Supporting Information

Targeted quantification of low ng/mL level proteins in human serum without immunoaffinity depletion

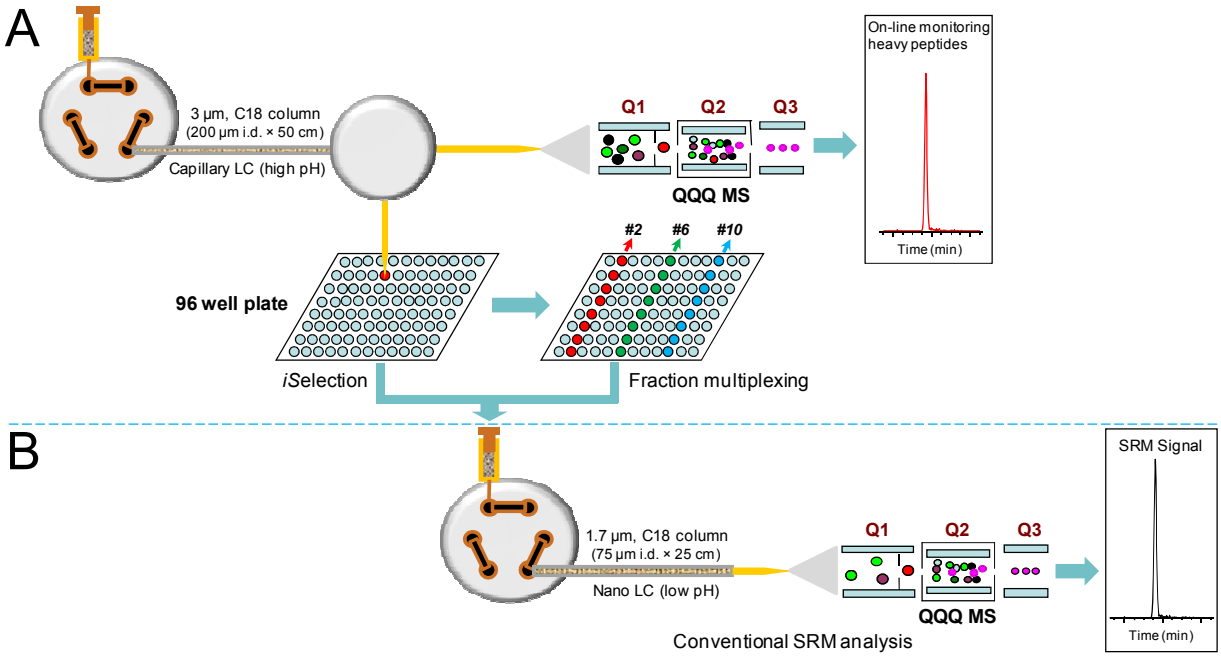
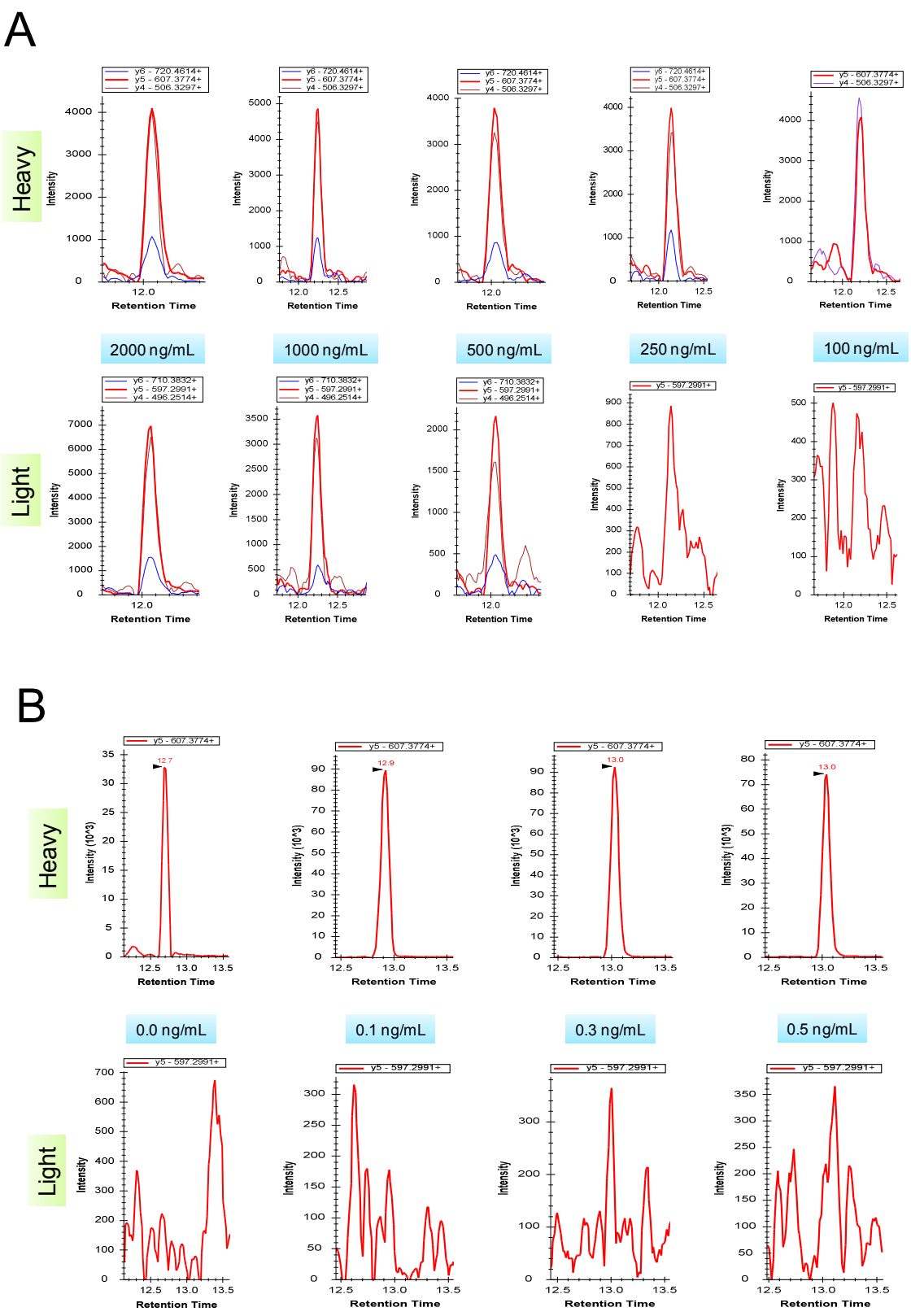


Figure S1. Schematic diagram of the PRISM-SRM workflow. (**A**) PRISM workflow. ~20 µg peptide sample spiked with internal standard (IS) heavy peptides was injected and separated by a high resolution reversed-phase cLC system using high pH mobile phases. The eluent from the cLC column at a flow rate of 3.3 µL/min was split into two flowing streams via a Tee union (the split ratio of flow rates is 1:10): a small fraction (9%) of the column eluent went to a triple quadrupole mass spectrometer for on-line SRM monitoring IS peptides; a large fraction (91%) of the column eluent was automatically collected every minute into a 96-well plate during a ~100-min LC run. The specific target peptide fractions were either selected based on the same elution times of IS being monitored by the on-line SRM (*i*Selection) or multiplexed. For example, 96 fractions collected along with the first dimensional LC separation can be multiplexed into 12 fractions by combining 8 fractions from the first dimensional LC separation into one fraction for downstream LC-SRM analyses, such as pooling fractions 2, 14, 26, 38, 50, 62, 74, and 86 (marked in red color) into one sample (#2) for the second dimensional LC-SRM. (**B**) Conventional LC-SRM workflow. Following *i*Selection, a target peptide fraction was either directly subjected to nanoLC-SRM with 4 µL sample per injection (~ 45 ng peptides on nanoLC column) or multiplexed with other target fractions with a final volume of 20 µL prior to nanoLC-SRM analysis.


A
Heavy
Light
2000 ng/mL
1000 ng/mL
500 ng/mL
250 ng/mL
100 ng/mL
y6 - 720.4614+
y5 - 607.3774+
y4 - 506.3297+
y6 - 710.3832+
y5 - 597.2991+
y4 - 496.2514+
Intensity
Retention Time
B
Heavy
Light
0.0 ng/mL
0.1 ng/mL
0.3 ng/mL
0.5 ng/mL
y5 - 607.3774+
y5 - 597.2991+
Intensity (10^3)
Intensity
Retention Time

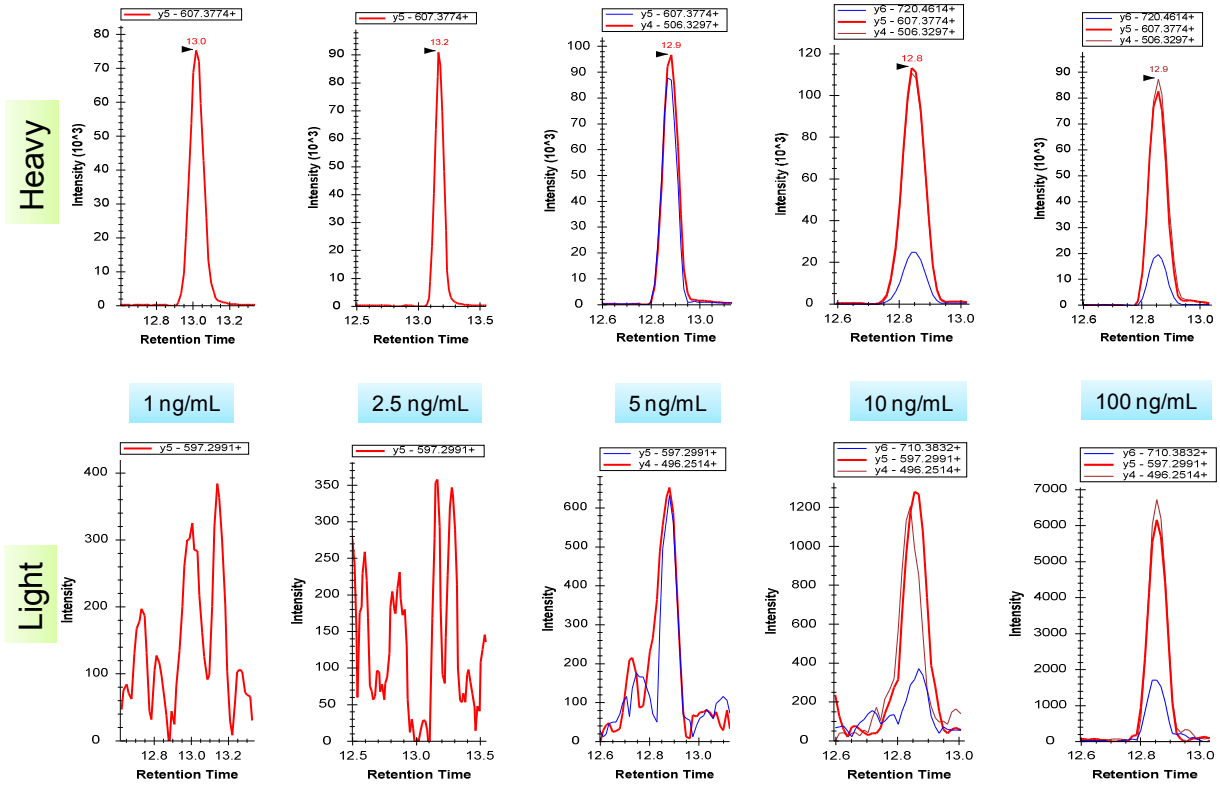


Figure S2.1. Extracted ion chromatograms of transitions monitored for DGPLTGTYR derived from bovine carbonic anhydrase. **(A)** Direct LC-SRM. **(B)** PRISM-SRM. Internal standards were spiked at 0.5 fmol/µL.

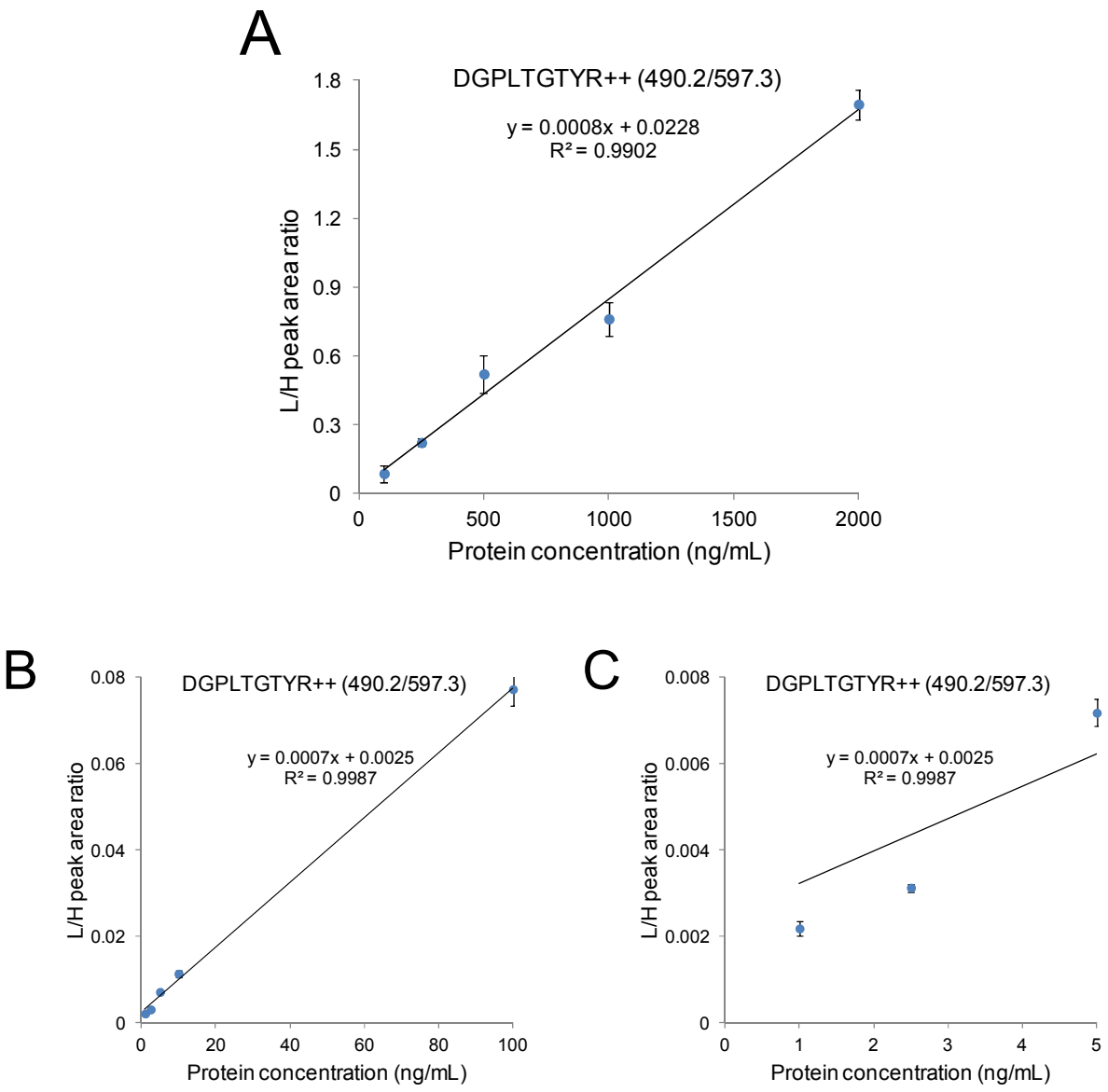


Figure S2.2. Calibration curves for quantifying bovine carbonic anhydrase. **(A)** Direct LC-SRM; **(B)** PRISM-SRM (target protein: 0-100 ng/mL); **(C)** PRISM-SRM (Zoom-in range of 0-5 ng/mL).

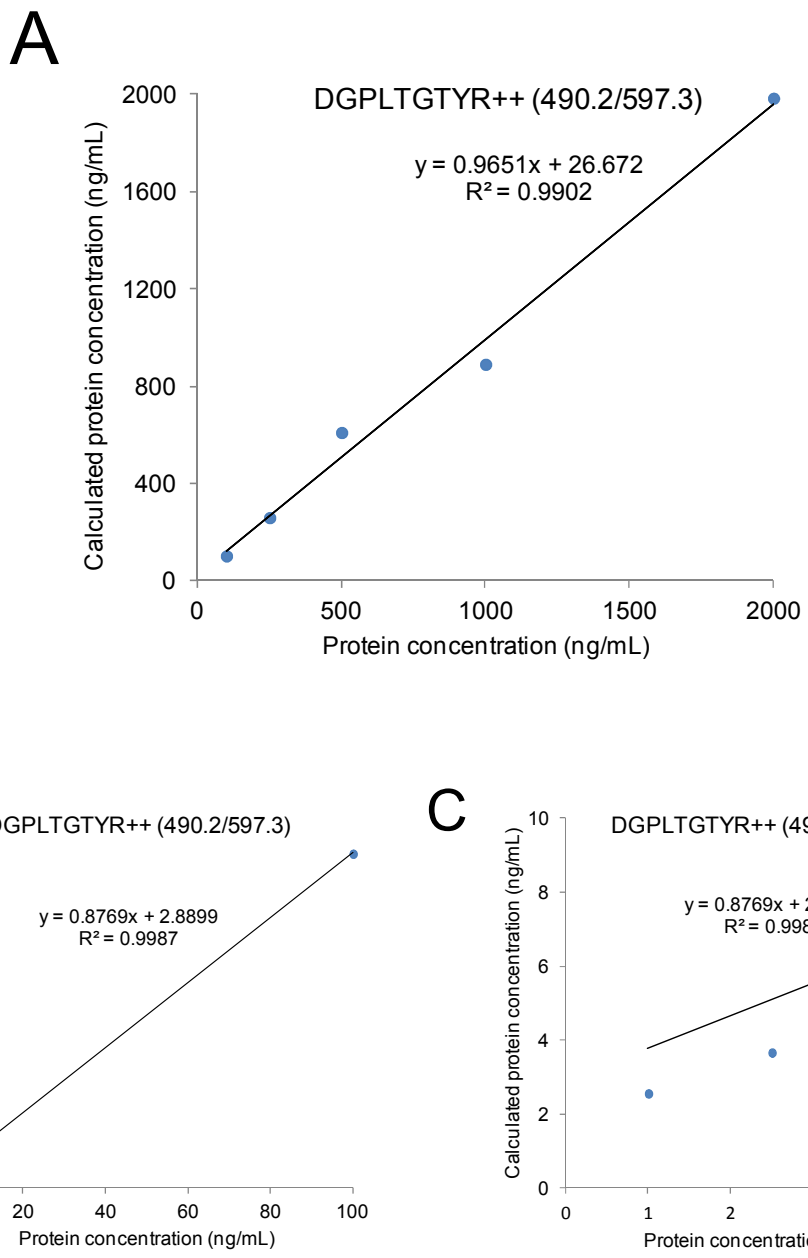
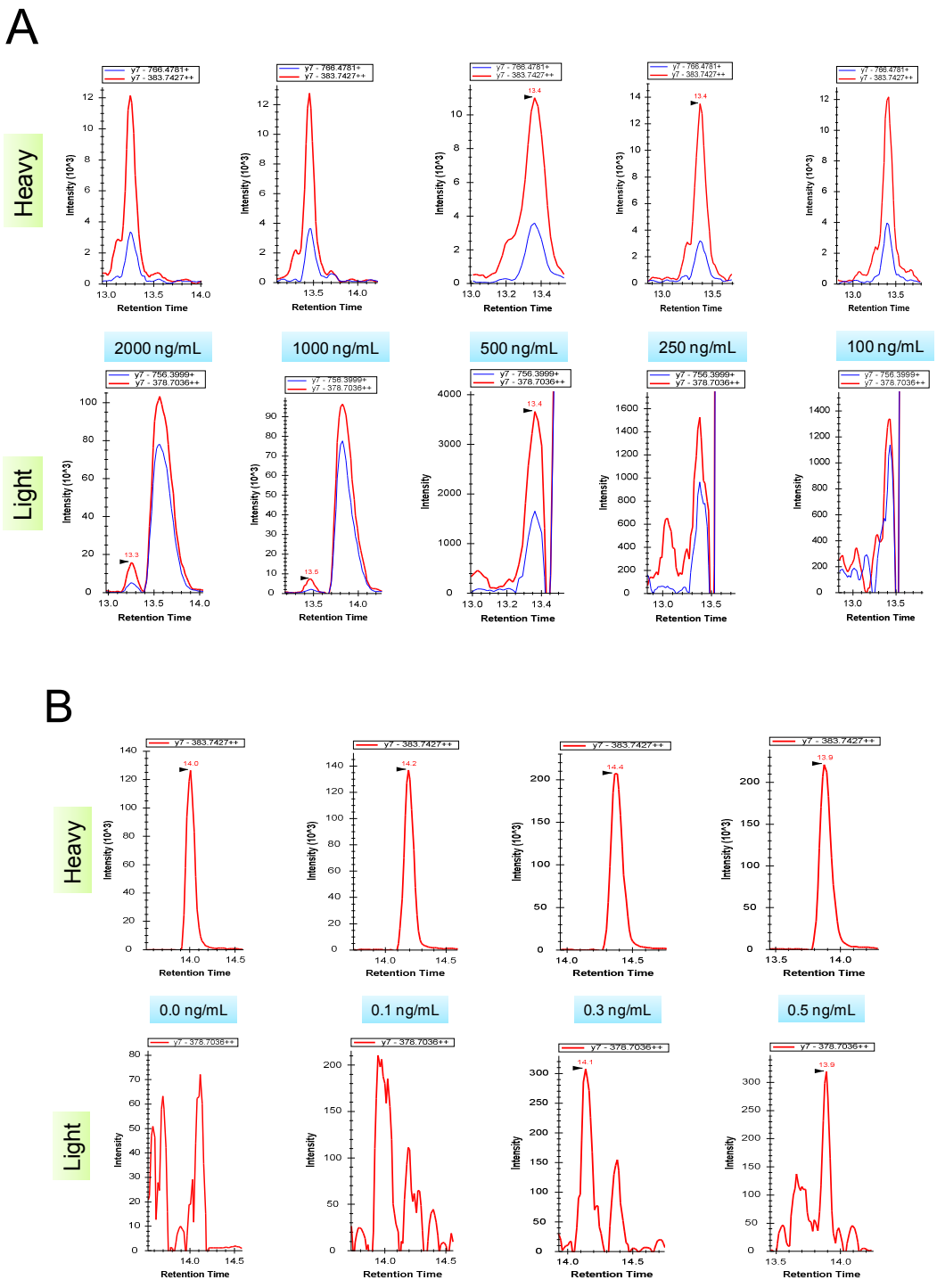


Figure S2.3. Correlation curves for quantifying bovine carbonic anhydrase. **(A) Direct LC-SRM.** Target protein concentrations at the range of 100-2000 ng/mL. **(B)** PRISM-SRM. Target protein concentrations at the range of 0-100 ng/mL. **(C)** PRISM-SRM (Zoom-in range of 0-5 ng/mL).


A
Heavy
Light
2000 ng/mL
1000 ng/mL
500 ng/mL
250 ng/mL
100 ng/mL
B
Heavy
Light
0.0 ng/mL
0.1 ng/mL
0.3 ng/mL
0.5 ng/mL
Retention Time
Intensity

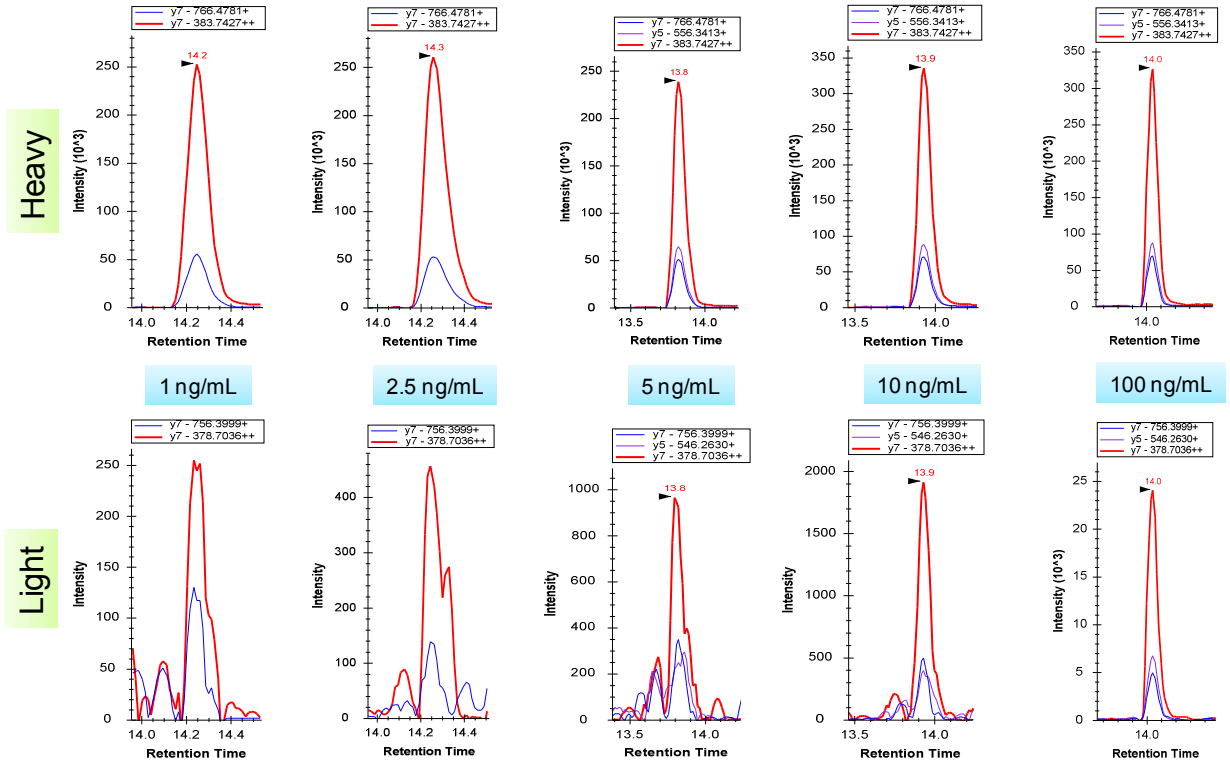


Figure S3.1. Extracted ion chromatograms of transitions monitored for DFPIANGER derived from bovine carbonic anhydrase. **(A)** Direct LC-SRM. **(B)** Direct PRISM-SRM. Internal standards were spiked at 0.5 fmol/µL.

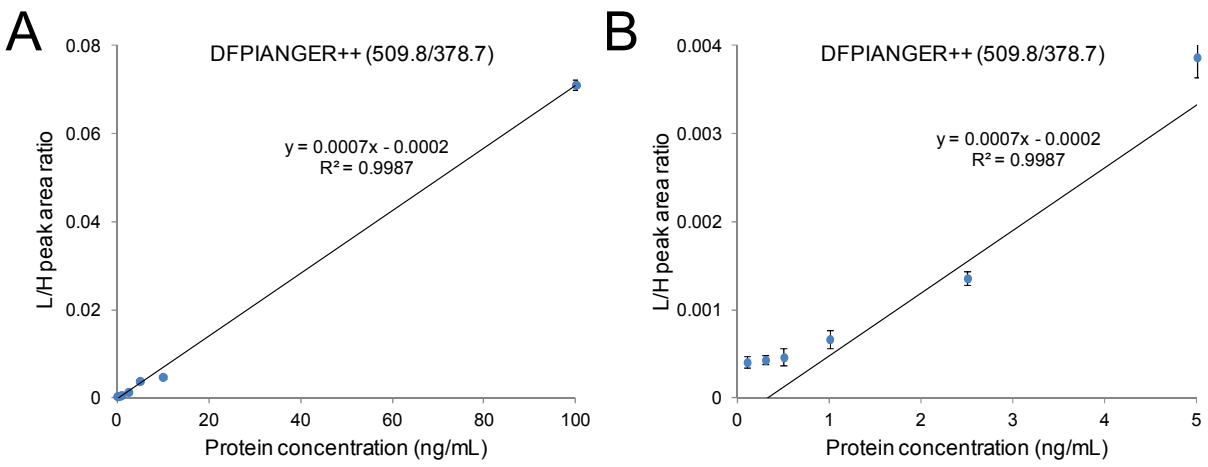


Figure S3.2. Calibration curves for quantifying bovine carbonic anhydrase. **(A)** PRISM-SRM (target protein: 0-100 ng/mL); **(B)** PRISM-SRM (Zoom-in range of 0-5 ng/mL).

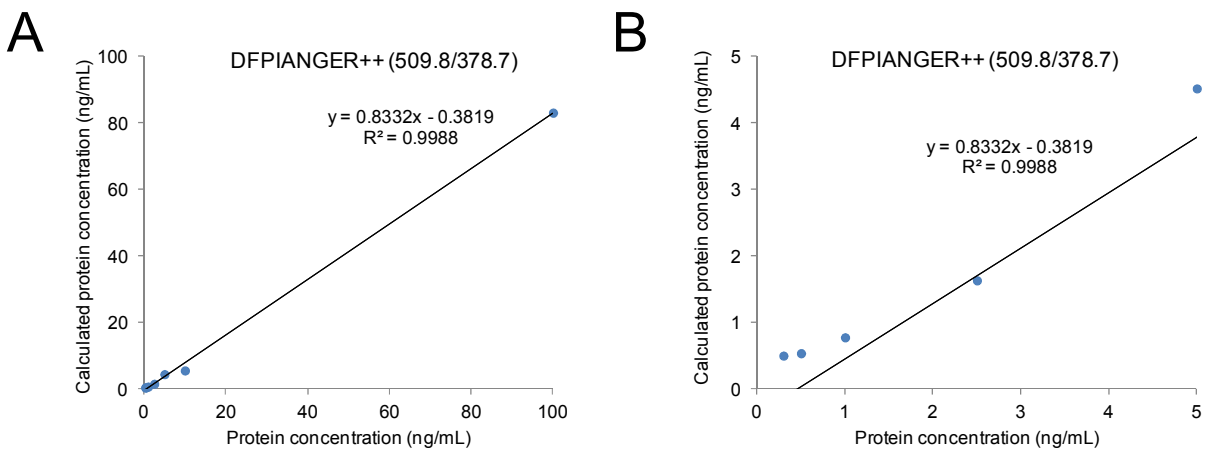
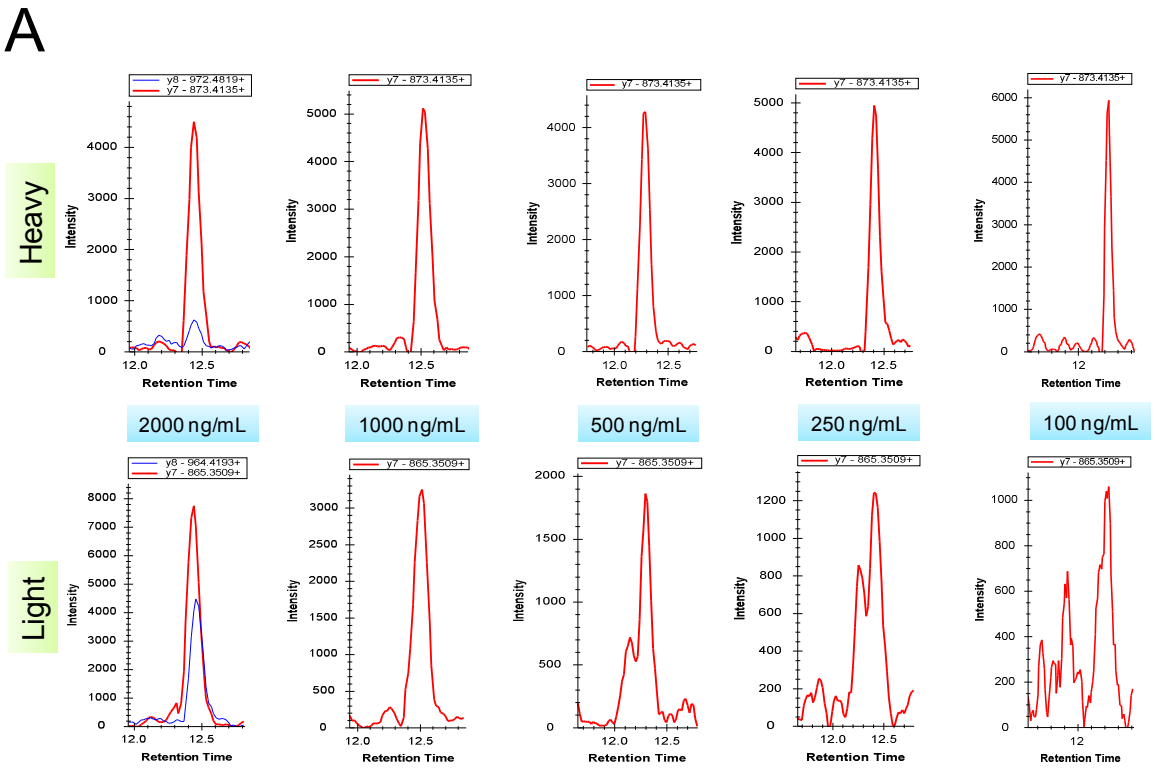
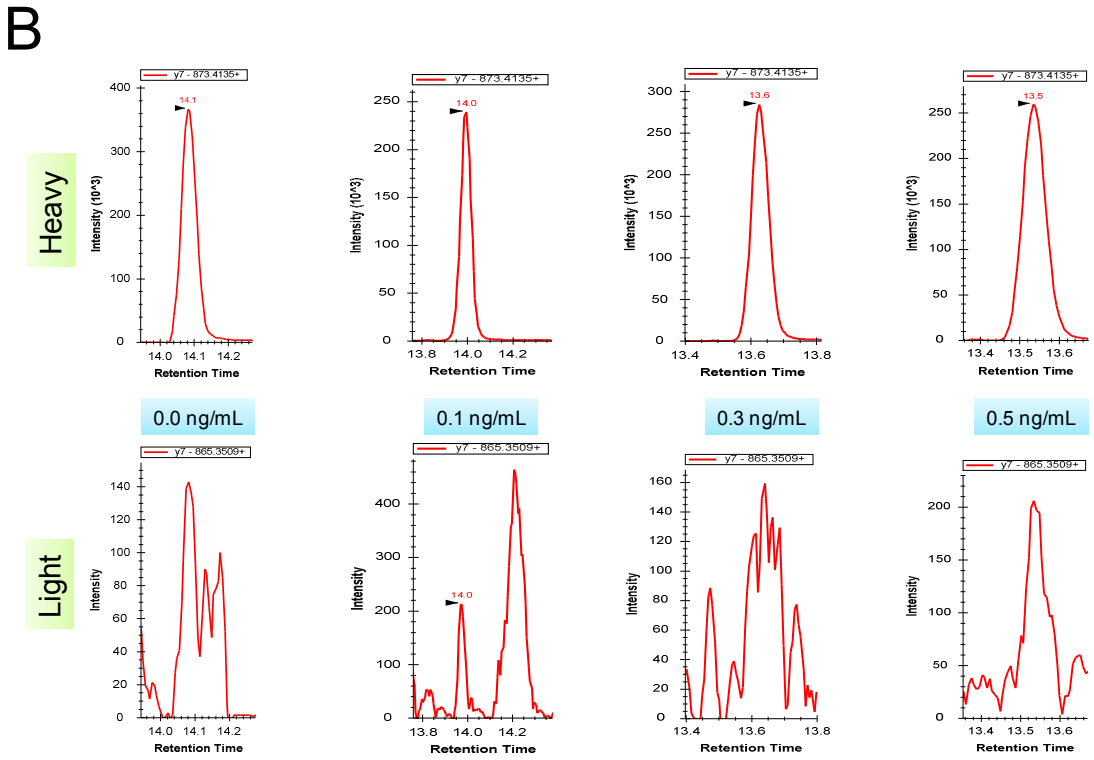


Figure S3.3. Correlation curves for quantifying bovine carbonic anhydrase. **(A)** PRISM-SRM. Target protein concentrations at the range of 0-100 ng/mL. **(B)** PRISM-SRM (Zoom-in range of 0-5 ng/mL).


A
Heavy
Light
y8 - 972.4819+
y7 - 873.4135+
y8 - 964.4193+
y7 - 865.3509+
Intensity
Retention Time
2000 ng/mL
1000 ng/mL
500 ng/mL
250 ng/mL
100 ng/mL


B
Heavy
Light
y7 - 873.4135+
y7 - 865.3509+
Intensity (10^3)
Intensity
Retention Time
0.0 ng/mL
0.1 ng/mL
0.3 ng/mL
0.5 ng/mL

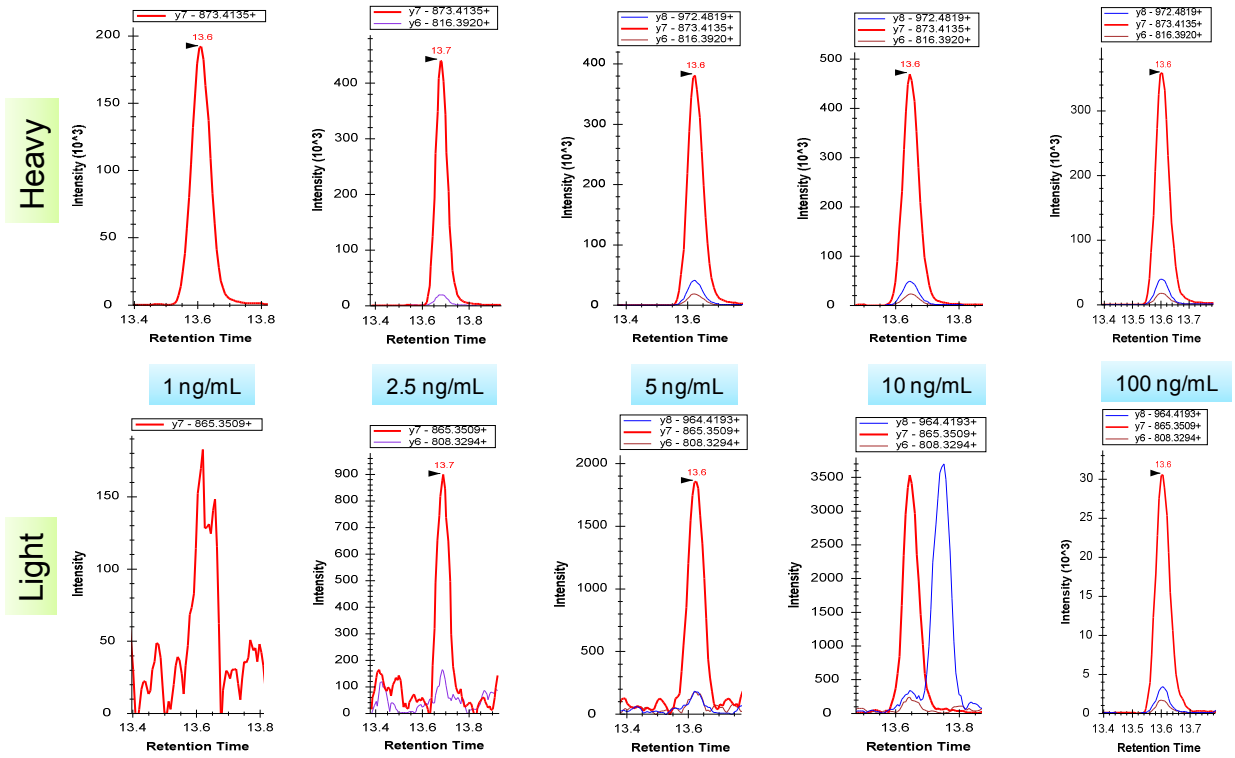


Figure S4.1. Extracted ion chromatograms of transitions monitored for IVGGWEC$_{cam}$EK derived from prostate-specific antigen. **(A)** Direct LC-SRM. **(B)** Direct PRISM-SRM. Internal standards were spiked at 0.5 fmol/µL.

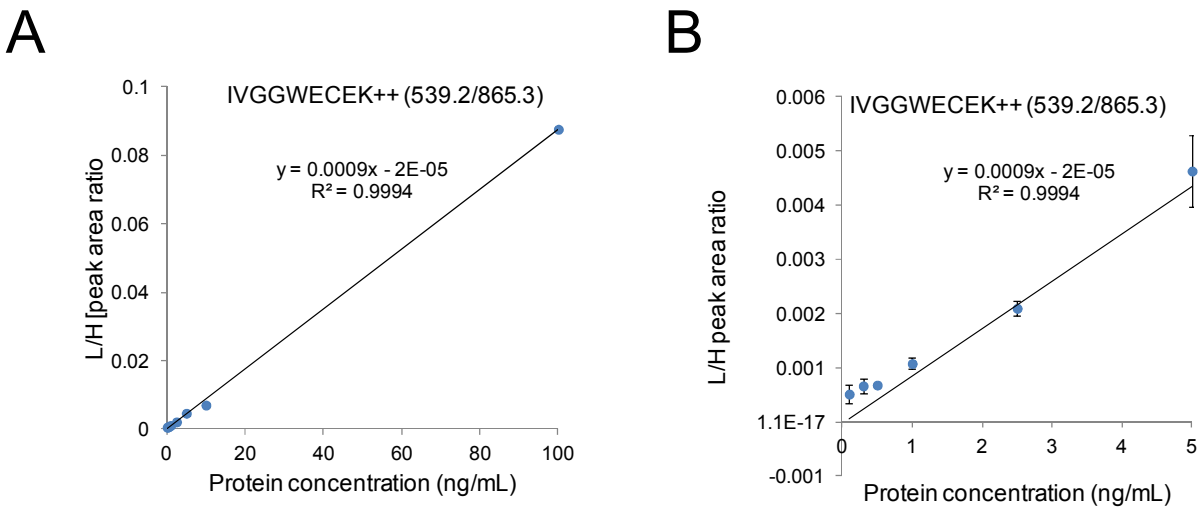


Figure S4.2. Calibration curves for quantifying prostate-specific antigen. **(A)** PRISM-SRM (target protein: 0-100 ng/mL); **(B)** PRISM-SRM (Zoom-in range of 0-5 ng/mL).

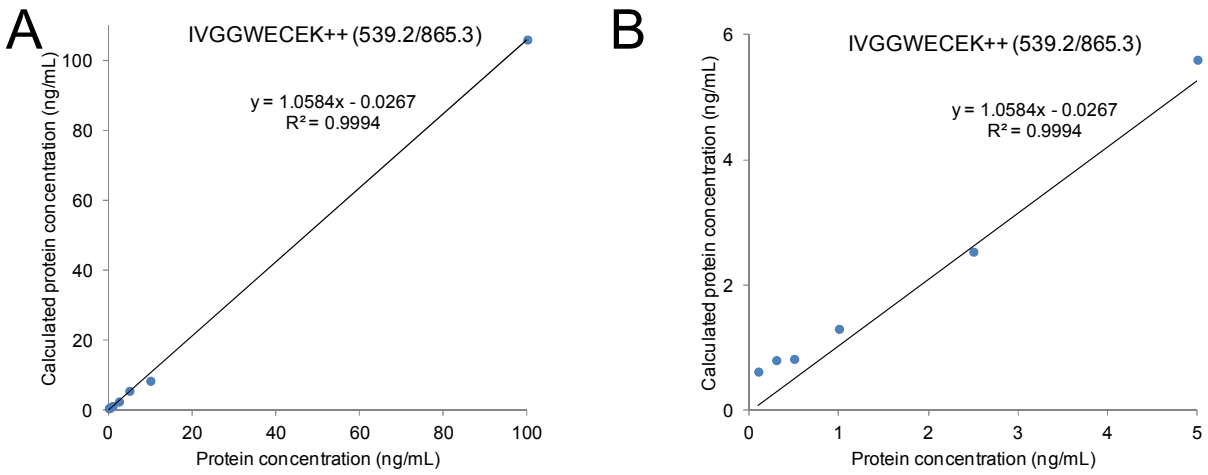
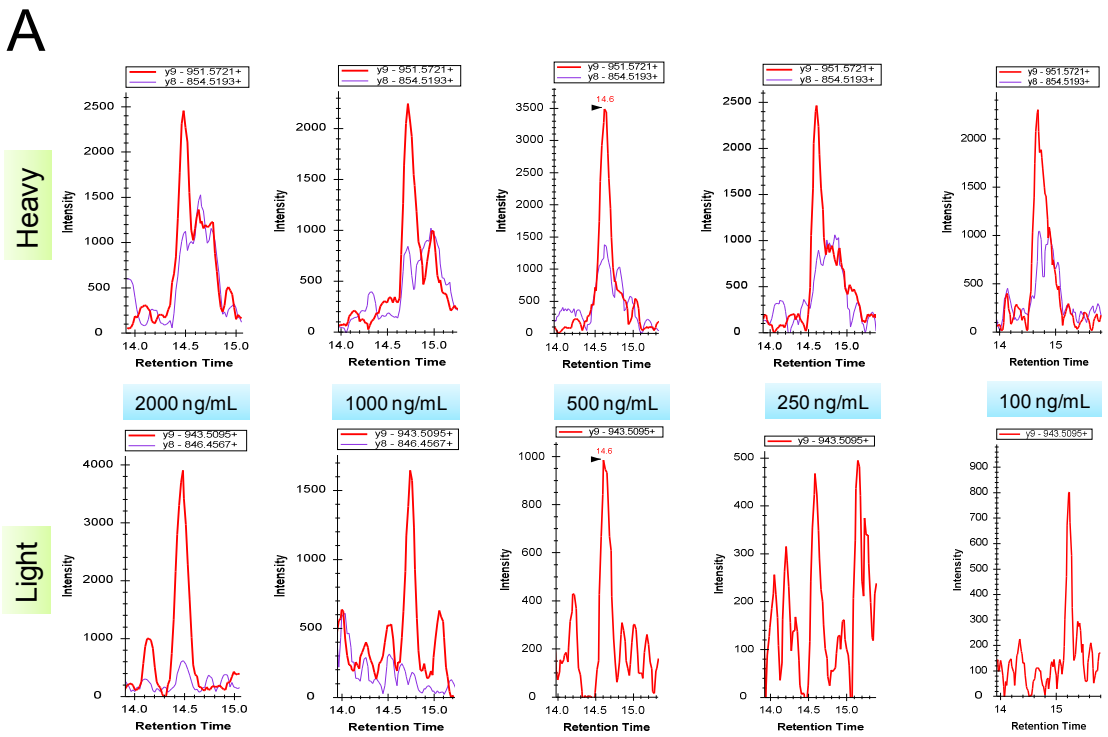
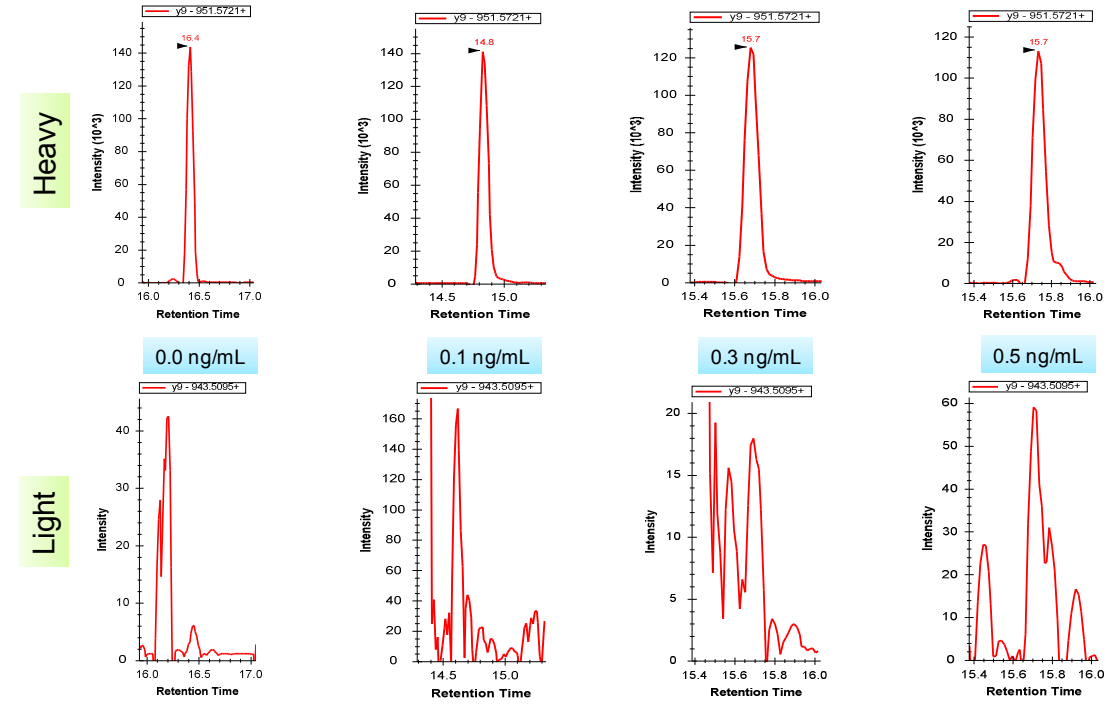


Figure S4.3. Correlation curves for quantifying prostate-specific antigen. **(A) PRISM-SRM.** Target protein concentrations at the range of 0-100 ng/mL. **(B)** PRISM-SRM (Zoom-in range of 0-5 ng/mL).


A
Heavy
Light
y9 - 951.5721+
y8 - 854.5193+
y9 - 943.5095+
y8 - 846.4567+
2000 ng/mL
1000 ng/mL
500 ng/mL
250 ng/mL
100 ng/mL
Intensity
Retention Time


B
Heavy
Light
y9 - 951.5721+
y9 - 943.5095+
0.0 ng/mL
0.1 ng/mL
0.3 ng/mL
0.5 ng/mL
Intensity (10^3)
Intensity
Retention Time

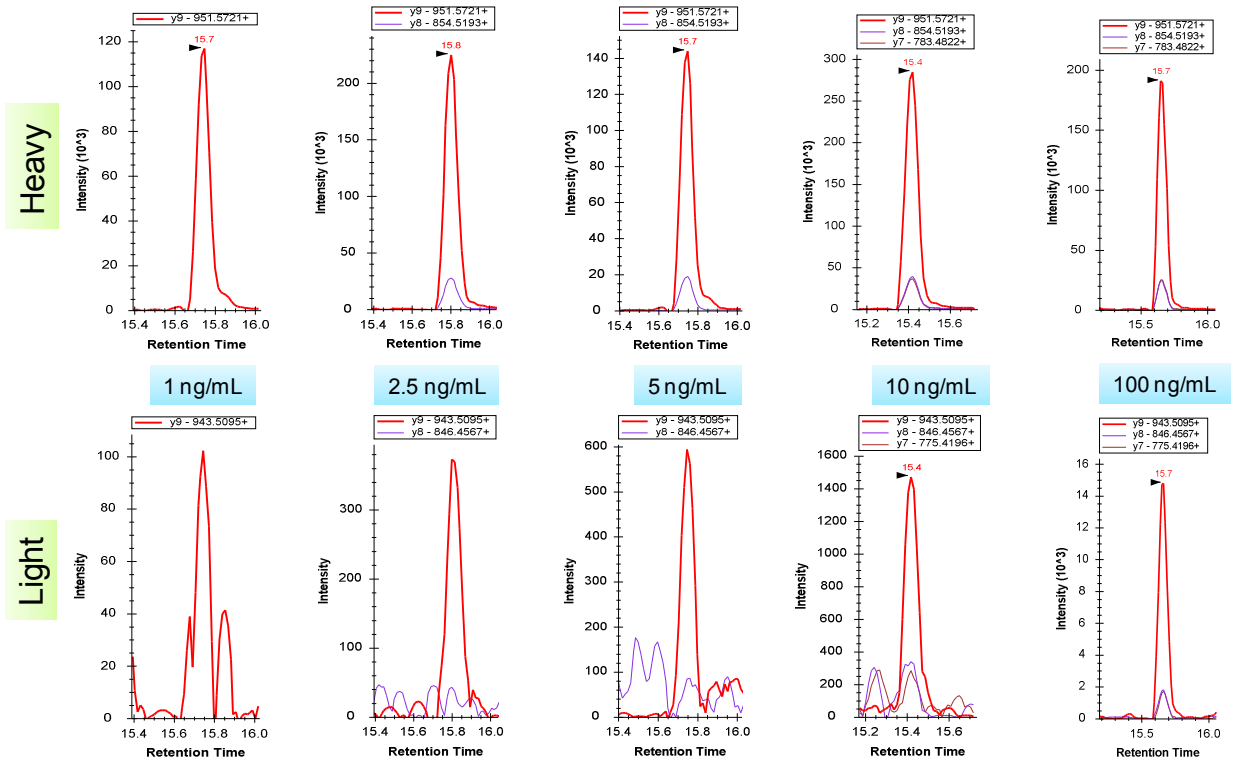


Figure S5.1. Extracted ion chromatograms of transitions monitored for LSEPAELTDAVK derived from prostate-specific antigen. **(A)** Direct LC-SRM. **(B)** Direct PRISM-SRM. Internal standards were spiked at 0.5 fmol/µL.

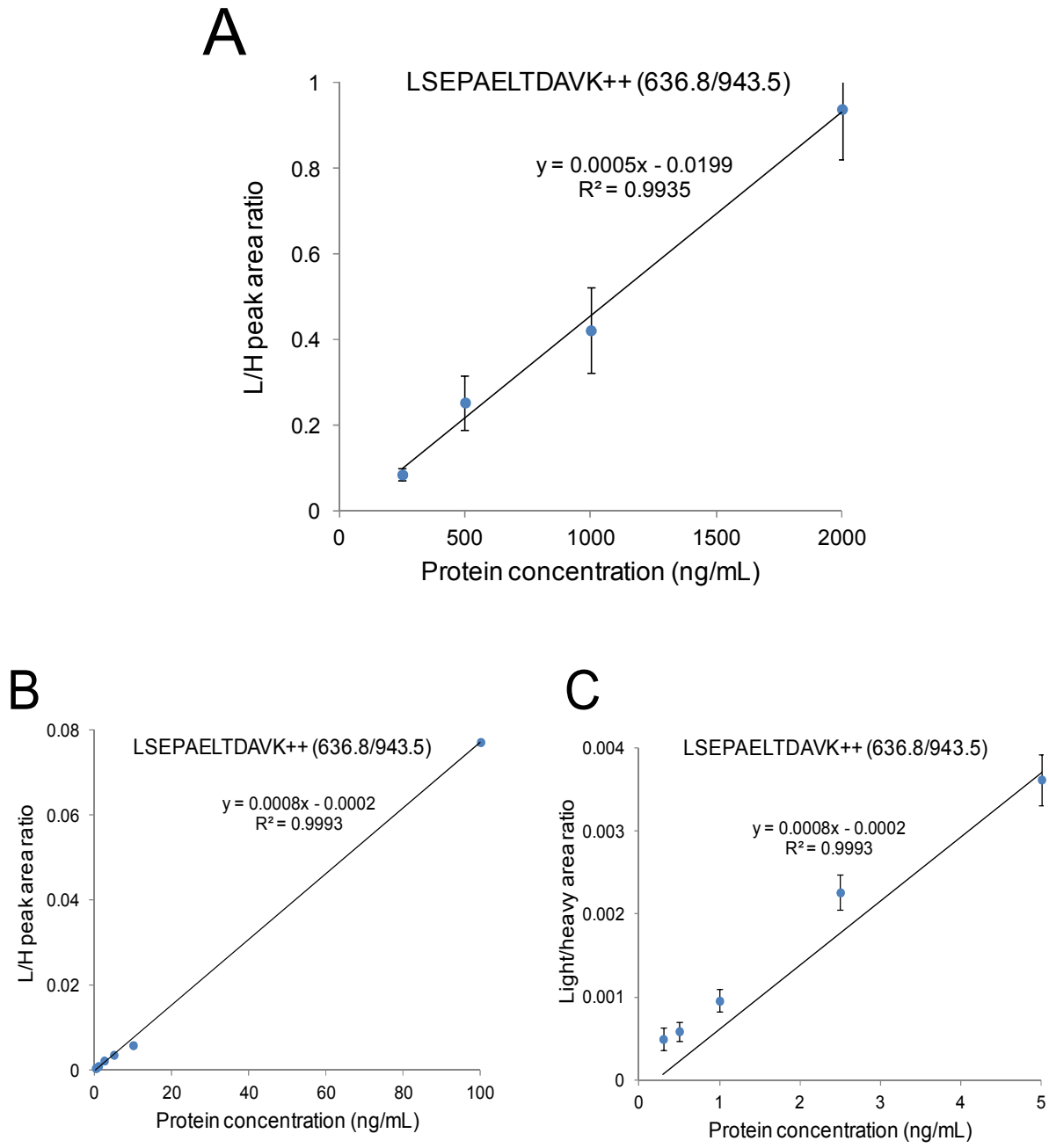


Figure S5.2. Calibration curves for quantifying prostate-specific antigen. **(A)** Direct LC-SRM; **(B)** PRISM-SRM (target protein: 0-100 ng/mL); **(C)** PRISM-SRM (Zoom-in range of 0-5 ng/mL).

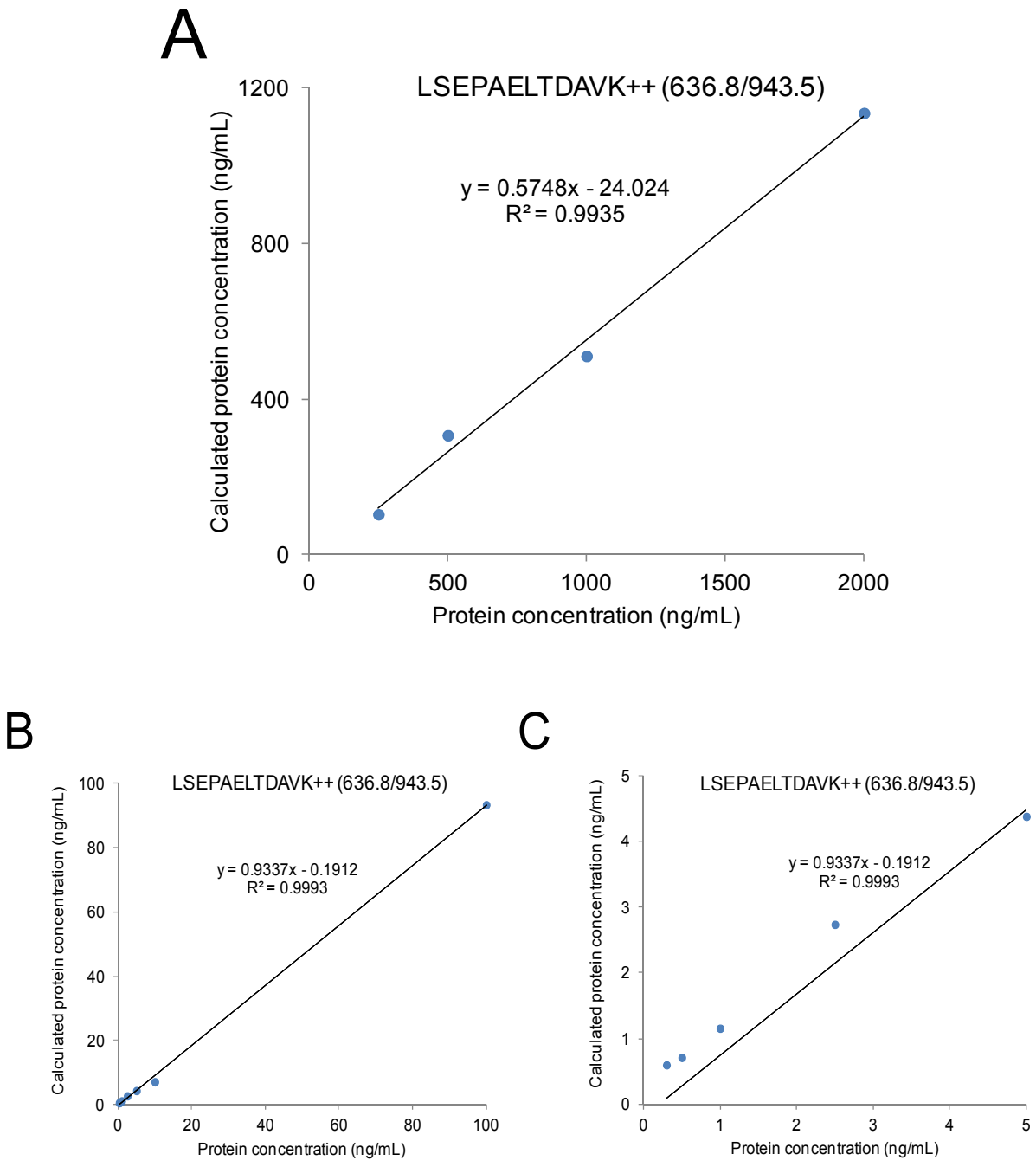
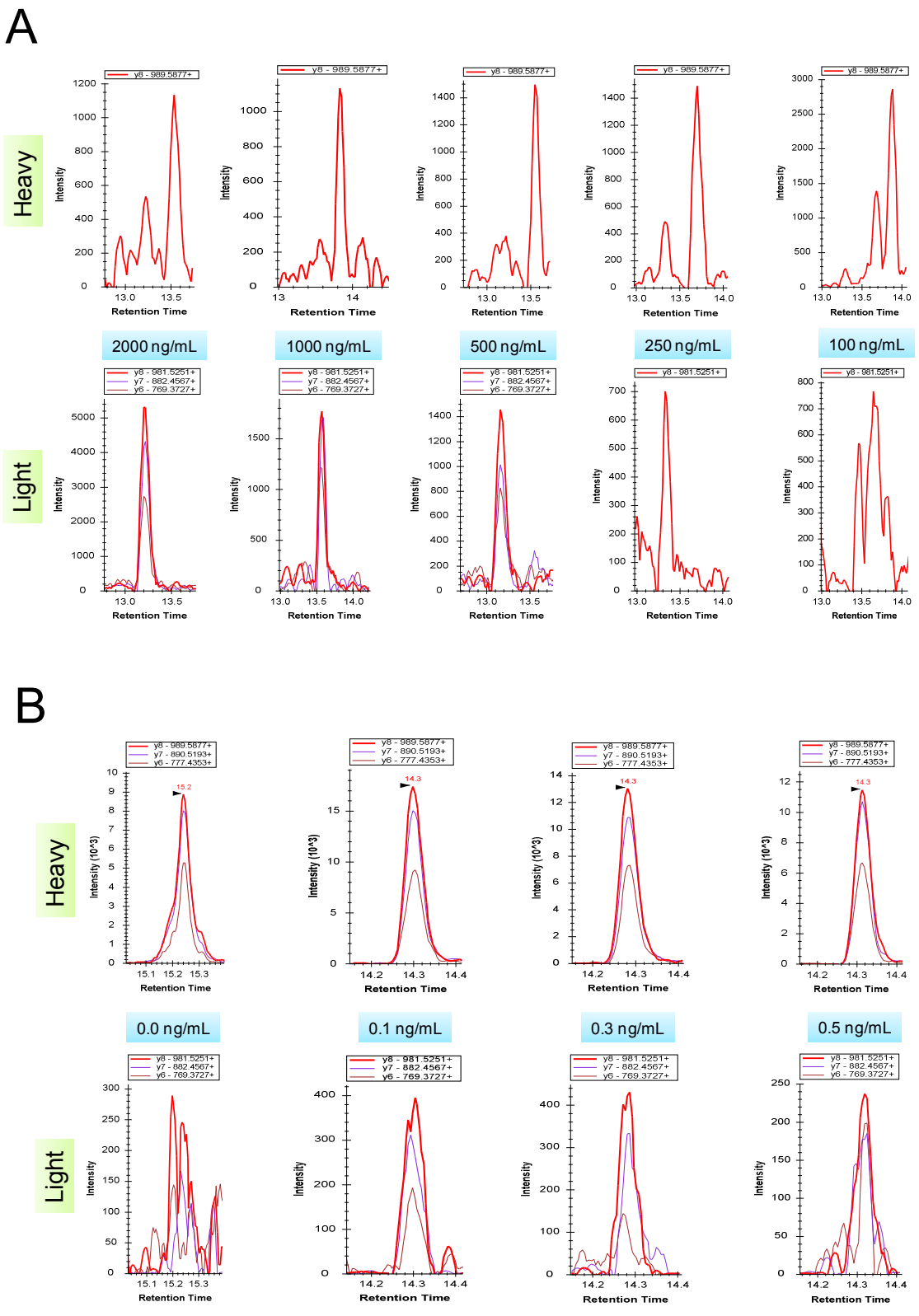


Figure S5.3. Correlation curves for quantifying prostate-specific antigen. **(A) Direct LC-SRM.** Target protein concentrations at the range of 100-2000 ng/mL. **(B)** PRISM-SRM. Target protein concentrations at the range of 0-100 ng/mL. **(C)** PRISM-SRM (Zoom-in range of 0-5 ng/mL).


A
Heavy
Light
2000 ng/mL
1000 ng/mL
500 ng/mL
250 ng/mL
100 ng/mL
y8 - 989.5877+
y8 - 981.5251+
y7 - 882.4567+
y6 - 769.3727+
Intensity
Retention Time
B
Heavy
Light
0.0 ng/mL
0.1 ng/mL
0.3 ng/mL
0.5 ng/mL
y8 - 989.5877+
y7 - 890.5193+
y6 - 777.4353+
y8 - 981.5251+
y7 - 882.4567+
y6 - 769.3727+
Intensity (10^3)
Retention Time

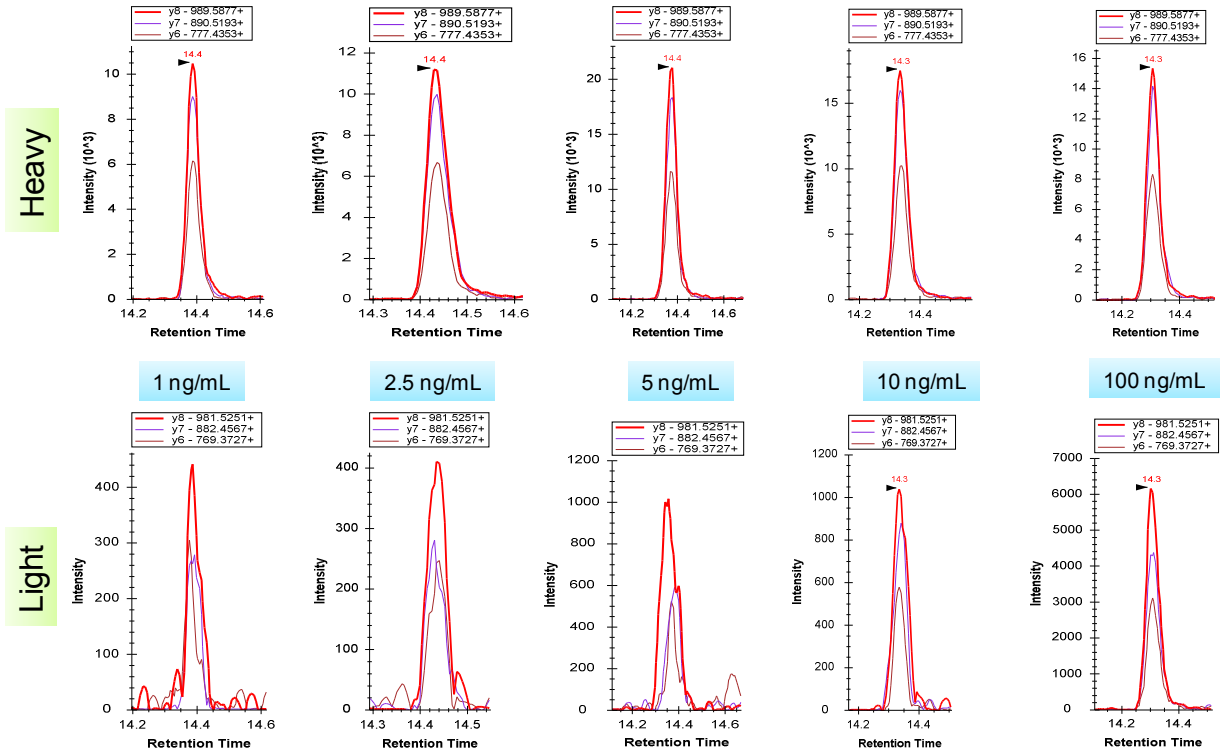


Figure S6.1. Extracted ion chromatograms of transitions monitored for VLVLDTDYKK derived from bovine beta-lactoglobulin. **(A)** Direct LC-SRM. **(B)** Direct PRISM-SRM. Internal standards were spiked at 0.5 fmol/µL.

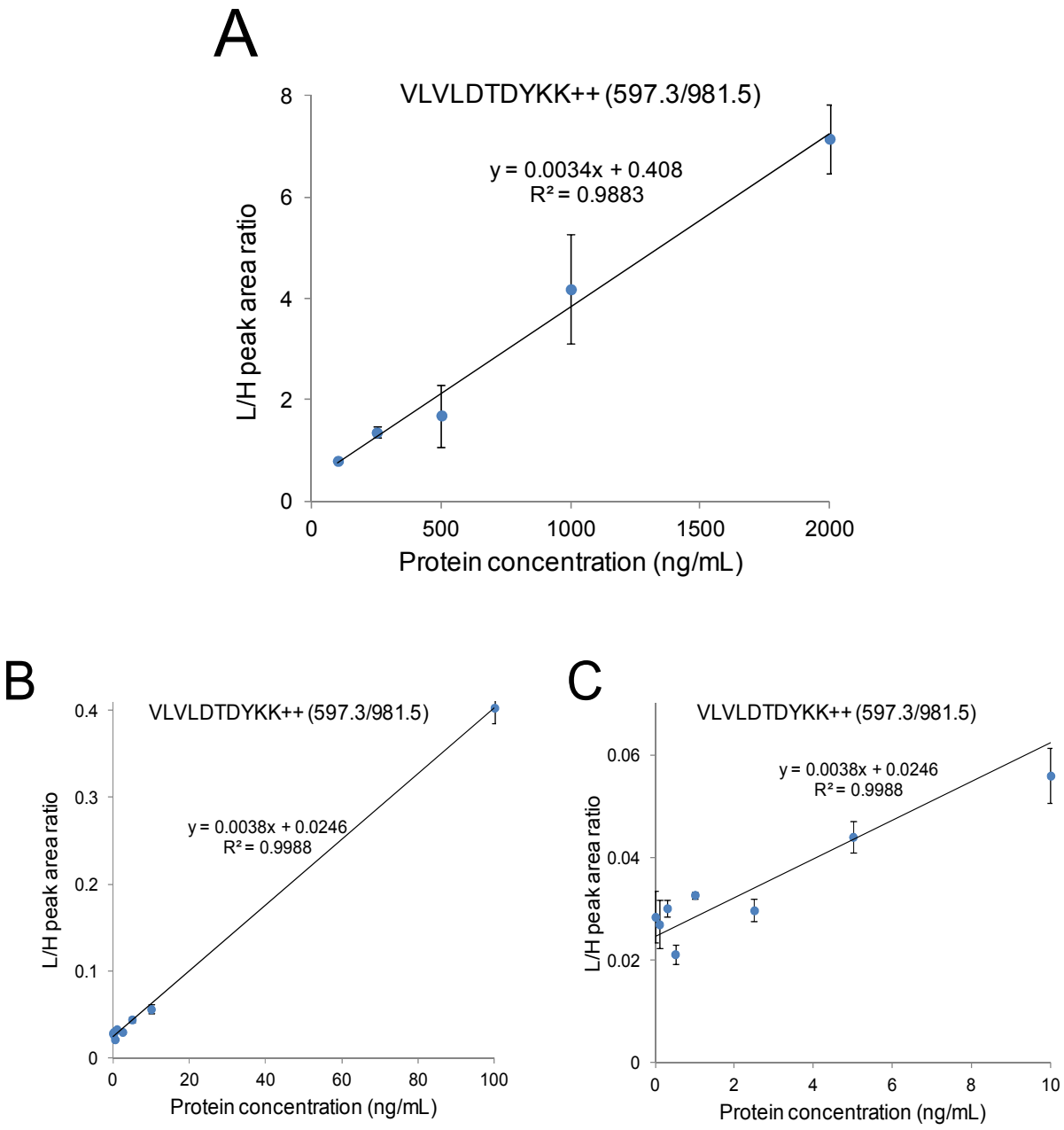


Figure S6.2. Calibration curves for quantifying bovine beta-lactoglobulin. **(A)** Direct LC-SRM; **(B)** PRISM-SRM (target protein: 0-100 ng/mL); **(C)** PRISM-SRM (Zoom-in range of 0-5 ng/mL).

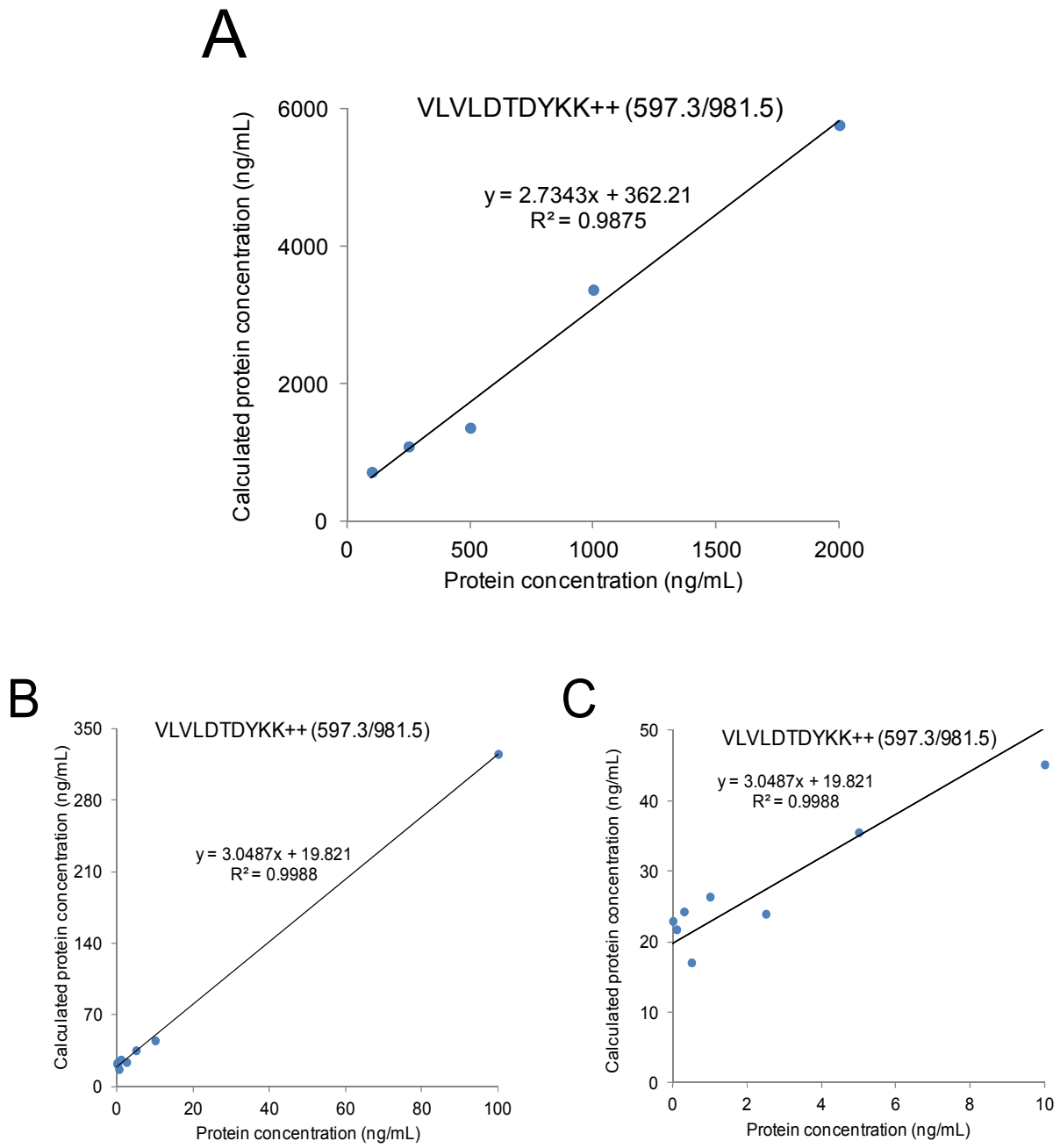
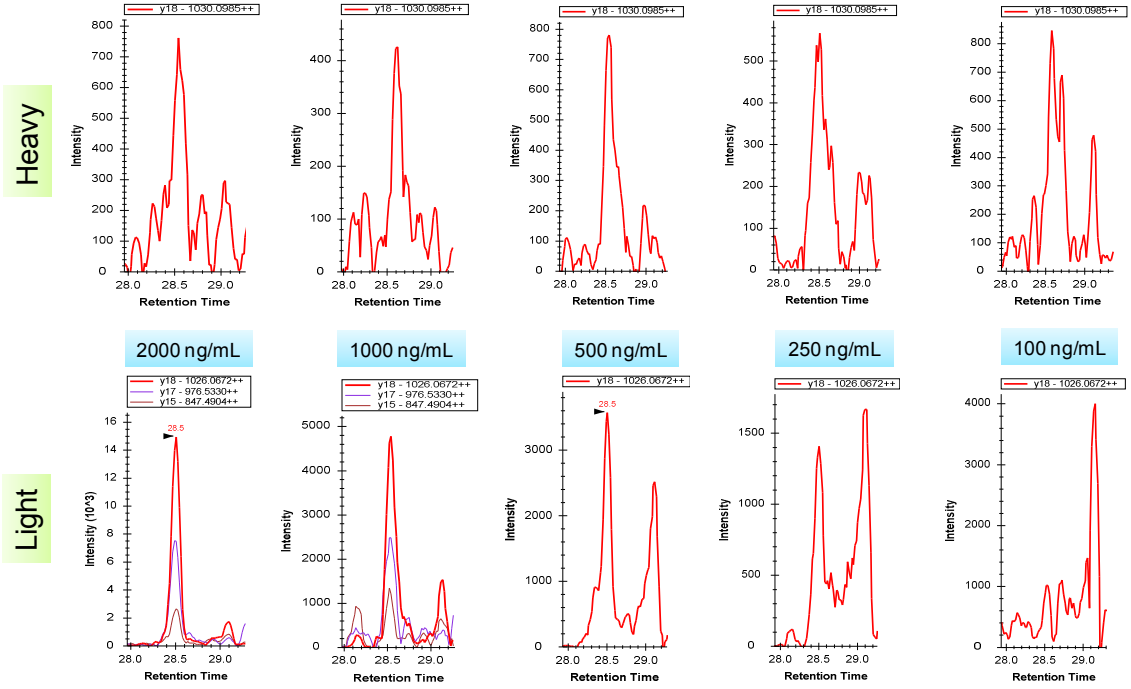
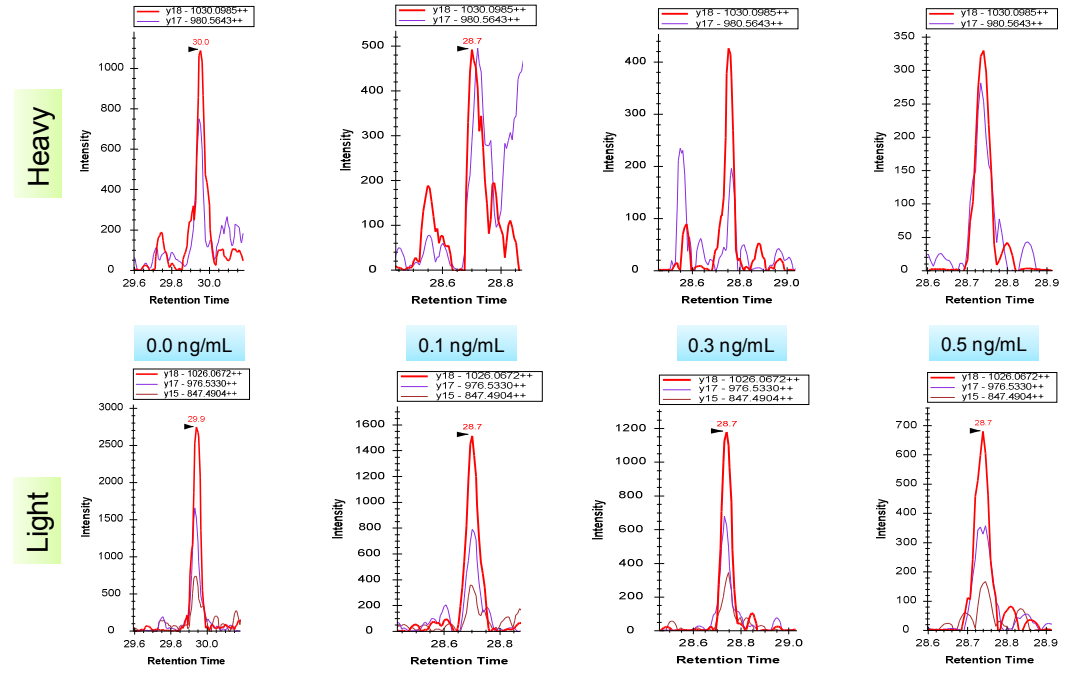


Figure S6.3. Correlation curves for quantifying bovine beta-lactoglobulin. **(A) Direct LC-SRM.** Target protein concentrations at the range of 100-2000 ng/mL. **(B)** PRISM-SRM. Target protein concentrations at the range of 0-100 ng/mL. **(C)** PRISM-SRM. Zoom-in range of 0-5 ng/mL.

A


Heavy
Light
y18 - 1030.0985++
y18 - 1026.0672++
y17 - 976.5330++
y15 - 847.4904++
2000 ng/mL
1000 ng/mL
500 ng/mL
250 ng/mL
100 ng/mL
Intensity
Intensity (10^3)
Retention Time

B


Heavy
Light
y18 - 1030.0985++
y17 - 980.5643++
y18 - 1026.0672++
y17 - 976.5330++
y15 - 847.4904++
0.0 ng/mL
0.1 ng/mL
0.3 ng/mL
0.5 ng/mL
Intensity
Retention Time

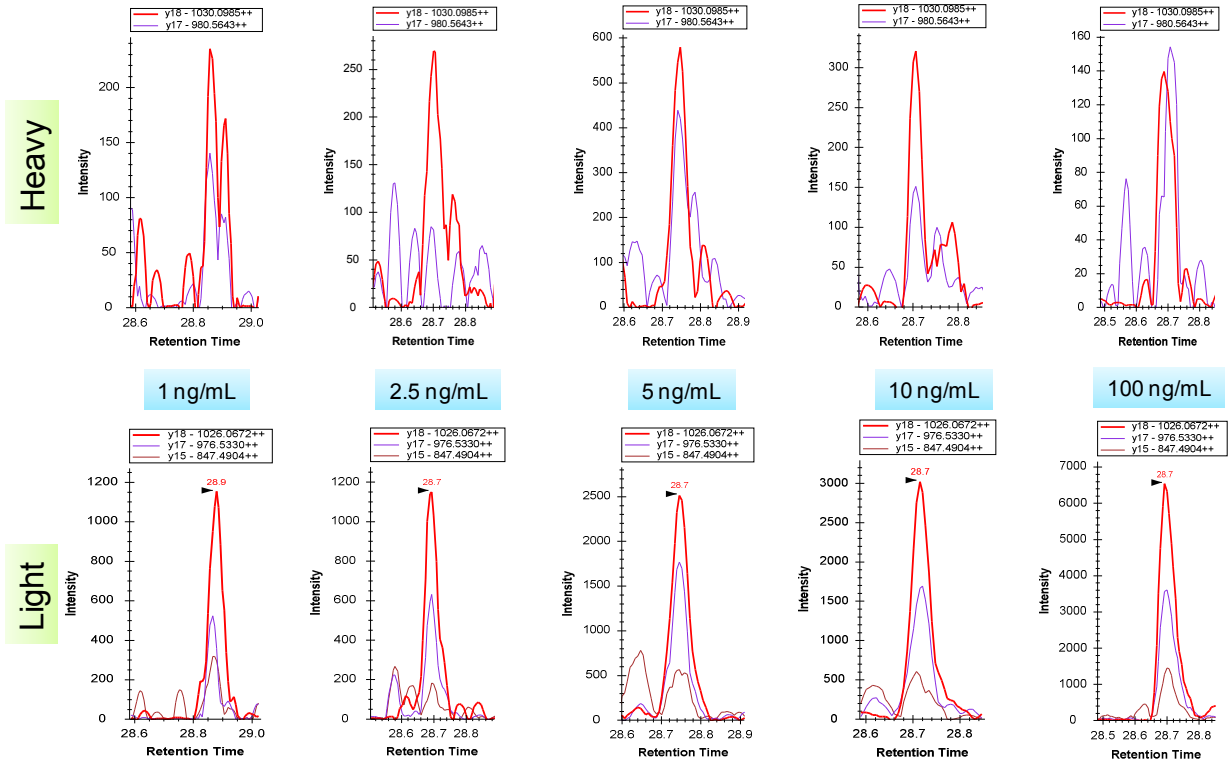


Figure S7.1. Extracted ion chromatograms of transitions monitored for VYVEELKPTPEGDLEILLQK derived from bovine beta-lactoglobulin. **(A)** Direct LC-SRM. **(B)** Direct PRISM-SRM. Internal standards were spiked at 0.5 fmol/µL.

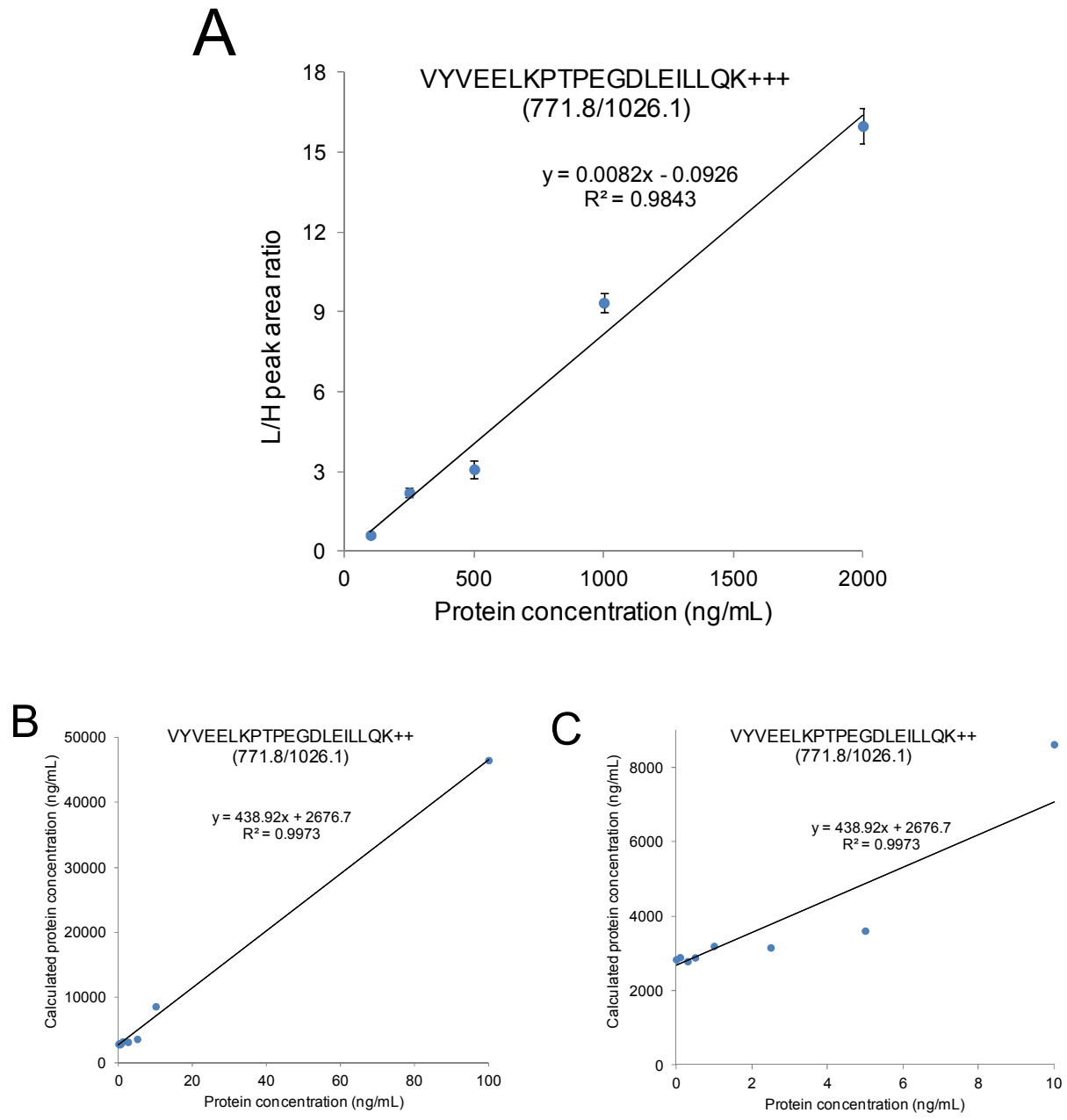


Figure S7.2. Calibration curves for quantifying bovine beta-lactoglobulin. **(A)** Direct LC-SRM; **(B)** PRISM-SRM (target protein: 0-100 ng/mL); **(C)** PRISM-SRM (Zoom-in range of 0-5 ng/mL).

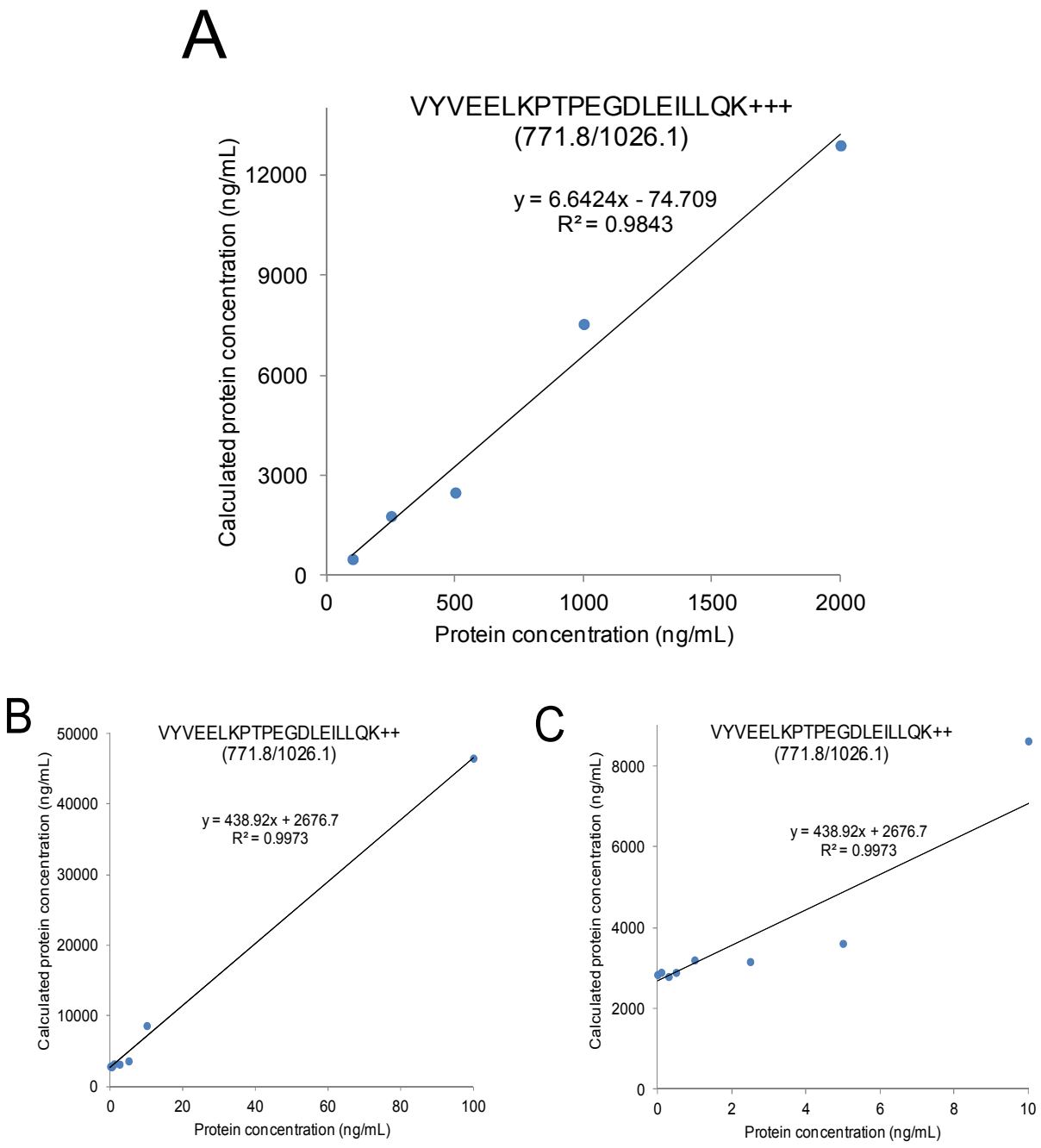


Figure S7.3. Correlation curves for quantifying bovine beta-lactoglobulin. **(A) Direct LC-SRM.** Target protein concentrations at the range of 100-2000 ng/mL. **(B)** PRISM-SRM. Target protein concentrations at the range of 0-100 ng/mL. **(C)** PRISM-SRM (Zoom-in range of 0-5 ng/mL).

Table S1. Quantitative SRM measurements of 3 target proteins in human female serum with direct LC-SRM. For each concentration point SRM measurements were performed in triplicate.

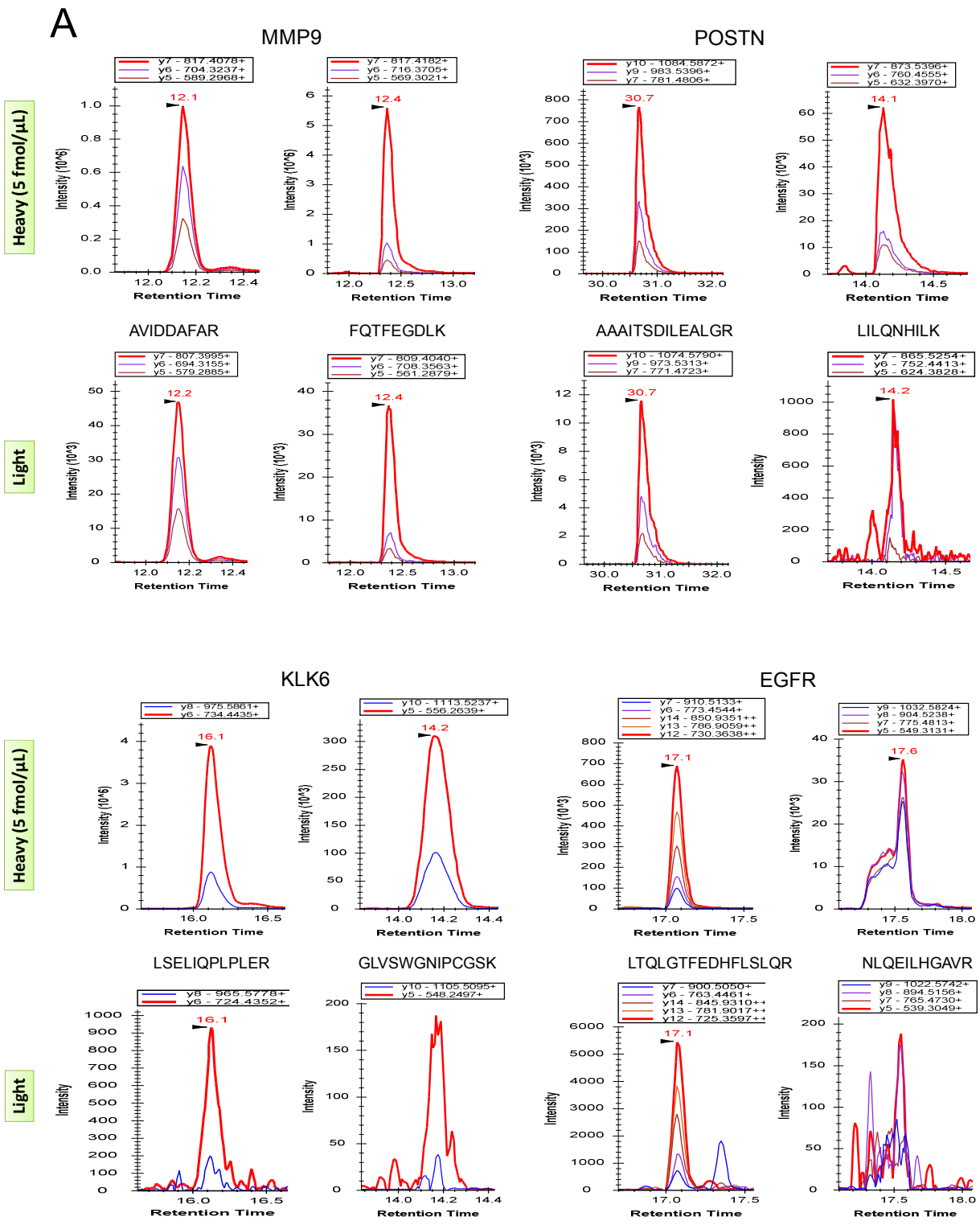
Target protein	Surrogate peptide	Transition		Target protein concentration (ng/mL)				
				100	**250**	**500**	**1000**	**2000**
Bovine carbonic anhydrase	DGPLTGTYR	490.2/496.3	%CV	42.4	7.2	15.9	9.6	3.8
			Calc. concentration[b]	**103**	**261**	**610**	**891**	**1984**
			%relative error[c]	3.2	4.2	22.1	10.9	0.8
			S/N[d]	4	7	12	17	21
	DFPIANGER	509.7/378.3	%CV				4.3	2.9
			Calc. concentration				**742**	**1449**
			%relative error				34.8	38.0
			S/N			< 3[e]	12	10
Bovine beta-lactoglobulin	VLVLDTDYKK	597.3/981.5	%CV	2.5	7.9	36.1	25.8	9.6
			Calc. concentration	**725**	**1098**	**1369**	**3377**	**5770**
			%relative error	625.2	339.1	173.7	237.7	188.5
			S/N	5	12	15	21	34
	VYVEELKPTPEGDLEILLQK	771.8/1026.1	%CV	31.7	38.9	46.7	35.8	18.1
			Calc. concentration	**495**	**1781**	**2490**	**7539**	**12893**
			%relative error	n/a	n/a	n/a	n/a	n/a
			S/N	6	6	10	27	150
Prostate-specific antigen (PSA)	IVGGWEC$_{cam}$EK[a]	539.2/865.3	%CV			12.7	7.3	7.0
			Calc. concentration			**656**	**1039**	**2473**
			%relative error			31.2	3.9	23.6
			S/N			3[e]	41	62
	LSEPAELTDAVK	636.8/943.5	%CV		16.7	24.9	23.7	12.6
			Calc. concentration		**104**	**308**	**511**	**1136**
			%relative error		58.3	38.5	48.9	43.2
			S/N	< 3	7	8	10	30

[a] Cysteine were synthesized as carbamidomethyl cysteine.
[b] The formula for obtaining calculated concentrations is described in **Supplemental Methods**.
[c] %relative error = (|target ng/mL – calculated ng/mL| / |target ng/mL|) × 100.
[d] Average S/N for 3 SRM measurements.
[e] Large co-eluting interference.

Table S2. Quantitative direct PRISM-SRM measurements of 3 target proteins in human female serum. For the target protein concentration at 2.5 ng/mL three process replicates followed by three injection replicates for SRM measurement were used to test reproducibility of the PRISM workflow. For other concentration points only three SRM injection replicates were performed without any process replicate.

Target protein	Surrogate peptide	Transition		Target protein concentration (ng/mL)								
				0	0.1	0.3	0.5	1	2.5[e]	5	10	100
Bovine carbonic anhydrase	DGPLTGTYR	490.2/496.3	%CV					8.0	7.9	4.3	6.2	4.9
			Calc. concentration[b]					**2.6**	**3.7**	**8.4**	**13.3**	**90.4**
			%relative error[c]					155.9	46.6	68.1	33.3	9.6
			S/N[d]		< 3	< 3	< 3	3	3	11	15	78
	DFPIANGER	509.7/378.3	%CV			11.8	20.7	15.0	5.1	5.9	8.7	1.6
			Calc. concentration			**0.50**	**0.54**	**0.78**	**1.6**	**4.5**	**5.6**	**83.1**
			%relative error			68.2	8.1	22.0	34.6	9.6	43.8	16.9
			S/N		< 3	8	10	17	17	18	25	281
Bovine beta-lactoglobulin	VLVLDTDYKK	597.3/981.5	%CV	17.8	17.4	5.4	8.9	1.7	7.3	7.0	9.5	4.5
			Calc. concentration	**23.0**	**21.8**	**24.3**	**17.1**	**26.4**	**23.8**	**35.5**	**45.2**	**325**
			%relative error		21666	7999	3314	2540	859	611	352	225
			S/N	5	89	131	75	24	83	172	116	1232
	VYVEELKPTPEGDLEILLQK	771.8/1026.1	%CV	8.4	29.6	44.1	35.1	15.7	15.0	19.3	8.4	3.3
			Calc. concentration	**2839**	**2896**	**2790**	**2891**	**3199**	**3158**	**3611**	**8622**	**46490**
			%relative error		n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
			S/N	35	28	29	26	136	35	60	33	111
Prostate-specific antigen (PSA)	IVGGWEC$_{cam}$EK[a]	539.2/865.3	%CV		31.5	20.7	5.4	9.7	10.7	14.3	8.0	0.8
			Calc. concentration		**0.62**	**0.80**	**0.82**	**1.3**	**2.5**	**5.6**	**8.5**	**106.0**
			%relative error		520.8	168.1	64.5	30.3	1.42	12.0	15.3	6.0
			S/N	< 3	4	6	13	12	26	33	166	186
	LSEPAELTDAVK	636.8/943.5	%CV			27.1	19.2	14.4	14.3	8.5	7.7	0.8
			Calc. concentration			**0.60**	**0.71**	**1.2**	**2.7**	**4.4**	**7.1**	**93.4**
			%relative error			99.6	42.4	15.6	9.4	12.5	28.9	6.6
			S/N		< 3	5	13	13	31	37	45	188

[a] Cysteine were synthesized as carbamidomethyl cysteine.
[b] The formula for obtaining calculated concentrations is described in **Supplemental Methods**.
[c] %relative error = (|target ng/mL – calculated ng/mL| / |target ng/mL|) × 100.
[d] Average S/N for 3 SRM measurements with the exception of 9 SRM measurements for the concentration point at 2.5 ng/mL.
[e] Three PRISM process replicates followed by three SRM injection replicates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MMP9
POSTN
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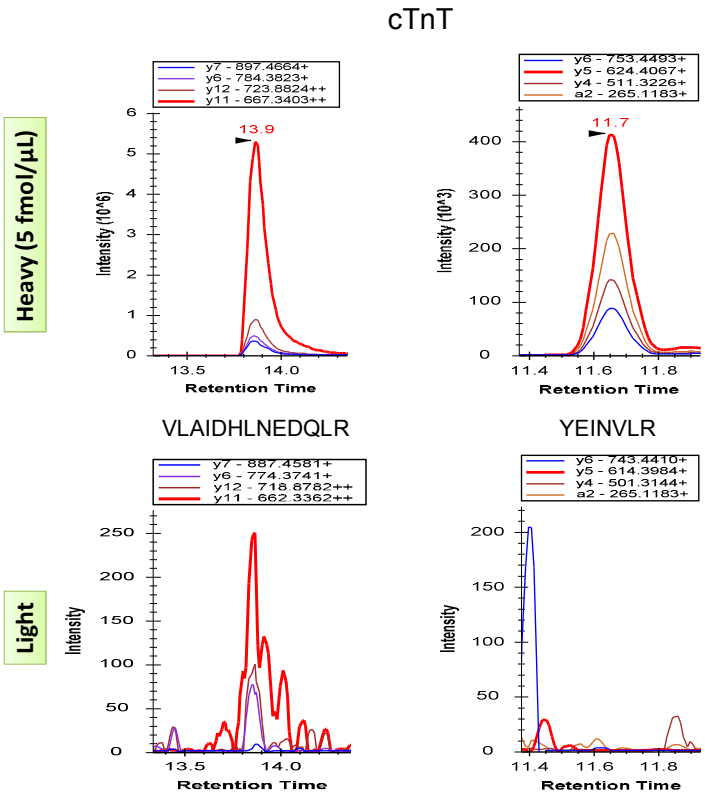
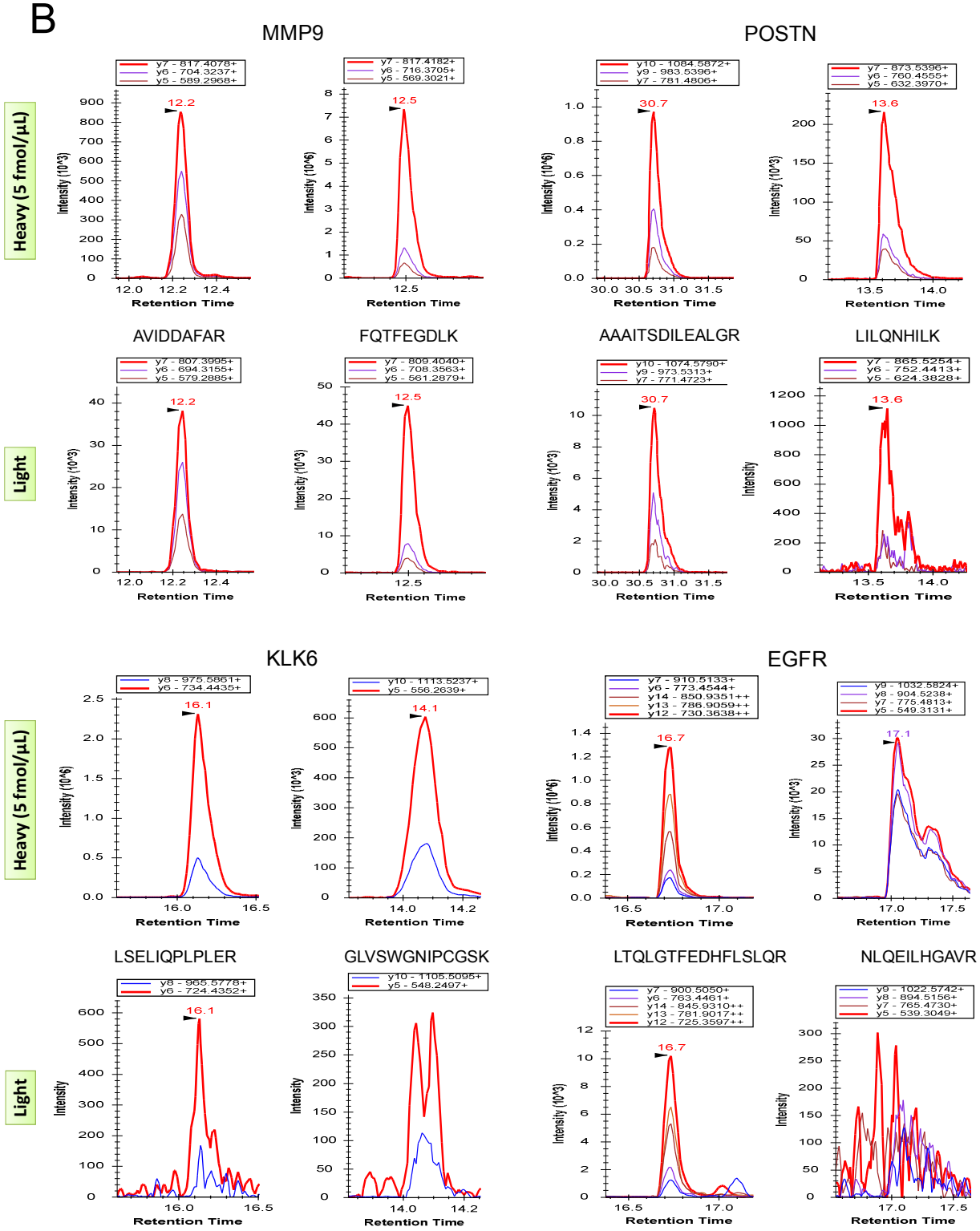
Light
AVIDDAFAR
FQTFEGDLK
AAAITSDILEALGR
LILQNHILK
KLK6
EGFR
LSELIQPLPLER
GLVSWGNIPCGSK
LTQLGTFEDHFLSLQR
NLQEILHGAVR
Intensity (10^6)
Intensity (10^3)
Intensity
Retention Time


cTnT
Heavy (5 fmol/µL)
y7 - 897.4664+
y6 - 784.3823+
y12 - 723.8824++
y11 - 667.3403++
13.9
Intensity (10^6)
Retention Time
y6 - 753.4493+
y5 - 624.4067+
y4 - 511.3226+
a2 - 265.1183+
11.7
Intensity (10^3)
Retention Time
VLAIDHLNEDQLR
YEINVLR
Light
y7 - 887.4581+
y6 - 774.3741+
y12 - 718.8782++
y11 - 662.3362++
Intensity
Retention Time
y6 - 743.4410+
y5 - 614.3984+
y4 - 501.3144+
a2 - 265.1183+
Intensity
Retention Time


B
MMP9
POSTN
AVIDDAFAR
FQTFEGDLK
AAAITSDILEALGR
LILQNHILK
KLK6
EGFR
LSELIQPLPLER
GLVSWGNIPCGSK
LTQLGTFEDHFLSLQR
NLQEILHGAVR
Heavy (5 fmol/µL)
Light
Intensity
Retention Time

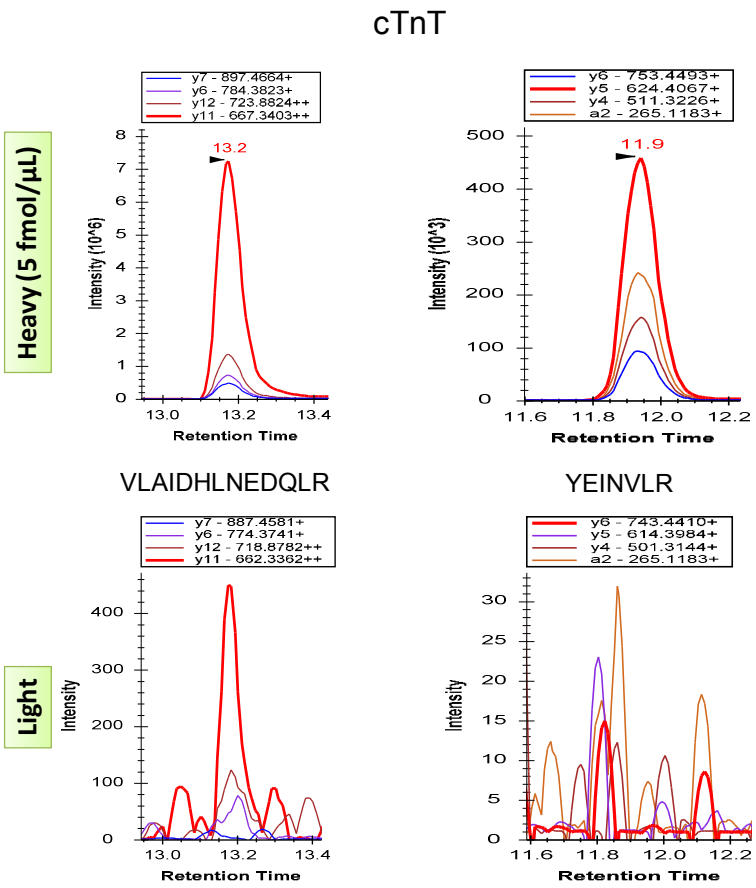


Figure S8. Extracted ion chromatograms of transitions monitored for quantification of endogenous proteins in human serum. **(A)** Direct PRISM-SRM measurements for clinical patient serum (Sample ID: 21). **(B)** Direct PRISM-SRM measurements for clinical patient serum (Sample ID: 6). Crude heavy peptides were used as internal standards with the spiking level at 5 fmol/µL in patient sera.

Table S3. The calculated six cancer-associated endogenous protein concentrations in the two clinical serum samples (see **Supplemental Methods: 2. endogenous protein concentration calculation in blinded clinical patient sera**).

Endogenous protein	Accession number	Molecular weight (kDa)	Surrogate peptide (best transition)	Heavy peptide concentration (fmol/μL)	L/H[c]	Calculated protein concentration (ng/mL)	
cTnT	P45379	34	VLAIDHLNEDQLR (512.6/662.3)	5	0.000054[d]	0.85[d]	0.17[d]
					0.000046[e]	0.91[e]	0.18[e]
KLK6	Q92876	26.9	LSELIQPLPLER (704.4/724.4)	5	0.000205[d]	2.5[d]	0.5[d]
					0.000212[e]	3.3[e]	0.66[e]
PSA	P07288	30	IVGGWEC$_{cam}$EK[a] (539.2/865.3)	0.5[b]	0.051679[d]	71.6[d]	
					0.004028[e]	7.0[e]	
EGFR	P00533	134.3	LTQLGTFEDHFLSLQR (635.7/725.4)	5	0.008395[d]	520.5[d]	104.1[d]
					0.0076[e]	590.0[e]	118.0[e]
MMP9	P14780	78.5	AVIDDAFAR (489.3/807.4)	5	0.043311[d]	1569.7[d]	313.9[d]
					0.048864[e]	2217.2[e]	443.4[e]
POSTN	Q15063	87	AAAITSDILEALGR (700.9/1074.6)	5	0.011428[d]	459.0[d]	91.8[d]
					0.016037[e]	806.5[e]	161.3[e]

[a] Cysteine were synthesized as carbamidomethyl cysteine.

[b] Besides PSA with pure heavy internal standards for the other five proteins crude heavy surrogate peptides were used as internal standards.

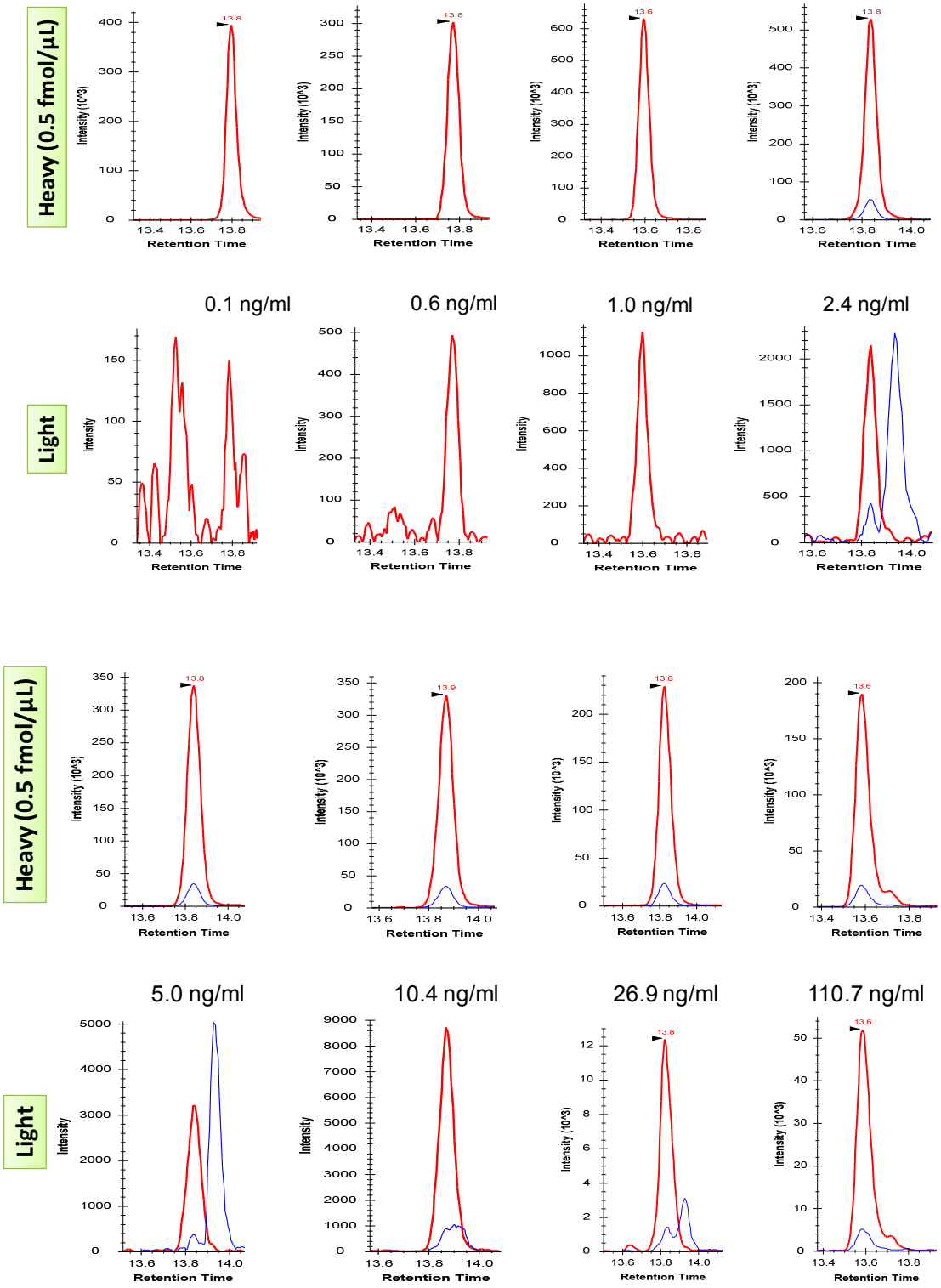
[c] Average L/H for 2 SRM measurements.

[d] Clinical patient sample from JHU (sample ID: 6).

[e] Clinical patient sample from JHU (sample ID: 21).

[f] Based on our experimental results (not published) the purity of crude peptides is around 20% . The detailed calculation can be found in Supplemental Material from the paper "Antibody-free, targeted mass-spectrometric approach for quantification of proteins at low picogram per milliliter levels in human plasma/serum. *Proc Natl Acad Sci U S A* 109, 15395-15400".

A


Heavy (0.5 fmol/µL)
Light
0.1 ng/ml
0.6 ng/ml
1.0 ng/ml
2.4 ng/ml
5.0 ng/ml
10.4 ng/ml
26.9 ng/ml
110.7 ng/ml
Intensity (10^3)
Intensity
Retention Time

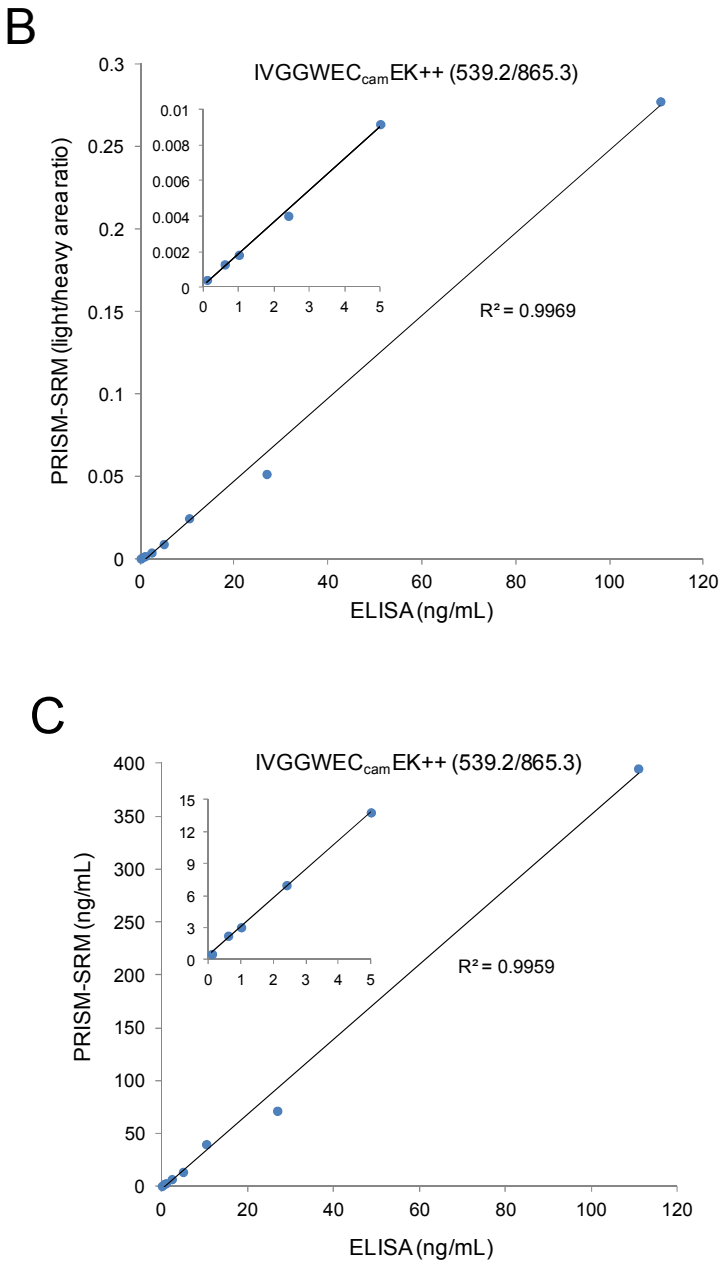
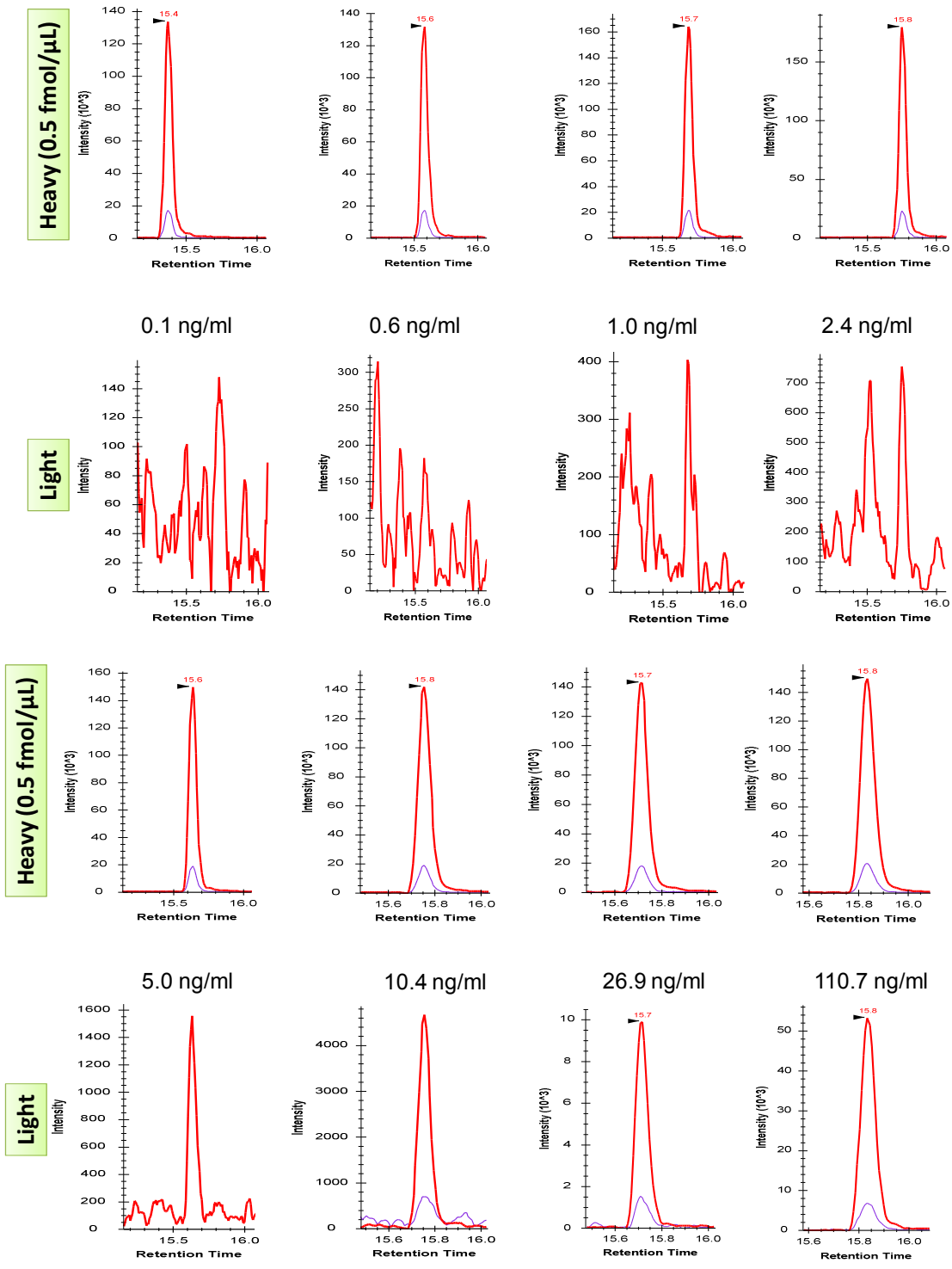


Figure S9. Direct PRISM-SRM versus ELISA for quantification of PSA in clinical patient samples. **(A)** XICs of transitions monitored for IVGGWEC$_{cam}$EK derived from PSA along with internal standard at 0.5 fmol/µL. IVGGWEC$_{cam}$EK: 539.2/865.3 (*red*), 539.2/964.4 (*blue*). PSA concentrations were obtained from the ELISA measurement. **(B)** Correlation curve between PRISM-SRM and ELISA measurements for PSA in patient serum. **(C)** Correlation curve between the calculated PSA concentrations based on the PRISM-SRM measurement and the ELISA values. Inset plots show the details of the low concentration range.

A


Heavy (0.5 fmol/µL)
Light
0.1 ng/ml
0.6 ng/ml
1.0 ng/ml
2.4 ng/ml
5.0 ng/ml
10.4 ng/ml
26.9 ng/ml
110.7 ng/ml
Intensity (10^3)
Intensity
Retention Time

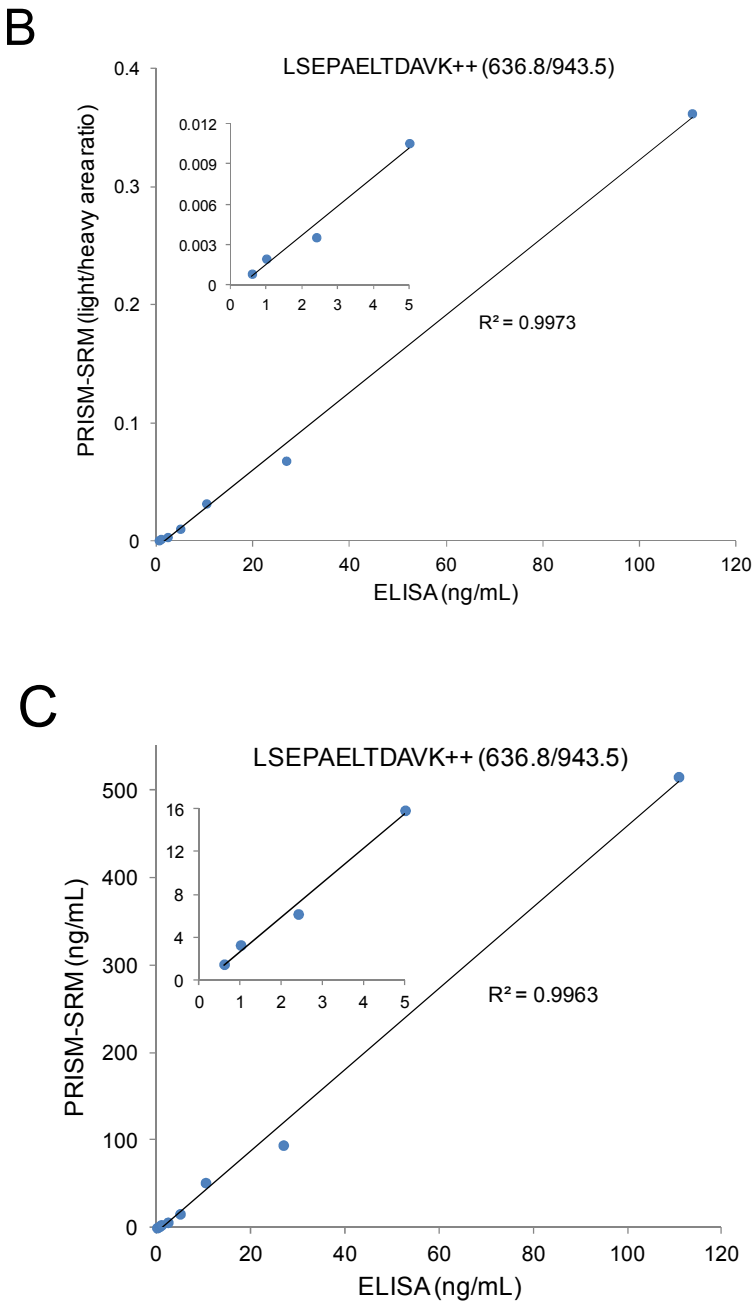


Figure S10. Direct PRISM-SRM versus ELISA for quantification of PSA in clinical patient samples. **(A)** XICs of transitions monitored for LSEPAELTDAVK derived from PSA along with internal standard at 0.5 fmol/µL. LSEPAELTDAVK: 636.8/943.5 (*red*), 636.8/846.5 (*purple*). PSA concentrations were obtained from the ELISA measurement. **(B)** Correlation curve between PRISM-SRM and ELISA measurements for PSA in patient serum. **(C)** Correlation curve between the calculated PSA concentrations based on the PRISM-SRM measurement and the ELISA values. Inset plots show the details of the low concentration range.

Table S4. Results of quantitative measurements from PRISM-SRM and ELISA.

Sample ID	6	13	20	21	23	29	30	33
ELISA (ng/mL)	26.9	5	10.4	2.4	0.6	110.8	0.1	1
PRISM-SRM (L/H ratio)	0.051679	0.009178	0.024814	0.004028	0.001301	0.277614	0.000428	0.001838
Calculated PSA concentration (ng/mL)	71.6	13.8	40.0	7.0	2.2	395.1	0.5	3.0

Supplemental Methods

1. Target protein concentration calculation

The detailed calculation, including digestion efficiency and SPE recovery, can be found in Supplemental Material from the paper "Antibody-free, targeted mass-spectrometric approach for quantification of proteins at low picogram per milliliter levels in human plasma/serum. *Proc Natl Acad Sci U S A* 109, 15395-15400".

2. Endogenous protein concentration calculation in blinded clinical patient sera

The detailed calculation, including digestion efficiency and SPE recovery, can be found in Supplemental Material from the paper "Antibody-free, targeted mass-spectrometric approach for quantification of proteins at low picogram per milliliter levels in human plasma/serum. *Proc Natl Acad Sci U S A* 109, 15395-15400".

3. Volume of raw female serum required for PRISM-SRM experiment

$V_{loading\ volume}$ = 45 µL (PRISM fractionation at a concentration of 1 µg/µL)

$$V_{total\ raw\ female\ serum} = 12.4\ \mu L\ (1\ mg\ female\ serum)$$

Following trypsin digestion and SPE cleanup, the peptide recovery is ~33%, which means that the amount of total peptide is 330 μg, i.e., the total volume will be 330 μL with the peptide concentration at 1 μg/μL.

$$V_{raw\ female\ serum}\ (direct\ PRISM\text{-}SRM) = 45\ \mu L \times 12.4\ \mu L/330\ \mu L = 1.69\ \mu l = \sim 2\ \mu L$$

4. On-column calculation of injected target protein

The detailed calculation can be found in Supplemental Material from the paper "Antibody-free, targeted mass-spectrometric approach for quantification of proteins at low picogram per milliliter levels in human plasma/serum. *Proc Natl Acad Sci U S A* 109, 15395-15400".

5. Coefficient of variation (CV) calculation

Precision was determined by CV (standard derivation divided by the mean) and expressed as a percentage. The concentration point at 2.5 ng/mL involved three process replicates. The mean value of the three process replicates was obtained from three average L/H area ratio values, and each average L/H value was based on three injection replicates per process replicate. The standard deviation was calculated with the three average L/H values and their mean value. For the other concentration points, CV calculation was simply based on three injection replicates of LC-SRM measurements.