, in which the magnitude of the peaks are color-coded on a continuous scale. In addition

to browsing functions such as zoom and scroll, the rank of X- and Y- position coordinates can be reorganized without the constraints of statistical correlation that are enforced by most HeatMap commercial software packages.

References

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Supplementary Table 1. *Age distribution, gender and clinical characteristics of 106 study samples used for serum peptide profiling*. All samples were collected, stored and handled at Memorial Sloan-Kettering Cancer Center following established protocol (Villanueva et al., 2005, J Proteome Res 4:1060-72). Abbreviations: PSA, prostate-specific antigen; LVI, lymphatic/vascular invasion; ER, estrogen receptor: PR, progesterone receptor; LN, lymph nodes; pT, pathologic stage.

Supplementary Table 2. *Data set of 68 selected biomarker m/z-peaks, derived from MALDI-TOF MS serum peptide profiling of three groups of cancer patients and healthy controls.* The three sub-tables contain: (A) averages plus (±) standard deviations, and medians (in brackets) of the intensities of each *m/z*-peak (i.e., serum peptide) within a particular data set derived from each of the three cancer patient groups and of the healthy controls. Intensities refer to normalized units that were calculated for each peak by dividing its raw intensity by the total of all the intensities in that spectrum (TIC – Total Ion Count). The resultant values were then multiplied by fixed scaling factor (1×10^7) to convert the data to a 'user-friendly' scale (i.e. most values ≥1). (B) Ratios were calculated by dividing the median normalized intensity of each *m/z*-peak in each cancer group by the median of the same *m/z*-peak in the control group. To avoid having to divide by zero, any median value of less than was converted to 1; this was applied to all groups. Data for a second, independent validation set of prostate cancer samples is also listed. (C) False discovery rate adjusted p-values were calculated for each *m/z*-peak using the Mann-Whitney rank sum test (for binary comparisons) or the Kruskal-

Wallis test (for multi-class comparisons). The group of 68 *m/z*-peaks listed here were derived from the original peak list, containing normalized ion intensities (and medians within a group, case/control ratios and adjusted p-values) for each of the 651 m/z-peaks for each of the 106 samples, by applying p-value and median intensity cutoff filters ($p<0.00001$; median intensity ≥500 'units') to. Entries which passed both filters in one or more cancer groups are color-coded: prostate cancer (14; blue), breast cancer (14; red) and bladder cancer (58; green).

Supplementary Table 3. *Total number of serum peptide sequences obtained in the course of current study.* Sequences are organized per overlapping cluster; and clusters are organized per precursor protein (NCBI ID nos. are given). Positions in the precursor proteins are indicate. Residues between brackets were not observed but are listed here to indicated the putative primary cleavage sites by endoproteases. Additional information is given, as for instance the relative position of adjacently located peptides or peptide clusters, identity of previously known serum petides (e.g., FPA, C3f), position of propeptides, and location of C-termini (C-t). Key: Met_{ox} or M_{ox}, oxidized methionine; $Pro_{hydroxyl}$, hydroxylated proline.

Supplementary Table 4. *Locations of sequenced serum peptides in the precursor proteins.* NCBI ID nos. are given, as well as the positions of known, processed serum proteins, peptides and propeptides. The peptide sequences obtained in the current study are shown in bold and are underlined.

Supplementary Figure 1. *MALDI-TOF mass spectral overlays of selected peaks derived from serum peptide profiling of three groups of cancer patients, and male and female healthy controls.* Spectra were obtained, aligned and normalized as described in the 'Methods' section, and are displayed using the Mass Spectra Viewer (MSV). Peptide-ions have been selected to illustrate cancer-specific differences of normalized intensities compared to controls. The nine overlays (not to scale) each show a binary comparison for all spectra from either the bladder cancer (*n*=20; green), or prostate cancer (*n*=32; blue) or breast cancer patient group (*n*=21; red) versus male control (n=14; black) and female control groups (*n*=19; yellow). The mono-isotopic mass (*m/z*) is shown for each peptide-ion peak.

TABLE S1: Study set of cancer patients and healthy controls (Villanueva *et al*.)

A. PROSTATE CANCER

n = 32 **TRAINING SET** **n = 41** **VALIDATION SET**

All Hormone-Refractory Chemo-Naïve		Age when Blood was Drawn	Highest MSKCC Gleason	PSA at time of Blood Draw	All Hormone-Refractory Chemo-Naïve		Age when Blood was Drawn	Highest MSKCC Gleason	PSA at time of Blood Draw
					Average:				
Average:		51	7	214	Age	67	52	9	247
Age	66	55	8	51	Gleason	7.9	53	10	51
Gleason	7.7	56	10	1380	PSA	400	54	7	5
PSA	232	57	7	95			57	None	538
		57	8	119			58	9	18
		58	9	585	**Median:**		59	9	29
Median:		59	6	94	Age	67	61	8	821
Age	66	60	8	74	Gleason	8	61	8	22
Gleason	8	60	9	10	PSA	133	62	7	329
PSA	66	61	9	232			62	8	55
		62	6	21			63	None	89
		62	8	417			63	9	15
		63	7	24			63	8	775
		64	7	495			64	8	26
		64	None	44			65	8	51
		64	None	68			65	7	622
		67	7	25			65	7	75
		67	9	11			66	7	531
		67	None	0.31			66	6	1845
		68	6	8			67	7	138.8
		70	None	48			67	9	87
		70	7	25			68	7	417
		71	7	305			68	8	87
		71	8	650			68	7	1737
		72	None	63			69	8	603
		73	6	43			69	8	1113
		73	9	42			69	7	428
		75	7	21			69	8	18
		75	8	796			70	9	133
		77	8	50			71	8	24
		78	None	1283			71	None	369
		81	8	131			73	10	91
							73	8	23.08
							75	8	255
							76	7	252
							76	9	24
							77	9	249
							77	7	58
							80	7	1715
							80	7	131
							81	8	2298

B. BREAST CANCER

n = 21

		AGE	INVASIVE TYPE	SIZE (cm)	HISTOL GRADE	NUCLEAR GRADE	LVI	ER	PR	Her-2	LN
Average:		28	DUCT/MUC	3.5	3	3	?	90	0	0	neg
Age	51.6	35	NONE	NA	na	na	na	na	na	na	neg
Size (cm)	1.4	40	DUCT	0.6 MAX	3	3	Y, ext	0	0	1	
		40	DUCT	1.2	3	2	sus	90	80	1+	neg
		42	DUCT	2.5	3	3	Y	100	30	2+	+
		43	DUCT	1.6	1 to 2	2	no	60	100	1+	neg
Median:		47	DUCT	1	2	2	no	100	90	1+	neg
Age	49	47	DUCT	2.1	3	2	no	90	50	0	neg
Size (cm)	1.3	48	DUCT	1.1	2	2	no	90	60	1+	neg
		49	LOB	1.3	na	na	no	60	60	1	neg
		49	DUCT	0.85	3	2	no	95	<5	1+	neg
		51	DUCT	1.4	3	3	no	0	0	0	2 of 26
		53	DUCT	0.5	3	3	no	100	100	1+	neg
		54	TUB/LOB	0.9	2	2	NO	90	90	0	2

55	DUCT	1.5	3	2	?	60	80	0	neg
56	DUCT	2	3	2	yes	100	40	0	neg
61	DUCT	0.9	3	2	NO	95	5	1	neg
67	DUCT	1.9	2 to 3	2	NO	0	0	1	neg
68	DUCT	0.5	2 to 3	2	NO	100	<5	1+	micromet
72	DUCT	1.7	3	3	NO	0	0	2 (ampl)	neg
79	DUCT	1.1	3	2	no	75	25	1+	neg

C. BLADDER CANCER

n = 20

		AGE	SEX	STAGE	GRADE	Vascular invasion
Average:		49	M	pT1	High	No
Age	66.1	50	M	pT4	High	Yes
		52	M	pTIS	High	No
Median:		53	F	pTIS	High	No
Age	67	58	M	pT3B	High	Yes
		60	M	pT1	High	Yes
		62	M	pTA	High	No
		63	M	pTIS	High	No
		64	M	pT3A	High	No
		67	M	pTA	Low	No
		67	M	pT3B	High	Yes
		70	M	pTA	Low	No
		71	M	pTA	High	No
		74	F	pT3B	High	Yes
		74	M	pT1	High	No
		75	M	pTIS	Low	No
		75	M	pT1	High	No
		79	M	pTIS	High	No
		79	M	pTA	Low	No
		80	M	pTIS	High	No

D. NON-CANCER CONTROLS

n=33

		AGE	SEX
Average:		23	F
Age	34.9	23	F
		23	M
Median:		24	M
Age	31	25	F
		25	M
		25	M
		26	F
		26	F
		27	F
		27	F
		27	M
		28	M
		29	F
		30	F
		31	F
		31	M
		32	M
		34	F
		35	M
		39	F
		40	F
		42	F
		42	F
		43	M
		44	F
		46	M
		47	F
		48	F
		51	F
		51	M
		52	M
		56	M

Table S2: Feature selection (Villanueva *et al.*)

A

Binary Comparisons Mean ± STD (Median)

M/Z-value	Prostate (14)	Breast (14)	Bladder (58)	Controls
823	252.99 ± 173.92, (204.02)	160.11 ± 69.57, (135.22)	1064.54 ± 726.55, (829.12)	195.36 ± 80.69, (177.91)
830	254.8 ± 166.72, (233.97)	143.99 ± 43.67, (142.23)	1156.92 ± 703.1, (1243.67)	176.1 ± 127.5, (149.42)
890	594.67 ± 338.63, (543.63)	309.71 ± 123.02, (280.38)	778.16 ± 225.79, (752.41)	404.31 ± 117.45, (371.2)
906	4561.2 ± 1727.3, (4511.86)	7338.56 ± 2182.97, (6910.05)	3272.39 ± 767.81, (3381.28)	4369.04 ± 539.06, (4265.11)
944	1161.36 ± 752.6, (943.64)	332.25 ± 102.72, (313.79)	2817.62 ± 707.49, (2808.31)	643.38 ± 420.99, (542)
1022	1581.47 ± 1259.27, (1121.19)	2769.8 ± 1905.14, (1894)	1207.52 ± 762.91, (1116.83)	4016.7 ± 464.05, (4018.76)
1046	444.12 ± 245.1, (371.99)	763.89 ± 221.41, (743.79)	155.23 ± 151.88, (160.86)	1002.57 ± 279.33, (894.87)
1057	274.25 ± 299.72, (226.73)	181.78 ± 247.01, (1)	939.21 ± 493.65, (1050.72)	25.32 ± 61.91, (1)
1062	2911.33 ± 1457.22, (3074.48)	8192.98 ± 2705.77, (7566.57)	1746.85 ± 958.92, (1694.1)	4216.94 ± 790.82, (3972.51)
1079	1880.88 ± 1044.4, (2014.18)	3688.8 ± 1709.02, (3515.63)	1909.45 ± 528.38, (1833.42)	3664.76 ± 401.86, (3702.34)
1100	438.06 ± 226.06, (362.24)	186.9 ± 89.59, (204.22)	600.4 ± 292.61, (581)	254.91 ± 72.11, (253.33)
1125	252.64 ± 146.67, (214.62)	477.82 ± 166.46, (445.61)	115.07 ± 77.82, (128.27)	521.36 ± 195.04, (515.14)
1209	2259.24 ± 1661.17, (2267.99)	3437.89 ± 2294.84, (3107.73)	2171.06 ± 864.61, (1970.59)	4558.92 ± 641.12, (4517.21)
1214	1460.59 ± 1300.61, (1391.49)	205.32 ± 398.04, (1)	2038.25 ± 989.02, (1912.04)	483.85 ± 741.93, (177.1)
1231	142.29 ± 127.3, (162.25)	177.67 ± 111.17, (222.86)	967.3 ± 495, (1016.21)	173.62 ± 133.9, (179.55)
1266	908.3 ± 670.36, (638.46)	1200.4 ± 1082.93, (755.41)	988.66 ± 727.44, (785.8)	3187.09 ± 640.76, (3271.91)
1280	315.68 ± 237.5, (251.92)	84.09 ± 166.11, (1)	1446.41 ± 708.12, (1406.33)	73.44 ± 278.74, (1)
1313	106.62 ± 251.67, (1)	36.1 ± 77.88, (1)	715.63 ± 501.13, (634.96)	38.84 ± 129.6, (1)
1353	2080.48 ± 1235.52, (2102.44)	2153.51 ± 1680.56, (1571.81)	2107.42 ± 835.37, (2086.29)	4545.13 ± 463.86, (4496.6)
1383	145.8 ± 87.14, (164.77)	781.38 ± 358.17, (795.73)	192.51 ± 178.45, (151.76)	173.54 ± 76.32, (183.51)
1426	193.04 ± 195.89, (174.45)	277.17 ± 80.82, (286.87)	1103.34 ± 806.59, (840.46)	320.38 ± 250.78, (261.41)
1453	2159.29 ± 1380.63, (1885.15)	390.67 ± 177.43, (437.78)	2751.62 ± 1140.1, (2646.51)	565.13 ± 755.93, (1)
1468	3240.85 ± 1805.38, (3378.1)	3949.53 ± 2241.27, (4081.59)	2915.25 ± 948.24, (2834.33)	5117.08 ± 720.94, (5133.73)
1502	100.31 ± 147.84, (1)	67.74 ± 113.81, (1)	831.64 ± 535, (809.48)	29.71 ± 81.34, (1)
1522	688.1 ± 334.43, (598.26)	1047.44 ± 452.72, (911.47)	269.71 ± 299.37, (242.68)	1465.61 ± 330.44, (1583.23)
1534	888.53 ± 546.08, (768.39)	944.93 ± 726.19, (746)	1915.21 ± 742.33, (2058.15)	866.59 ± 492.35, (788.51)
1540	511.92 ± 415.35, (568.44)	548.63 ± 337.96, (526.19)	63.06 ± 247.7, (1)	904.68 ± 283.76, (979.16)
1566	866.76 ± 701.1, (620.9)	448.06 ± 107.93, (424.35)	1121.01 ± 631.83, (892.96)	769.93 ± 167, (788.67)
1619	494.63 ± 434.51, (345.33)	411.56 ± 270.5, (308.13)	183.73 ± 118.29, (179.97)	565.78 ± 195.43, (533.41)
1630	284.69 ± 140.7, (298.72)	810.04 ± 377.15, (764.04)	318.86 ± 183.28, (240.99)	249.83 ± 113.53, (274.41)
1694	1190.9 ± 1175.42, (795.86)	158.28 ± 125, (197.94)	1801.44 ± 1248.81, (1346.59)	264.99 ± 200.67, (196.44)
1743	868.29 ± 507.68, (764.05)	2528.75 ± 1182.77, (2817.04)	904.21 ± 659.21, (669.96)	985.98 ± 272.35, (1020.07)
1757	175.76 ± 150.02, (199.37)	139.54 ± 140.04, (149.67)	660.76 ± 269.38, (625.98)	90.96 ± 76.14, (109.77)
1782	1755.48 ± 1517.47, (1015.47)	206.3 ± 110.37, (222.49)	2209.42 ± 1326.83, (1789.4)	348.39 ± 261.82, (232.33)
1868	3335.21 ± 1759.63, (2660.63)	444.93 ± 164.29, (369.54)	3975.98 ± 897.39, (4057.03)	1387.84 ± 932.62, (1219.19)
1899	1159.19 ± 551.08, (1042.22)	2549.48 ± 1183.38, (2415.09)	2569.25 ± 761.88, (2732.08)	817.45 ± 243.86, (819.5)
1931	182.49 ± 219.7, (78.66)	6.36 ± 29.14, (1)	694.31 ± 390.38, (671.27)	40.47 ± 95.75, (1)
1975	45.77 ± 110.64, (1)	56.5 ± 85.43, (1)	749.54 ± 588.68, (640.68)	31.52 ± 79, (1)
1981	465.51 ± 303.8, (401.9)	253.16 ± 94.16, (252.61)	509.78 ± 192.1, (503.42)	280.44 ± 83.61, (291.52)
2119	423.08 ± 416.02, (307.12)	938.9 ± 524.4, (1011.77)	1196.66 ± 694.48, (1166)	104.39 ± 139.48, (95.37)
2145	147.6 ± 116.77, (147.98)	175.22 ± 110.49, (164.93)	566.47 ± 246.56, (587.13)	88.45 ± 42.79, (85.25)
2189	444.65 ± 193.51, (399.04)	700.19 ± 340.28, (659.4)	442.72 ± 223.55, (418.33)	248.71 ± 72.2, (233.35)
2273	1179.54 ± 966.02, (810.1)	396.38 ± 501.88, (258.68)	578.94 ± 448.48, (467.27)	198.98 ± 126.21, (176.84)
2341	225.26 ± 140.74, (197.02)	235.01 ± 143.67, (236.19)	771.06 ± 852.04, (548.12)	118.8 ± 96.8, (112.58)
2358	179.83 ± 285.35, (99.81)	534.86 ± 312.47, (531.1)	75.2 ± 76.55, (72.58)	26.81 ± 49.77, (1)
2383	602.55 ± 380.24, (549.5)	628.11 ± 218.93, (600.55)	98.26 ± 72.59, (76.29)	329.12 ± 105.17, (350.32)
2414	207.25 ± 264.92, (96.71)	97.34 ± 70.28, (109.24)	1818.24 ± 1301.3, (2124.24)	84.07 ± 281.49, (1)
2513	215.6 ± 379.84, (160.91)	854.57 ± 405.59, (928.01)	151.76 ± 74.32, (140.08)	102.04 ± 60.83, (116.58)
2570	63.36 ± 287.03, (1)	0 ± 0, (1)	960.6 ± 826.63, (901.87)	54.15 ± 259.92, (1)
2615	565.34 ± 322.95, (476.37)	686.64 ± 335.04, (792.37)	389.77 ± 315.07, (385.25)	182.64 ± 73.01, (167.56)
2729	310.64 ± 437.03, (162.51)	152.86 ± 60.24, (152.26)	530.95 ± 268.79, (573.43)	109.58 ± 77.01, (92.98)
2760	162.39 ± 302.21, (98.96)	83.07 ± 70.13, (109.19)	1333.1 ± 1073.2, (1104.71)	107.96 ± 178.88, (89.45)
2773	1374.94 ± 769.55, (1402.29)	1729.28 ± 915.47, (1767.74)	230.77 ± 460.16, (57.11)	1844.05 ± 626.78, (1810.76)
2937	2849.34 ± 1600.28, (3138.65)	2460.84 ± 1085.04, (2461.74)	449 ± 566.03, (327.68)	2535.87 ± 532.94, (2536.62)
3162	490.75 ± 219.94, (460.99)	218.04 ± 232.1, (178.22)	1378.69 ± 774.81, (1435.63)	146.55 ± 76.97, (134.48)
3188	185.07 ± 351.68, (96.45)	116.96 ± 71.53, (130.31)	994.5 ± 724.4, (814.41)	146.72 ± 110.92, (130.05)
3195	1230.27 ± 914.13, (1140.9)	1968.93 ± 882.4, (2048.72)	163.96 ± 352.72, (1)	2238.86 ± 540.43, (2188.87)
3267	1694.49 ± 1337.71, (1340.86)	3845.22 ± 1607.33, (3737.23)	363.36 ± 435.27, (308.7)	4053.35 ± 608.94, (4055.38)
3278	241 ± 545.8, (1)	109.86 ± 331.01, (1)	1187.65 ± 600.05, (1277)	35.09 ± 153.42, (1)
3960	1089.65 ± 699.43, (905.2)	693.97 ± 377.07, (590.35)	1740.34 ± 956.75, (1677.38)	694.26 ± 210.11, (682.01)
3976	92.08 ± 242.92, (1)	274.17 ± 624.47, (1)	1142.42 ± 880.3, (957.31)	81.4 ± 165.25, (1)
4197	885.71 ± 594.73, (876.96)	1599.54 ± 367.22, (1684.09)	68.51 ± 256.55, (1)	1384.26 ± 345.28, (1260.57)
4270	815.44 ± 772.4, (661.24)	461.83 ± 1014.05, (205.45)	2619.7 ± 1488.91, (2851.85)	268.04 ± 572.24, (1)
4631	950.45 ± 378.46, (819.99)	994.7 ± 271.21, (986.77)	664.56 ± 335.09, (747.07)	1191.67 ± 292.57, (1194.98)
4990	188.71 ± 488.44, (1)	0 ± 0, (1)	828.21 ± 487.82, (739.13)	41.29 ± 139.31, (1)
5052	431.3 ± 291.5, (440.99)	547.41 ± 189.68, (524.93)	229.54 ± 409.22, (1)	606.09 ± 264.85, (571.51)
5888	1054.81 ± 627.53, (1017.01)	2596.05 ± 1033.3, (2365.27)	243.27 ± 400.35, (146.37)	1850.13 ± 522.29, (1871.1)
9265	880.23 ± 381.87, (736.72)	1376.35 ± 358.29, (1379.52)	613.52 ± 282.95, (612.02)	1230.99 ± 312.11, (1224.81)

B

Ratio: Group/Control

M/Z	Prostate	Breast	Bladder	Prostate2
823	1.15	0.76	4.66	0.52
830	1.57	0.95	8.32	0.58
890	1.46	0.76	2.03	1.03
906	1.06	1.62	0.79	2.09
944	1.74	0.58	5.18	2.01
1022	0.28	0.47	0.28	0.15
1046	0.42	0.83	0.18	0.40
1057	226.73	1.00	1050.72	191.06
1062	0.77	1.90	0.43	1.16
1079	0.54	0.95	0.50	0.59
1100	1.43	0.81	2.29	0.99
1125	0.42	0.87	0.25	0.28
1209	0.50	0.69	0.44	0.19
1214	7.86	0.01	10.80	7.39
1231	0.90	1.24	5.66	0.33
1266	0.20	0.23	0.24	0.11
1280	251.92	1.00	1406.33	231.96
1313	1.00	1.00	634.96	21.25
1353	0.47	0.35	0.46	0.25
1383	0.90	4.34	0.83	0.42
1426	0.67	1.10	3.22	0.40
1453	1885.15	437.78	2646.51	2217.87
1468	0.66	0.80	0.55	0.33
1502	1.00	1.00	809.48	98.91
1522	0.38	0.58	0.15	0.33
1534	0.97	0.95	2.61	0.73
1540	0.58	0.54	0.00	0.31
1566	0.79	0.54	1.13	0.60
1619	0.65	0.58	0.34	0.55
1630	1.09	2.78	0.88	0.89
1694	4.05	1.01	6.85	4.92
1743	0.75	2.76	0.66	1.23
1757	1.82	1.36	5.70	1.34
1782	4.37	0.96	7.70	6.79
1868	2.18	0.30	3.33	3.89
1899	1.27	2.95	3.33	1.97
1931	78.66	1.00	671.27	158.45
1975	1.00	1.00	640.68	52.59
1981	1.38	0.87	1.73	1.52
2119	3.22	10.61	12.23	2.84
2145	1.74	1.93	6.89	1.28
2189	1.71	2.83	1.79	2.17
2273	4.58	1.46	2.64	6.30
2341	1.75	2.10	4.87	1.58
2358	99.81	531.10	72.58	112.76
2383	1.57	1.71	0.22	3.46
2414	96.71	109.24	2124.24	64.14
2513	1.38	7.96	1.20	1.69
2570	1.00	1.00	901.87	170.85
2615	2.84	4.73	2.30	5.86
2729	1.75	1.64	6.17	3.64
2760	1.11	1.22	12.35	5.42
2773	0.77	0.98	0.03	1.61
2937	1.24	0.97	0.13	2.32
3162	3.43	1.33	10.68	4.23
3188	0.74	1.00	6.26	0.01
3195	0.52	0.94	0.00	0.94
3267	0.33	0.92	0.08	0.74
3278	1.00	1.00	1277.00	399.45
3960	1.33	0.87	2.46	1.88
3976	1.00	1.00	957.31	302.46
4197	0.70	1.34	0.00	1.51
4270	661.24	205.45	2851.85	753.39
4631	0.69	0.83	0.63	1.45
4990	1.00	1.00	739.13	63.32
5052	0.77	0.92	0.00	0.84
5888	0.54	1.26	0.08	1.48
9265	0.60	1.13	0.50	1.72

C

p-value

M/Z	Prostate	Breast	Bladder	Prostate2	Multiclass
823	0.11	7.42E-03	1.30E-12	3.27E-06	3.24E-15
830	2.53E-05	0.558	1.06E-09	1.21E-04	1.85E-15
890	1.55E-04	9.54E-03	8.72E-10	0.808	1.64E-14
906	0.593	7.39E-10	3.12E-07	5.68E-07	3.65E-14
944	1.01E-03	3.56E-05	7.65E-13	9.67E-06	6.77E-21
1022	1.34E-11	0.0323	2.80E-13	6.29E-13	1.67E-13
1046	2.20E-11	1.22E-03	1.80E-13	8.34E-05	9.04E-24
1057	5.29E-07	8.13E-03	1.80E-13	2.35E-09	1.22E-13
1062	4.89E-04	1.03E-11	1.03E-12	0.272	9.04E-24
1079	2.94E-11	0.683	7.65E-13	9.69E-05	1.97E-14
1100	2.96E-06	0.0356	5.55E-08	0.665	7.10E-15
1125	6.84E-08	0.676	7.65E-13	3.28E-04	3.51E-18
1209	4.18E-08	0.0654	1.30E-12	1.91E-10	1.31E-09
1214	8.63E-04	0.251	9.43E-08	4.13E-07	8.69E-11
1231	0.523	0.867	1.10E-10	0.02	9.53E-11
1266	6.48E-17	2.75E-07	1.05E-10	4.47E-20	2.74E-16
1280	4.81E-09	0.434	1.95E-13	5.19E-13	5.74E-20
1313	0.193	0.834	2.94E-11	6.81E-05	2.40E-12
1353	1.01E-15	4.82E-05	4.50E-13	6.26E-17	1.38E-14
1383	0.245	5.56E-13	0.506	3.29E-07	6.74E-13
1426	5.65E-05	0.901	8.63E-06	6.96E-11	8.37E-11
1453	6.86E-07	0.852	6.37E-10	1.40E-12	1.98E-14
1468	1.44E-05	0.128	1.25E-11	4.92E-08	1.88E-07
1502	0.0525	0.429	2.93E-15	1.71E-10	5.31E-15
1522	2.20E-11	1.04E-03	2.16E-12	1.76E-18	1.18E-19
1534	0.977	0.864	9.76E-06	0.513	1.33E-05
1540	1.08E-04	1.38E-04	2.52E-10	1.26E-11	5.10E-12
1566	0.46	6.15E-10	0.0904	2.90E-10	3.64E-08
1619	0.023	0.0178	1.05E-10	2.33E-04	4.80E-09
1630	0.288	6.69E-12	1	0.821	4.28E-10
1694	1.69E-04	0.4	8.73E-11	1.22E-15	4.77E-13
1743	0.0758	3.92E-06	0.129	0.0835	2.84E-08
1757	0.0103	0.389	4.50E-13	8.46E-04	9.48E-13
1782	5.29E-07	0.522	1.06E-09	1.18E-18	1.85E-15
1868	1.80E-05	1.39E-05	3.34E-11	1.18E-18	2.17E-19
1899	0.0153	1.04E-08	4.50E-13	6.05E-07	9.14E-17
1931	0.0241	0.12	6.60E-14	6.03E-09	1.47E-16
1975	0.989	0.424	4.10E-09	4.41E-07	1.89E-10
1981	2.15E-03	0.683	7.70E-06	1.03E-07	1.15E-07
2119	2.36E-07	8.19E-10	2.75E-11	6.62E-09	5.82E-18
2145	0.0189	5.27E-05	7.65E-13	0.131	3.45E-14
2189	6.59E-07	1.49E-08	2.21E-04	1.65E-07	9.87E-11
2273	7.63E-12	0.0981	5.29E-06	1.67E-13	3.08E-13
2341	3.07E-04	2.40E-04	1.05E-10	1.91E-04	1.18E-11
2358	3.68E-04	4.07E-12	4.39E-03	5.38E-12	8.65E-13
2383	4.37E-04	1.26E-07	1.25E-10	8.74E-11	1.54E-16
2414	8.82E-04	6.77E-03	3.52E-12	9.67E-06	7.39E-13
2513	0.0101	5.56E-13	0.0929	1.74E-05	5.60E-14
2570	0.532	0.105	2.81E-07	9.19E-10	4.26E-13
2615	1.43E-08	2.08E-07	0.0317	6.55E-19	6.60E-10
2729	5.99E-06	4.31E-04	4.48E-09	3.88E-11	9.52E-13
2760	0.605	0.533	1.95E-07	4.07E-08	3.06E-07
2773	0.0514	0.695	8.42E-11	2.30E-03	8.37E-11
2937	0.416	0.937	4.19E-11	4.55E-08	5.39E-09
3162	2.64E-12	0.198	7.04E-10	2.48E-15	1.74E-19
3188	0.0228	0.923	7.64E-09	7.31E-08	1.38E-09
3195	7.74E-07	0.499	1.95E-13	0.429	3.76E-17
3267	7.63E-12	0.875	1.82E-13	0.0218	6.77E-21
3278	0.0122	0.887	2.44E-14	2.65E-11	2.82E-14
3960	0.0514	0.546	1.48E-06	4.32E-07	4.78E-06
3976	1	0.29	5.98E-09	7.70E-08	2.68E-09
4197	5.73E-04	0.105	1.95E-13	4.14E-04	3.89E-16
4270	2.83E-04	0.276	4.92E-09	5.54E-07	1.36E-09
4631	3.58E-03	0.0323	3.33E-08	2.48E-03	4.27E-07
4990	0.288	0.322	5.15E-14	2.63E-06	5.90E-18
5052	8.63E-04	0.424	5.52E-08	0.0799	1.35E-08
5888	5.28E-06	0.0186	1.25E-11	0.0449	1.90E-19
9265	4.16E-04	0.256	2.03E-09	1.30E-04	1.71E-11

Table S3: Serum Peptide Identifications (Villanueva *et al.*)

m/z	MH+	Δ(Da)	AA sequence	
1) Fibrinopeptide A (FPA); NCBI # 229185 – Fibrinogen alpha; NCBI # 4033511 (Res. 20-35)				
758.45	758.41	(0.04)	LAEGGGVR	
905.50	905.48	(0.02)	FLAEGGGVR	
1020.47	1020.51	(0.04)	DFLAEGGGVR	
1077.53	1077.53	(0.00)	GDFLAEGGGVR	
1206.57	1206.58	(0.01)	EGDFLAEGGGVR	
1263.60	1263.59	(0.01)	GEGDFLAEGGGVR	
1350.64	1350.63	(0.01)	SGEGDFLAEGGGVR	
1465.65	1465.65	(0.00)	DSGEGDFLAEGGGVR	
1536.68	1536.69	(0.01)	ADSGEGDFLAEGGGVR	= FPA
Fibrinogen alpha; NCBI # 4033511 (Res. 548-574 and 576-604, separated by K575; 605-629)				
2816.25	2816.31	(0.06)	(R) GSESGIFTNTKESSSHHPGIAEFPSRG (K)	
2553.01	2553.09	(0.08)	(K) SSSYSKQFTSSTSYNRGDSTFES	
2768.26	2768.22	(0.04)	(K) SSSYSKQFTSSTSYNRGDSTFESKS	
2931.20	2931.28	(0.08)	(K) SSSYSKQFTSSTSYNRGDSTFESKSY	
3190.36	3190.42	(0.06)	(K) SSSYSKQFTSSTSYNRGDSTFESKSYKM	
3261.43	3261.45	(0.02)	(K) SSSYSKQFTSSTSYNRGDSTFESKSYKMA	
2379.03	2379.03	(0.00)	.SSYSKQFTSSTSYNRGDSTFE	
3206.34	3190.42[Met_{ox}] = 3206.41			
3277.39	3261.45[Met_{ox}] = 3277.44			
gsesgiftntkessshhpgiaefpsrgkssyskqftsstsynrgdstfesksykma (548-604)				
2659.03	2659.24	(0.21)	.....DEAGSEADHEGTHSTKRGHAKSRPV (R)	
3239.22	3239.51	(0.29)	SYKMADEAGSEADHEGTHSTKRGHAKSRPV (R)	

2) Complement C3f (C3f); NCBI #226159 – COMPLEMENT C3; NCBI # 68766 (Res. 1304-1320)

942.43	942.47	(0.04)	HWESASLL.	
1055.60	1055.55	(0.05)	IHWESASLL.	
1211.70	1211.65	(0.05)	RIHWESASLL.	
1348.70	1348.71	(0.01)	HRIHWESASLL.	
1449.76	1449.76	(0.00)	THRIHWESASLL.	
1562.84	1562.84	(0.00)	ITHRIHWESASLL.	
1690.90	1690.93	(0.03)	KITHRIHWESASLL.	
1777.93	1777.97	(0.04)	SKITHRIHWESASLL.	
1864.95	1864.99	(0.04)	SSKITHRIHWESASLL.	
2021.06	2021.10	(0.04)	SSKITHRIHWESASLLR	= C3f
1751.88	1751.91	(0.03)	SSKITHRIHWESASL..	

3) Complement C4 precursor; NCBI # 20141171 -- all peptides map to C4-alpha (Res. 1337-1351 and 1353-1382, separated by R1352)

1498.91	1498.78	(0.13)	NGFKSHALQLNNR..		
1626.85	1626.84	(0.01)	NGFKSHALQLNNRQ.		
1739.93	1739.92	(0.01)	NGFKSHALQLNNRQI	(R)	
1895.99	1896.02	(0.03)	RNGFKSHALQLNNRQI	(R)	
1762.87	1762.92	(0.05)		(R)	GLEEELQFSLGSKINV
2305.20	2305.19	(0.01)		(R)	GLEEELQFSLGSKINVKVGGNS
2704.13	2704.44	(0.31)		(R)	GLEEELQFSLGSKINVKVGGNSKGTL
3200.52	3200.79	(0.27)		(R)	GLEEELQFSLGSKINVKVGGNSKGTLKVLR

(Res. 957-979)

2551.06	2551.16	(0.10)		(R)	TLEIPGNSDPNMIPDGDFNSYVR

4) Inter-alpha-trypsin inhibitor heavy chain H4 (ITIH4); NCBI # 13432192

(Res. 650-688)

842.40	842.39	(0.01)		HAAYHPF.	
1786.86	1786.85	(0.01)		GLPGPPDVPDHAAYHPF.	
2028.01	2027.99	(0.02)		QLGLPGPPDVPDHAAYHPF.	
2271.14	2271.12	(0.02)		SRQLGLPGPPDVPDHAAYHPF.	
2358.09	2358.15	(0.06)		SSRQLGLPGPPDVPDHAAYHPF.	
2627.48	2627.34	(0.14)		GVLSSRQLGLPGPPDVPDHAAYHPF.	
2724.48	2724.38	(0.10)		PGVLSSRQLGLPGPPDVPDHAAYHPF.	= 'propeptide' (minus R)
3272.50	3272.63	(0.13)		MNFRPGVLSSRQLGLPGPPDVPDHAAYHPF.	
3970.97	3970.97	(0.00)	(R)	QAGAAGSRMNFRPGVLSSRQLGLPGPPDVPDHAAYHPF.	
2183.91	2184.09	(0.18)		QLGLPGPPDVPDHAAYHPFR	
998.45	998.49	(0.04)		HAAYHPFR	

(Res. 617-644)

3156.52	3156.61	(0.09)	(R)	NVHSGSTFFKYYLQGAKIPKPEASFSPR

ITIH4 splice variant: PRO1851; NCBI # 7770149 (Res. 347-367)

2115.01	2115.04	(0.03)	(R)	NVHSAGAAGSRMNFRPGVLSS (R)

5) Apolipoprotein A-I; NCBI # 4557321 (Res. 220-238 and 240-267, separated by R239)

2052.89 2053.07 (0.18) (K) ATEHLSTLSEKAKPALEDL (R)
3182.46 3182.72 (0.26) (R) QGLLPVLESFKVSFLSALEEYTKKLNTQ|C-t
1971.16 1971.04 (0.12) VSFLSALEEYTKKLNTQ|C-t

(Res. 148-176)

1807.78 1807.92 (0.14) ELQEGARQKLHELQE
3377.45 3377.71 (0.26) (R) AELQEGARQKLHELQEKLSPLGEEM$_{ox}$RDRA (R)

6) Apolipoprotein A-IV; NCBI # 114006 (Res. 256-278 and 280-304, separated by K279)

2508.16 2508.35 (0.19) ISASAEELRQRLAPLAEDVRGNL (K)
1771.81 1771.84 (0.03) SLAELGGHLDQQVEEF.
2599.18 2599.25 (0.07) (K) GNTEGLQKSLAELGGHLDQQVEEF.
2755.20 2755.35 (0.15) (K) GNTEGLQKSLAELGGHLDQQVEEFR
1927.94 1927.94 (0.00) SLAELGGHLDQQVEEFR

7) Apolipoprotein C-I; NCBI # 114016

2778.15 2778.44 (0.29) DVSSALDKLKEFGNTLEDKARELIS (R)

8) Apolipoprotein E; NCBI # 114039 (Res. 210-233)

2267.07 2267.18 (0.11) TVGSLAGQPLQERAQAWGERL.
2409.13 2409.26 (0.13) (R) AATVGSLAGQPLQERAQAWGERL.
2565.45 2565.36 (0.09) (R) AATVGSLAGQPLQERAQAWGERLR

9) CLUSTERIN precursor; NCBI # 42716297 (Res. 269-278; C-t of beta-chain, minus R279)

822.41 822.43 (0.02) HFFFPK
1277.71 1277.71 (0.00) HFFFPKSRIV (R)

10) Bradykinin (and des-Arg bradykinin) -- HMW Kininogen; NCBI # 125507 (Res. 381-389)

```
 904.48    904.46 (0.02)           RPPGFSPF.
1060.57   1060.57 (0.00)           RPPGFSPFR

 920.41    904.46[Pro_hydroxyl] = 920.45
1076.53   1060.57[Pro_hydroxyl] = 1076.56
```

HMW Kininogen; NCBI # 125507 (Res. 438-456 and 458-477, separated by R457)

```
1943.88   1943.90 (0.02)       NLGHGHKHERDQGHGHQ (R)
2209.08   2209.05 (0.03) (R) KHNLGHGHKHERDQGHGHQ (R)
2126.94   2127.00 (0.06)                         (R) GHGLGHGHEQQHGLGHGHKF (K)
```

11) FACTOR XIIIa; NCBI 119720 (Res. 14-38 of precursor; C-t of PROPEPTIDE)

```
2602.15   2602.30 (0.15)      (R) AVPPNNSNAAEDDLPTVELQGVVPR
```

12) TRANSTHYRETIN precursor ('prealbumin'); NCBI # 136464 (Res. 101-123)

```
2451.11   2451.19 (0.08)      (K) ALGISPFHEHAEVVFTANDSGPR
```

13) PLATELET BASIC PROTEIN Precursor; NCBI # 129874 (Res. 108-127; C-t)

(Mature peptides: NAP-2; CTAP-III; CXCL7; beta-thromboglobulin; LA-PF4;...)

```
2279.18   2279.26 (0.08)           PDAPRIKKIVQKKLAGDESAD|C-t
```

Table S4: Serum Peptide Precursor Proteins (Villanueva *et al.*)

1) FIBRINOGEN ALPHA (NCBI # 4033511) -- **FPA** = 20-35

```
  1 mfsmrivclv lsvvgtawtA DSGEGDFLAE GGGVRgprvv erhqsackds dwpfcsdedw
 61 nykcpsgcrm kglidevnqd ftnrinklkn slfeyqknnk dshslttnim eilrgdfssa
121 nnrdntynrv sedlrsriev lkrkviekvq hiqllqknvr aqlvdmkrle vdidikirsc
181 rgscsralar evdlkdyedq qkqleqviak dllpsrdrqh lplikmkpvp dlvpgnfksq
241 lqkvppewka ltdmpqmrme lerpggneit rggstsygtg setesprnps sagswnsgss
301 gpgstgnrnp gssgtggtat wkpgssgpgs tgswnsgssg tgstgnqnpg sprpgstgtw
361 npgssergsa ghwtsessvs gstgqwhses gsfrpdspgs gnarpnnpdw gtfeevsgnv
421 spgtrreyht eklvtskgdk elrtgkekvt sgsttttrrs csktvtktvi gpdghkevtk
481 evvtsedgsd cpeamdlgtl sgigtldgfr hrhpdeaaff dtastgktfp gffspmlgef
541 vsetesrgse sgiftntkes sshhpgiaef psrgksssys kqftsstsyn rgdstfesks
601 ykmadeagse adhegthstk rghaksrpvr gihtsplgkp slsp
```

2) COMPLEMENT C3 (NCBI # 68766) -- **C3f** = 1304-1320

```
   1 mgptsgpsll lllthlpla lgspmysiit pnilrlesee tmvleahdaq gdvpvtvtvh
  61 dfpgkklvls sektvltpat nhmgnvtfti panrefksek grnkfvtvqa tfgtqvvekv
 121 vlvslqsgyl fiqtdktiyt pgstvlyrif tvnhkllpvg rtvmvnienp egipvkqdsl
 181 ssqnqlgvlp lswdipelvn mgqwkirayy enspqqvfst efevkeyvlp sfevivepte
 241 kfyyiynekg levtitarfl ygkkvegtaf vifgiqdgeq rislpeslkr ipiedgsgev
 301 vlsrkvlldg vqnlraedlv gkslyvsatv ilhsgsdmvq aersgipivt spyqihftkt
 361 pkyfkpgmpf dlmvfvtnpd gspayrvpva vqgedtvqsl tqgdgvakls inthpsqkpl
 421 sitvrtkkqe lseaeqatrt mqalpystvg nsnnylhlsv lrtelrpget lnvnfllrmd
 481 raheakiryy tylimnkgrl lkagrqvrep gqdlvvlpls ittdfipsfr lvayytliga
 541 sgqrevvads vwvdvkdscv gslvvksgqs edrqpvpgqq mtlkiegdhg arvvlvavdk
 601 gvfvlnkknk ltqskiwdvv ekadigctpg sgkdyagvfs dagltftsss gqqtaqrael
 661 qcpqpaarrr rsvqltekrm dkvgkypkel rkccedgmre npmrfscqrr trfislgeac
 721 kkvfldccny itelrrqhar ashlglarsn ldediiaeen ivsrsefpes wlwnvedlke
 781 ppkngistkl mniflkdsit tweilavsms dkkgicvadp fevtvmqdff idlrlpysvv
 841 rneqveirav lynyrqnqel kvrvellhnp afcslattkr rhqqtvtipp ksslsvpyvi
 901 vplktglqev evkaavyhhf isdgvrkslk vvpegirmnk tvavrtldpe rlgregvqke
 961 dippadlsdq vpdtesetri llqgtpvaqm tedavdaerl khlivtpsgc geqnmigmtp
1021 tviavhylde teqwekfgle krqgalelik kgytqqlafr qpssafaafv krapstwlta
1081 yvvkvfslav nliaidsqvl cgavkwlile kqkpdgvfqe dapvihqemi gglrnnnekd
1141 maltafvlis lqeakdicee qvnslpgsit kagdfleany mnlqrsytva iagyalaqmg
1201 rlkgpllnkf lttakdknrw edpgkqlynv eatsyallal lqlkdfdfvp pvvrwlneqr
1261 yygggygstq atfmvfqala qyqkdapdhq elnldvslql psrSSKITHR IHWESASLLR
1321 seetkenegf tvtaegkgqg tlsvvtmyha kakdqltcnk fdlkvtikpa petekrpqda
1381 kntmileict ryrgdqdatm sildismmtg fapdtddlkq langvdryis kyeldkafsd
1441 rntliiyldk vshseddcla fkvhqyfnve liqpgavkvy ayynleesct rfyhpekedg
1501 klnklcrdel crcaeencfi qksddkvtle erldkacepg vdyvyktrlv kvqlsndfde
1561 yimaieqtik sgsdevqvgq qrtfispikc realkleekk hylmwglssd fwgekpnlsy
1621 iigkdtwveh wpeedecqde enqkqcqdlg aftesmvvfg cpn
```

3) COMPLEMENT C4 PRECURSOR (NCBI # 201411771) -- **C4-alpha** = 680-1446

```
   1 mrllwgliwa ssfftlslqk prlllfspsv vhlgvplsvg vqlqdvprgq vvkgsvflrn
  61 psrnnvpcsp kvdftlsser dfallslqvp lkdakscglh qllrgpevql vahspwlkds
 121 lsrttniqgi nllfssrrgh lflqtdqpiy npgqrvryrv faldqkmrps tdtitvmven
 181 shglrvrkke vympssifqd dfvipdisep gtwkisarfs dglesnsstq fevkkyvlpn
 241 fevkitpgkp yiltvpghld emqldiqary iygkpvqgva yvrfgllded gkktffrgle
 301 sqtklvngqs hislskaefq daleklnmgi tdlqglrlyv aaaiiespgg emeeaeltsw
 361 yfvsspfsld lsktkrhlvp gapfllqalv remsgspasg ipvkvsatvs spgsvpevqd
 421 iqqntdgsgq vsipiiipqt iselqlsvsa gsphpaiarl tvaappsggp gflsierpds
 481 rpprvgdtln lnlravgsga tfshyyymil srgqivfmnr epkrtltsvs vfvdhhlaps
 541 fyfvafyyhg dhpvanslrv dvqagacegk lelsvdgakq yrngesvklh letdslalva
 601 lgaldtalya agskshkpln mgkvfeamns ydlgcgpggg dsalqvfqaa glafsdgdqw
 661 tlsrkrlscp kekttrkkrN VNFQKAINEK LGQYASPTAK RCCQDGVTRL PMMRSCEQRA
 721 ARVQQPDCRE PFLSCCQFAE SLRKKSRDKG QAGLQRALEI LQEEDLIDED DIPVRSFFPE
 781 NWLWRVETVD RFQILTLWLP DSLTTWEIHG LSLSKTKGLC VATPVQLRVF REFHLHLRLP
 841 MSVRRFEQLE LRPVLYNYLD KNLTVSVHVS PVEGLCLAGG GGLAQQVLVP AGSARPVAFS
 901 VVPTAAAAVS LKVVARGSFE FPVGDAVSKV LQIEKEGAIH REELVYELNP LDHRGRTLEI
 961 PGNSDPNMIP DGDFNSYVRV TASDPLDTLG SEGALSPGGV ASLLRLPRGC GEQTMIYLAP
1021 TLAASRYLDK TEQWSTLPPE TKDHAVDLIQ KGYMRIQQFR KADGSYAAWL SRDSSTWLTA
1081 FVLKVLSLAQ EQVGGSPEKL QETSNWLLSQ QQADGSFQDP CPVLDRSMQG GLVGNDETVA
1141 LTAFVTIALH HGLAVFQDEG AEPLKQRVEA SISKANSFLG EKASAGLLGA HAAAITAYAL
1201 SLTKAPVDLL GVAHNNLMAM AQETGDNLYW GSVTGSQSNA VSPTPAPRNP SDPMPQAPAL
1261 WIETTAYALL HLLLHEGKAE MADQASAWLT RQGSFQGGFR STQDTVIALD ALSAYWIASH
1321 TTEERGLNVT LSSTGRNGFK SHALQLNNRQ IRGLEEELQF SLGSKINVKV GGNSKGTLKV
1381 LRTYNVLDMK NTTCQDLQIE VTVKGHVEYT MEANEDYEDY EYDELPAKDD PDAPLQPVTP
1441 LQLFEGrrnr rrreapkvve eqesrvhytv ciwrngkvgl sgmaiadvtl lsgfhalrad
1501 lekltslsdr yvshfetegp hvllyfdsvp tsrecvgfea vqevpvglvq pasatlydyy
1561 nperrcsvfy gapsksrlla tlcsaevcqc aegkcprqrr alerglqded gyrmkfacyy
1621 prveygfqvk vlredsraaf rlfetkitqv lhftkdvkaa anqmrnflvr ascrlrlepg
1681 keylimgldg atydleghpq ylldsnswie empserlcrs trqraacaql ndflqeygtq
1741 gcqv
```

4) ITIH4 (NCBI # 13432192) — **PROPEPTIDE** = 662-688

```
   1 mkpprpvrtc skvlvllsll aihqtttaek ngidiysltv dsrvssrfah tvvtsrvvnr
  61 antvqeatfq melpkkafit nfsmnidgmt ypgiikekae aqaqysaava kgksaglvka
 121 tgrnmeqfqv svsvapnaki tfelvyeell krrlgvyell lkvrpqqlvk hlqmdihife
 181 pqgisflete stfmtnqlvd alttwqnktk ahirfkptls qqqkspeqqe tvldgnliir
 241 ydvdraisgg siqiengyfv hyfapegltt mpknvvfvid ksgsmsgrki qqtrealiki
 301 lddlsprdqf nlivfsteat qwrpslvpas aenvnkarsf aagiqalggt nindamlmav
 361 qlldssnqee rlpegsvsli illtdgdptv getnprsiqn nvreavsgry slfclgfgfd
 421 vsyaflekla ldngglarri hedsdsalql qdfyqevanp lltavtfeyp snaveevtqn
 481 nfrllfkgse mvvagklqdr gpdvltatvs gklptqnitf qtessvaeqe aefqspkyif
 541 hnfmerlway ltiqqlleqt vsasdadqqa lrnqalnlsl aysfvtplts mvvtkpddqe
 601 qsqvaekpme gesrnrnvhs gstffkyylq gakipkpeas fsprrgwnrq agaagsrmnf
 661 rPGVLSSRQL GLPGPPDVPD HAAYHPFRrl ailpasappa tsnpdpavsr vmnmkieett
 721 mttqtpapiq apsailplpg qsverlcvdp rhrqgpvnll sdpeqgvevt gqyerekagf
 781 swievtfknp lvwvhaspeh vvvtrnrrss aykwketlfs vmpglkmtmd ktgllllsdp
 841 dkvtigllfw dgrgeglrll lrdtdrfssh vggtlgqfyq evlwgspaas ddgrrtlrvq
 901 gndhsatrer rldyqegppg veiscwsvel
```

5) PRO1851 (NCBI # 7770149) – ITIH4 alternative spliced form

```
  1 mpknvvfvid ksgsmsgrki qqtrealiki lddlsprdqf nlivfsteat qwrpslvpas
 61 aenvnkarsf aagiqalggt nindamlmav qlldssnqee rlpegsvsli illtdgdptv
121 getnprsiqn nvreavsgry slfclgfgfd vsyaflekla ldngglarri hedsdsalql
181 qdfyqevanp lltavtfeyp snaveevtqn nfrllfkgse mvvagklqdr gpdvltatvs
241 gklptqnitf qtessvaeqe aefqspkyif hnfmerlway ltiqqlleqt vsasdadqqa
301 lrnqalnlsl aysfvtplts mvvtkpddqe qsqvaekpme gesrnrnvhs agaagsrmnf
361 rpgvlssrql glpgppdvpd haayhpfrrl ailpasappa tsnpdpavsr vmnmkieett
421 mttqtpacps csrsrapavp apiqapsail plpgqsverl cvdprhrqgp vnllsdpeqg
481 vevtgqyere kagfswievt fknplvwvha spehvvvtrn rrssaykwke tlfsvmpglk
541 mtmdktglll lsdpdkvtig llfwdgrgeg lrlllrdtdr fsshvggtlg qfyqevlwgs
601 paasddgrrt lrvqgndhsa trerrldyqe gppgveiscw svel
```

6) APOLIPOPROTEIN A-I (NCBI # 4557321)

1 *mkaavltlav lfltgsqarh fwqqdeppqs pwdr*vkdlat vyvdvlkdsg rdyvsqfegs

61 algkqlnlkl ldnwdsvtst fsklreqlgp vtqefwdnle keteglrqem skdleevkak

121 vqpylddfqk kwqeemelyr qkveplr**ael qegarqklhe lqeklsplge emrdra**rahv

181 dalrthlapy sdelrqrlaa rlealkengg arlaeyhak**a tehlstlsek akpaledl**r**q**

241 **gllpvlesfk vsflsaleey tkklntq**

7) APOLIPOPROTEIN A-IV (NCBI # 114006)

1 mflkavvltl alvavagara evsadqvatv mwdyfsqlsn nakeavehlq kseltqqlna

61 lfqdklgevn tyagdlqkkl vpfatelher lakdseklke eigkeleelr arllphanev

121 sqkigdnlre lqqrlepyad qlrtqvntqa eqlrrqltpy aqrmervlre nadslqaslr

181 phadelkaki dqnveelkgr ltpyadefkv kidqtveelr rslapyaqdt qeklnhqleg

241 ltfqmkknae elkar**isasa eelrqrlapl aedvrgnl**k**g nteglqksla elgghldqqv**

301 **eefr**rrvepy genfnkalvq qmeqlrqklg phagdveghl sflekdlrdk vnsffstfke

361 kesqdktlsl peleqqqeqq qeqqqeqvqm lapels

8) APOLIPOPROTEIN E (NCBI # 114039)

```
   1 mkvlwaallv tflagcqakv eqavetepep elrqqtewqs gqrwelalgr fwdylrwvqt
  61 lseqvqeell ssqvtqelra lmdetmkelk aykseleeql tpvaeetrar lskelqaaqa
 121 rlgadmedvc grlvqyrgev qamlgqstee lrvrlashlr klrkrllrda ddlqkrlavy
 181 qagaregaer glsairerlg plveqgrvra atvgslaggp lqeraqawge rlrarmeemg
 241 srtrdrldev keqvaevrak leeqaqqirl qaeafqarlk swfeplvedm qrqwaglvek
 301 vqaavgtsaa pvpsdnh
```

9) CLUSTERIN precursor; 'APO J' (NCBI # 42716297)

BETA-CHAIN = 192-279; *alpha-chain* = 280-325

```
  1 mqvcsqpqrg cvreqsaint appsahnaas pggarghrvp lteackdsri ggmmktlllf
 61 vgllltwesg qvlgdqtvsd nelqemsnqg skyvnkeiqn avngvkqikt liektneerk
121 tllsnleeak kkkedalnet resetklkel pgvcnetmma lweeckpclk qtcmkfyarv
181 crsgsglvgr qLEEFLNQSS PFYFWMNGDR IDSLLENDRQ QTHMLDVMQD HFSRASSIID
241 ELFQDRFFTR EPQDTYHYLP FSLPHRRPHF FFPKSRIVRs lmpfspyepl nfhamfqpfl
301 emiheaqqam dihfhspafq hpptefireg dddrtvcrei rhnstgclrm kdqcdkcrei
361 lsvdcstnnp sqaklrreld eslqvaerlt rkynellksy qwkmlntssl leqlneqfnw
421 vsrlanltqg edqyylrvtt vashtsdsdv psgvtevvvk lfdsdpitvt vpvevsrknp
481 kfmetvaeka lqeyrkkhre e
```

10) HMW KININOGEN (NCBI # 125507) – contains **BRADYKININ** (381-389)

```
  1 mklitilflc srlllsltqe sqseeidcnd kdlfkavdaa lkkynsqnqs nnqfvlyrit
 61 eatktvgsdt fysfkyeike gdcpvqsgkt wqdceykdaa kaatgectat vgkrsstkfs
121 vatqtcqitp aegpvvtaqy dclgcvhpis tqspdlepil rhgiqyfnnn tqhsslfmln
181 evkraqrqvv aglnfritys ivqtncsken flfltpdcks lwngdtgect dnayidiqlr
241 iasfsqncdi ypgkdfvqpp tkicvgcprd iptnspelee tlthtitkln aennatfyfk
301 idnvkkarvq vvagkkyfid fvarettcsk esneeltesc etkklgqsld cnaevyvvpw
361 ekkiyptvnc qplgmislmk RPPGFSPFRs srigeikeet tvspphtsma paqdeerdsg
421 keqghtrrhd wghekqrkhn lghghkherd qghghqrghg lghgheqghg lghghkfkld
481 ddlehqgghv ldhghkhkhg hghgkhknkg kkngkhngwk tehlasssed sttpsaqtqe
541 ktegptpips lakpgvtvtf sdfqdsdlia tmmppispap iqsdddwipd iqtdpnglsf
601 npisdfpdtt spkcpgrpwk svseinpttq mkesyyfdlt dgls
```

11) FACTOR XIIIa (NCBI # 119720) -- *PROPEPTIDE* = 2-38

```
   1 m*SETSRTAFG GRR****AVPPNNS NAAEDDLPTV ELQGVVPR****gv nlqeflnvts vhlfkerwdt
  61 nkvdhhtdky ennklivrrg qsfyvqidfs rpydprrdlf rveyvigryp qenkgtyipv
 121 pivselqsgk wgakivmred rsvrlsiqss pkcivgkfrm yvavwtpygv lrtsrnpetd
 181 tyilfnpwce ddavyldnek ereeyvlndi gvifygevnd iktrswsygq fedgildtcl
 241 yvmdraqmdl sgrgnpikvs rvgsamvnak ddegvlvgsw dniyaygvpp sawtgsvdil
 301 leyrssenpv rygqcwvfag vfntflrclg iparivtnyf sahdndanlq mdifleedgn
 361 vnskltkdsv wnyhcwneaw mtrpdlpvgf ggwqavdstp qensdgmyrc gpasvqaikh
 421 ghvcfqfdap fvfaevnsdl iyitakkdgt hvvenvdath igklivtkqi ggdgmmditd
 481 tykfqegqee erlaletalm ygakkplnte gvmksrsnvd mdfevenavl gkdfklsitf
 541 rnnshnryti taylsanitf ytgvpkaefk ketfdvtlep lsfkkeavli qageymgqll
 601 eqaslhffvt arinetrdvl akqkstvlti peiiikvrgt qvvgsdmtvt vqftnplket
 661 lrnvwvhldg pgvtrpmkkm freirpnstv qweevcrpwv sghrkliasm ssdslrhvyg
 721 eldvqiqrrp sm
```

12) TRANSTHYRETIN precursor (NCBI # 136464)

1 *mashrllllc laglvfvsea* gptgtgeskc plmvkvldav rgspainvav hvfrkaaddt

61 wepfasgkts esgelhgltt eeefvegiyk veidtksywk **algispfheh aevvftands**

121 **gpr**rytiaal lspysystta vvtnpke

13) Platelet basic protein precursor (NCBI # 129874)

Signal peptide (1-34)

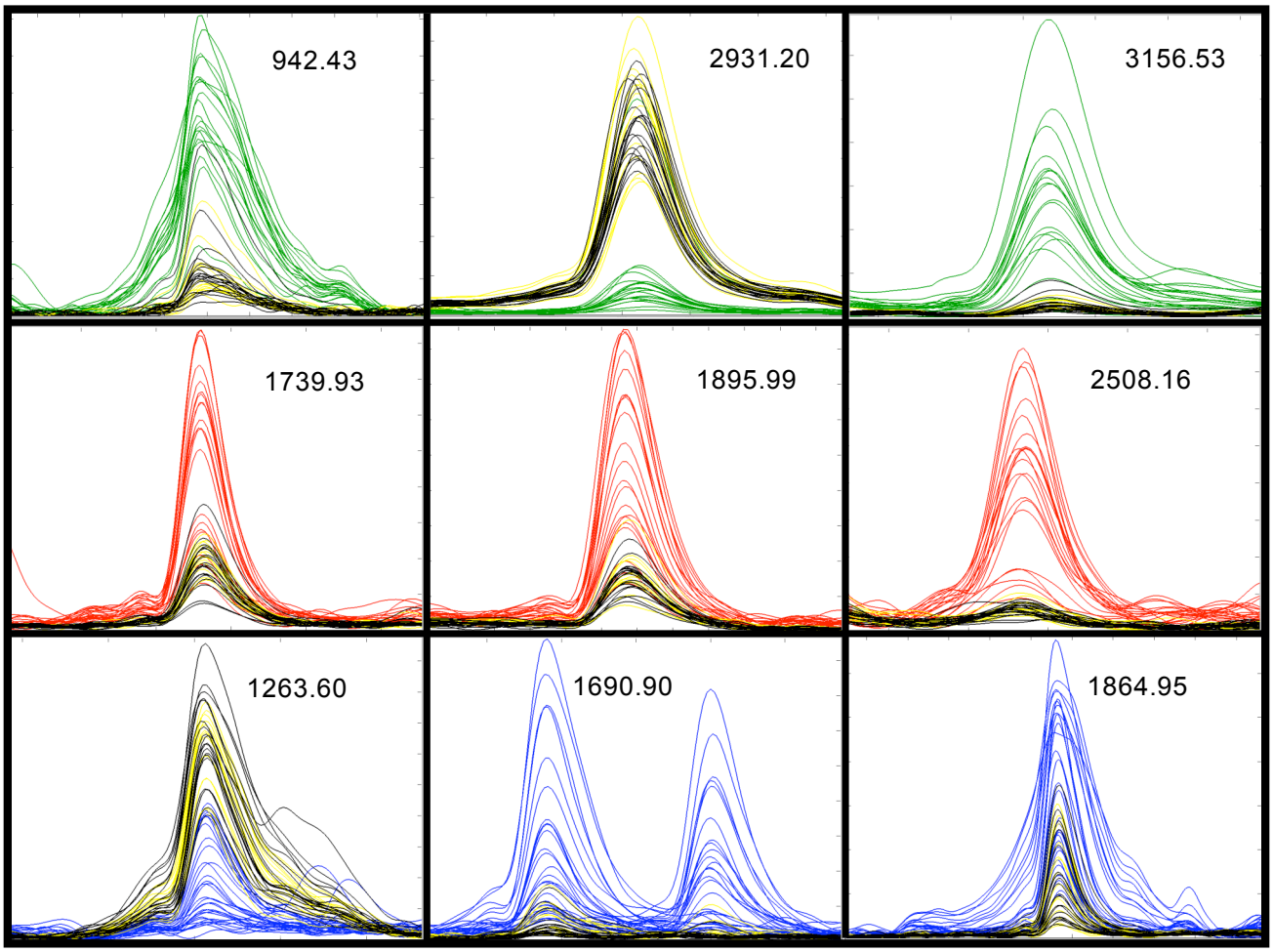
CTAP-III, TC-2 (44-128); beta-TG (48-128); NAP-2 (55-128); CXCL7 (?-128)

1 *mslrldttps cnsarplhal qvllllslll tala*sstkgq tkrnlakgke esldsdlyae

61 lrcmciktts gihpkniqsl evigkgthcn qveviatlkd grkicld**pda prikkivqkk**

121 **lagdesad**

Figure S1


942.43
2931.20
3156.53
1739.93
1895.99
2508.16
1263.60
1690.90
1864.95