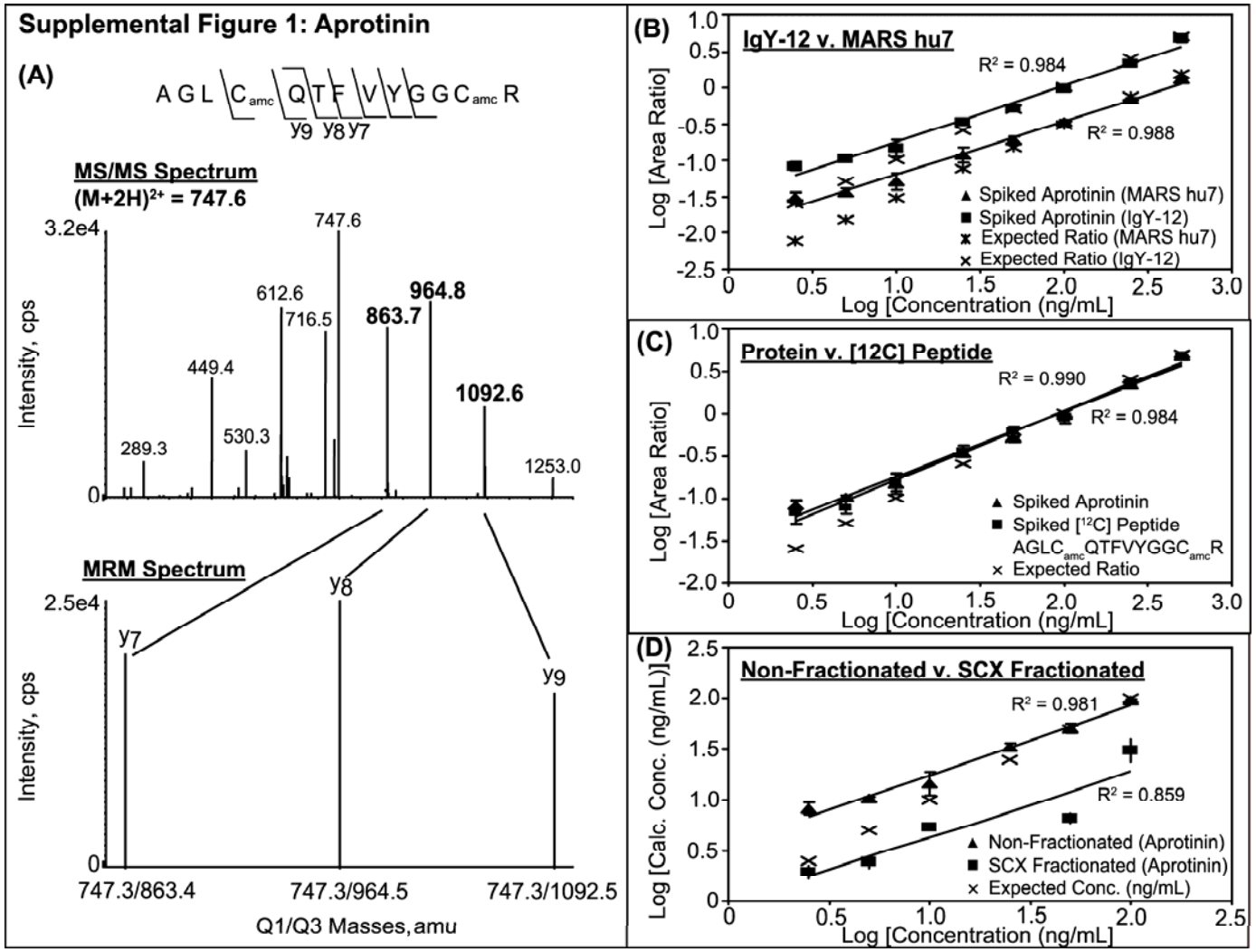
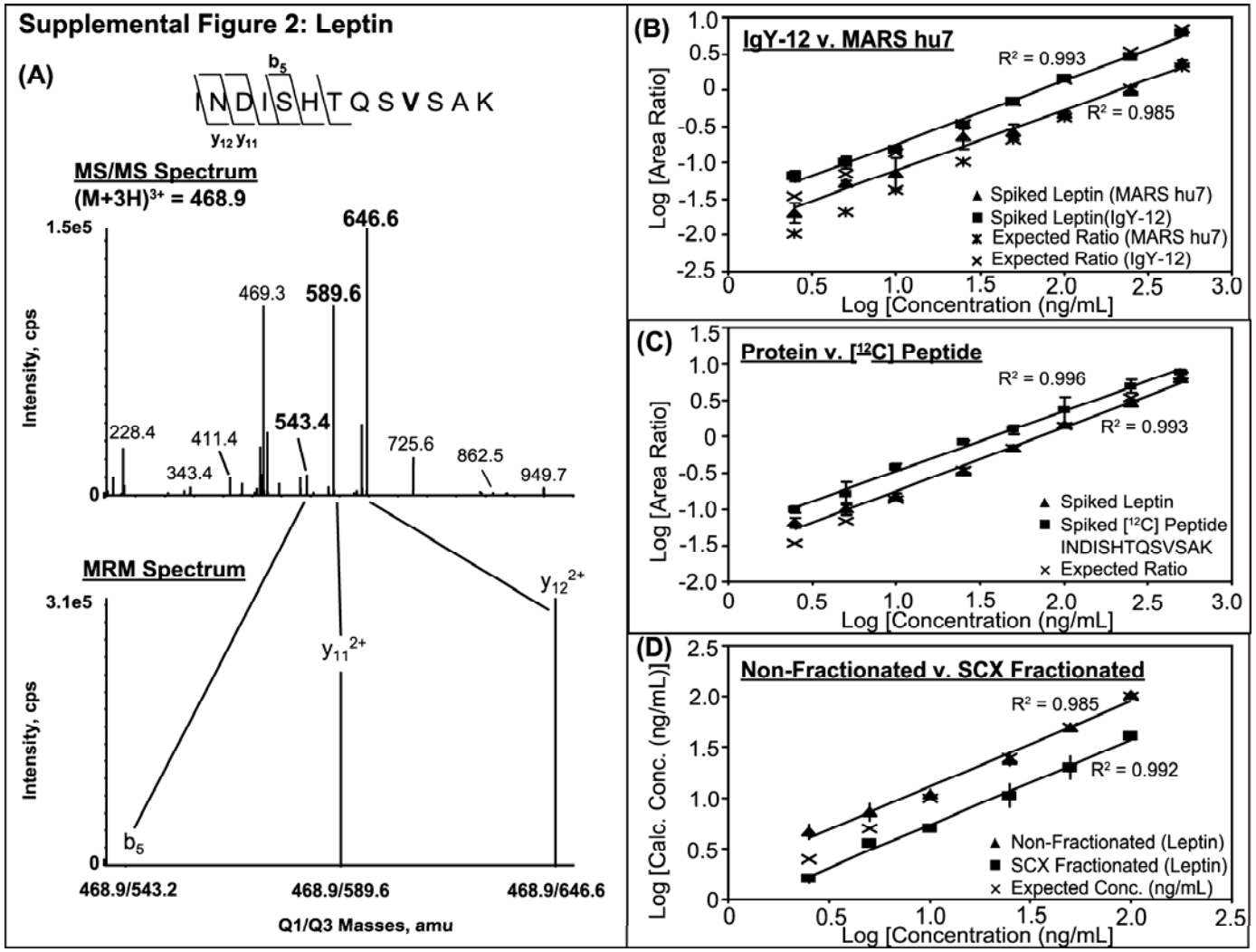
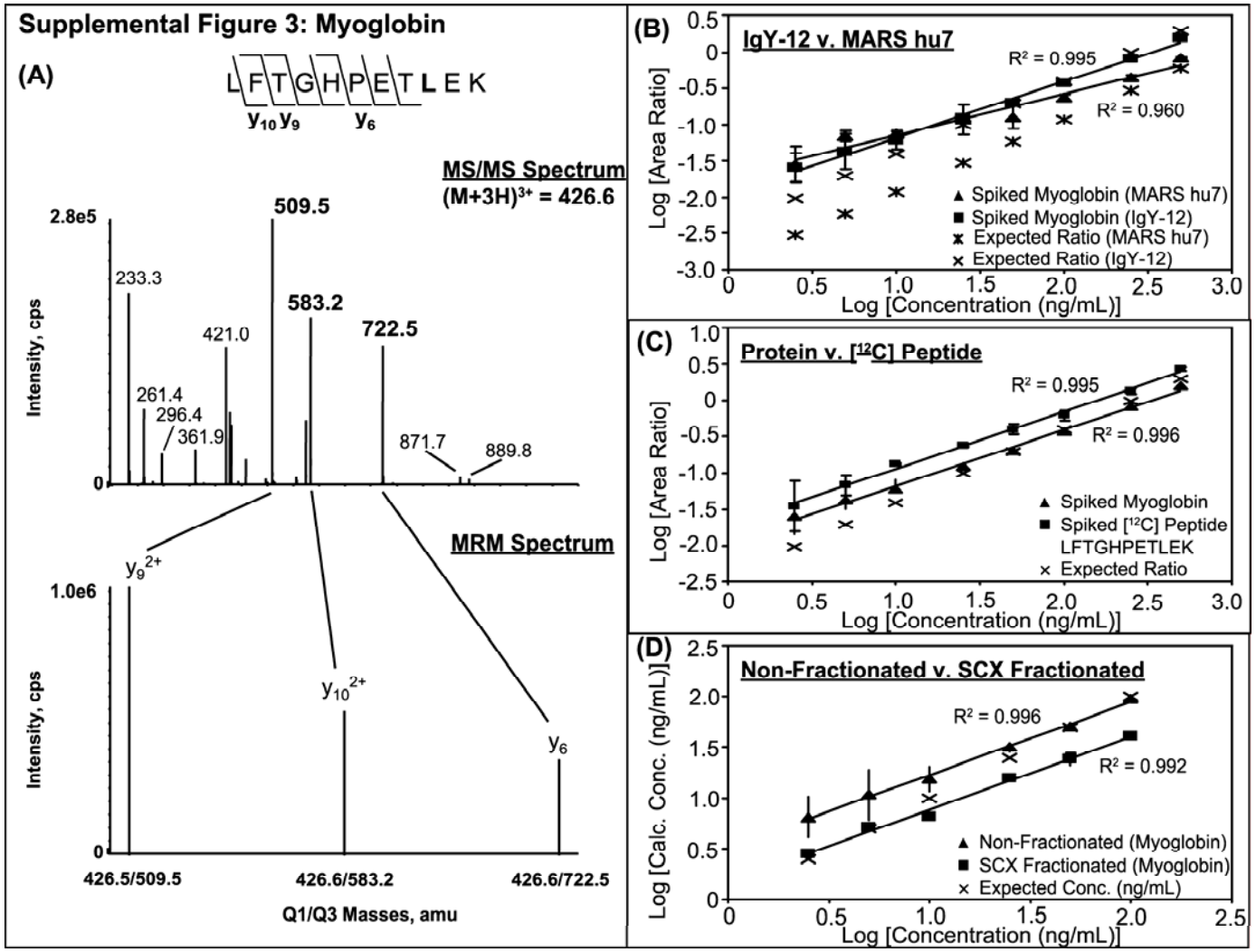


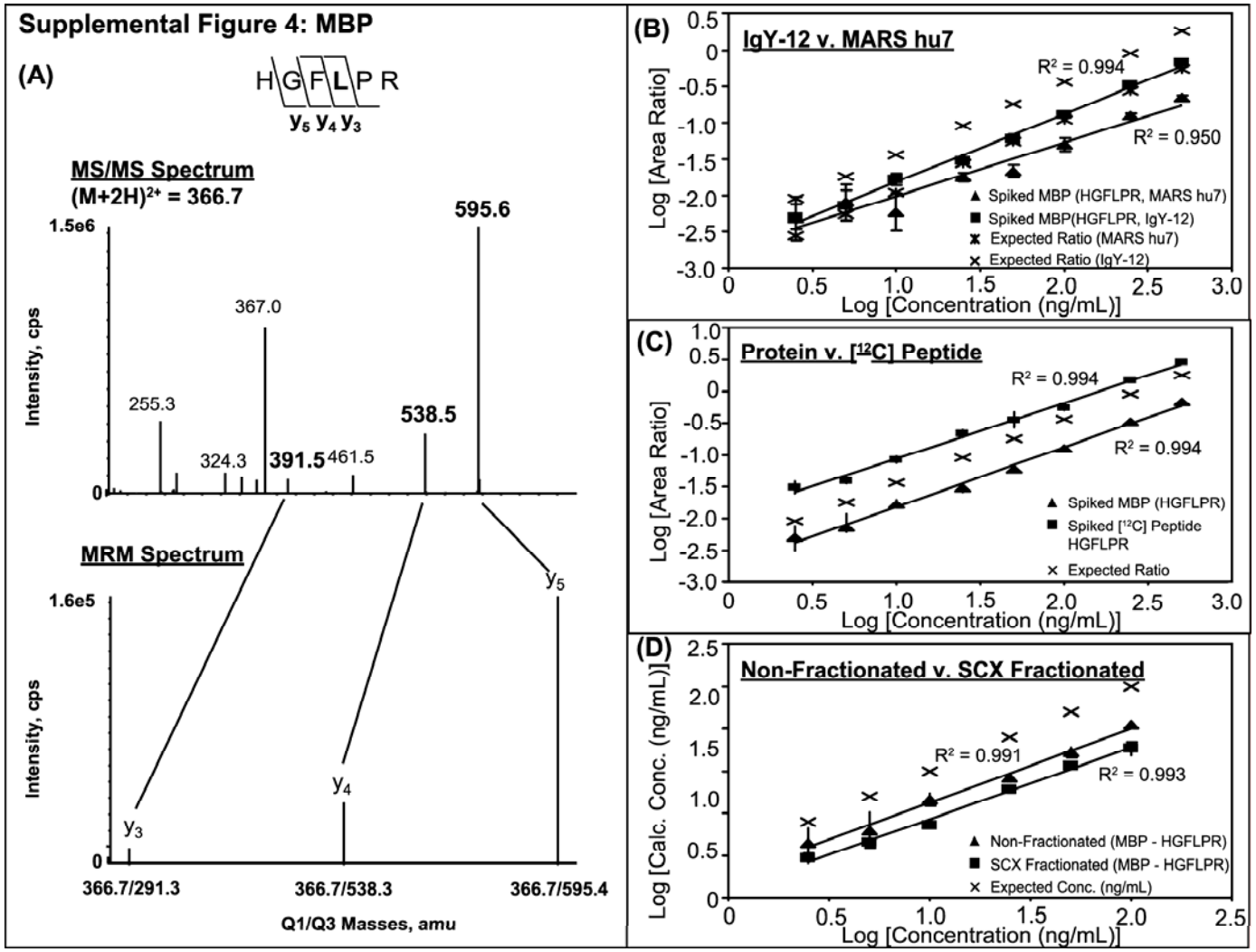
Supplemental Figure 1. **(A)** Full scan MS/MS (top) and optimized MRM transitions (bottom) of the [^{13}C] labeled peptide derived from aprotinin in buffer. Calibration curves obtained on the aprotinin peptide **(B)** in IgY-12 and MARS hu7 matrices; **(C)** with intact protein added prior to digestion or [^{12}C] synthetic peptide added to digested plasma; and **(D)** in non-fractionated and SCX fractionated plasma.



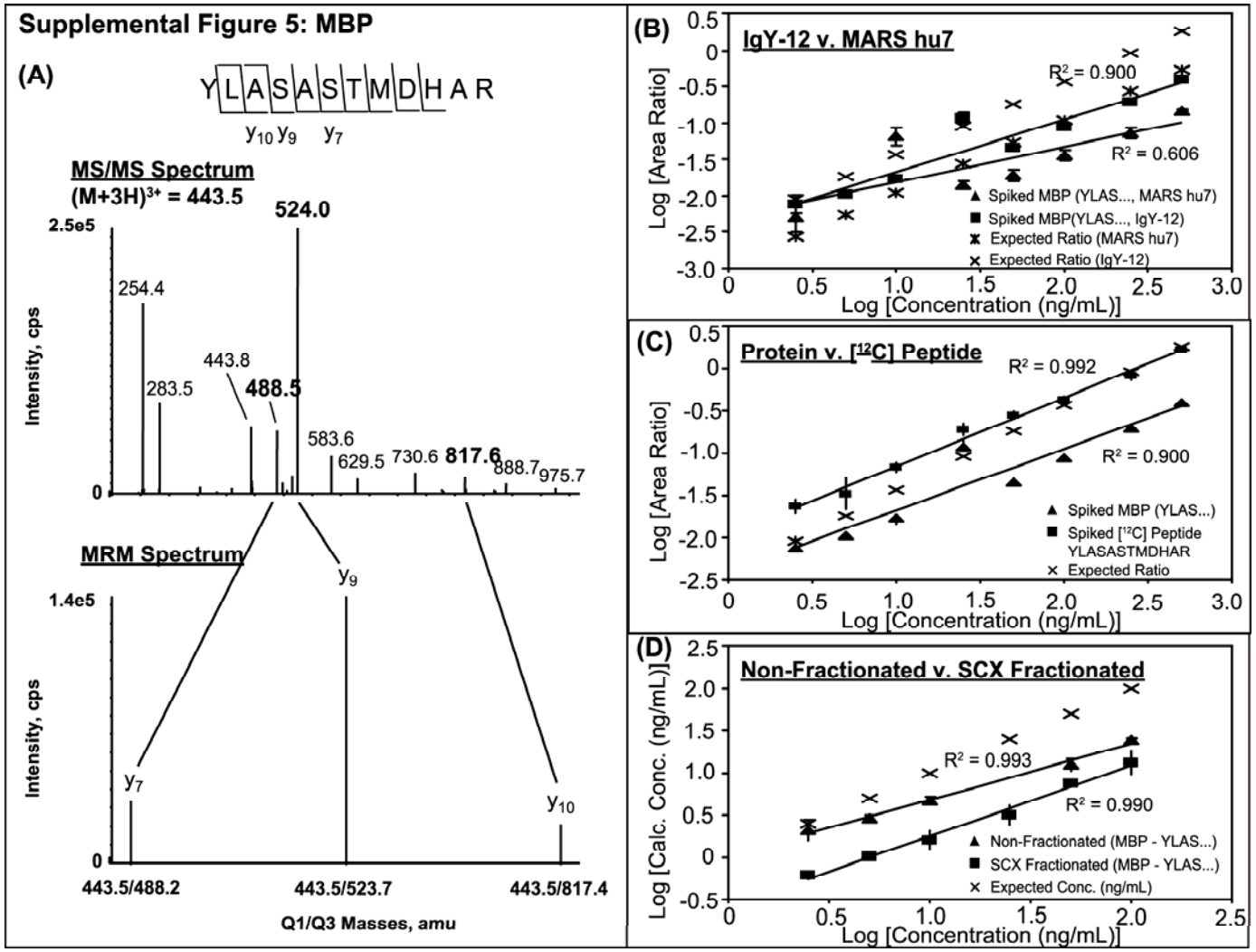
Supplemental Figure 2. **(A)** Full scan MS/MS (top) and optimized MRM transitions (bottom) of the [^{13}C] labeled peptide derived from leptin in buffer. Calibration curves obtained on the leptin peptide **(B)** in IgY-12 and MARS hu7 matrices; **(C)** with intact protein added prior to digestion or [^{12}C] synthetic peptide added to digested plasma; and **(D)** in non-fractionated and SCX fractionated plasma.



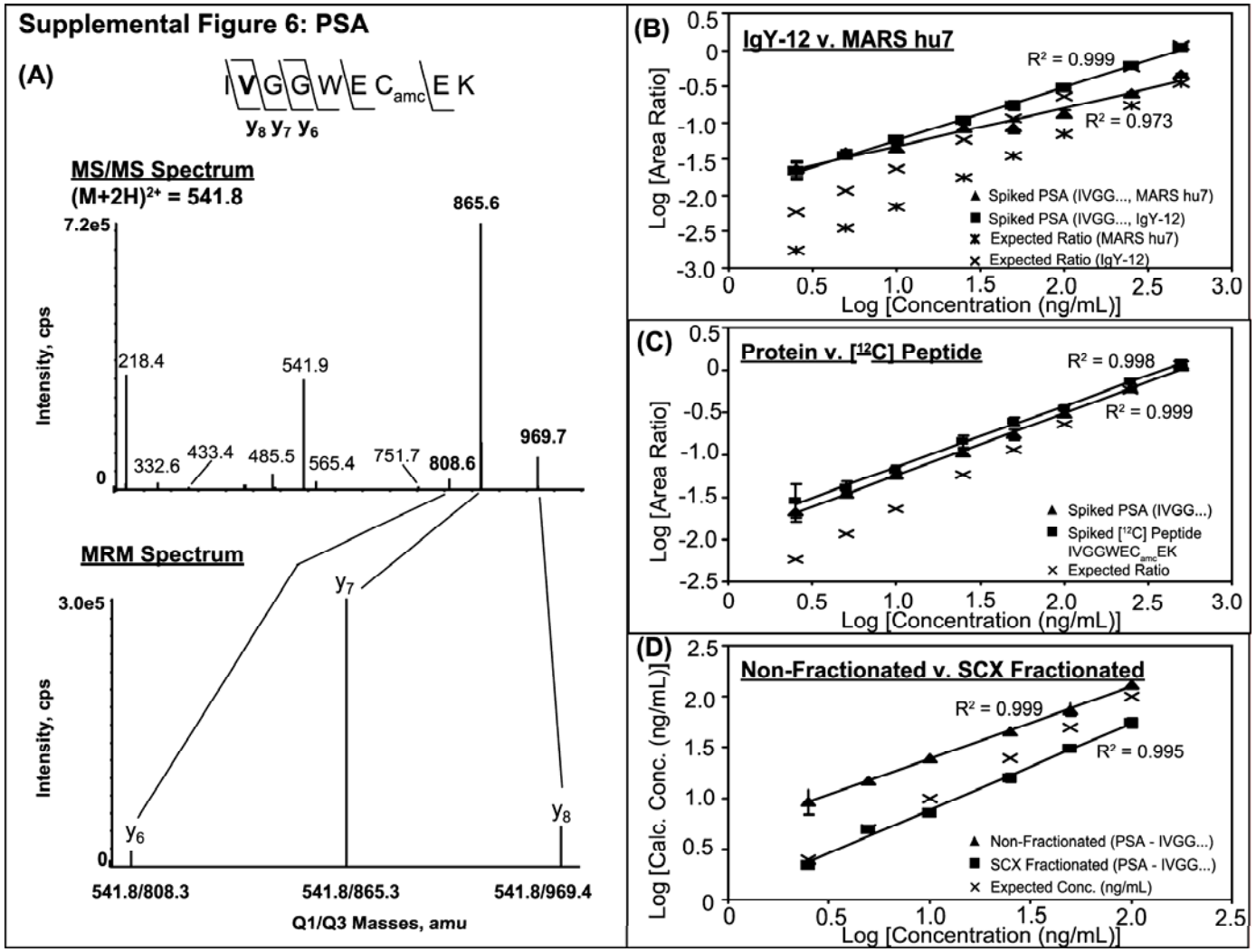
Supplemental Figure 3. **(A)** Full scan MS/MS (top) and optimized MRM transitions (bottom) of the [^{13}C] labeled peptide derived from myoglobin in buffer. Calibration curves obtained on the myoglobin peptide **(B)** in IgY-12 and MARS hu7 matrices; **(C)** with intact protein added prior to digestion or [^{12}C] synthetic peptide added to digested plasma; and **(D)** in non-fractionated and SCX fractionated plasma.



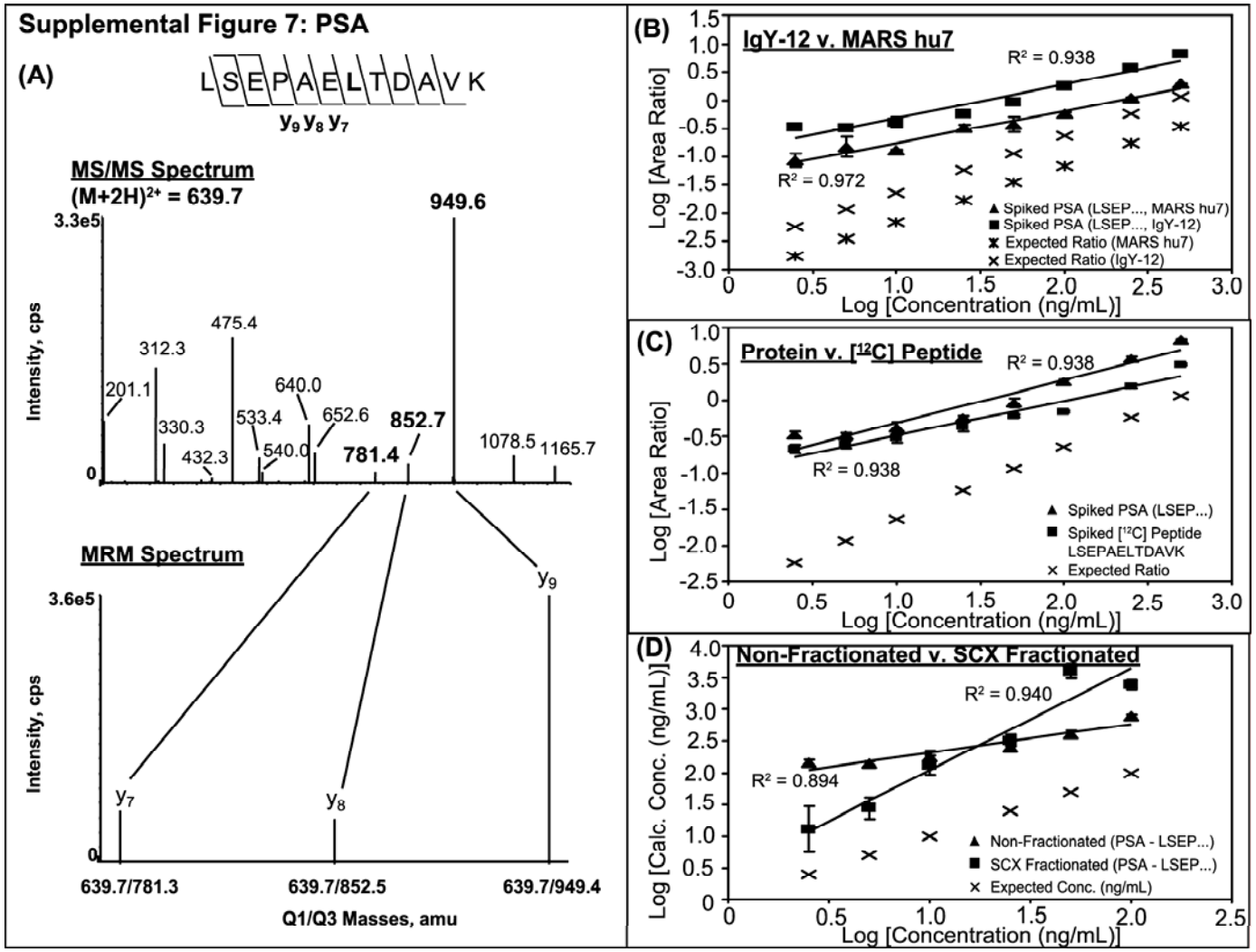
Supplemental Figure 4. **(A)** Full scan MS/MS (top) and optimized MRM transitions (bottom) of the [^{13}C] labeled HGFLPR peptide derived from MBP in buffer. Calibration curves obtained on the HGFLPR peptide **(B)** in IgY-12 and MARS hu7 matrices; **(C)** with intact protein added prior to digestion or [^{12}C] synthetic peptide added to digested plasma; and **(D)** in non-fractionated and SCX fractionated plasma.



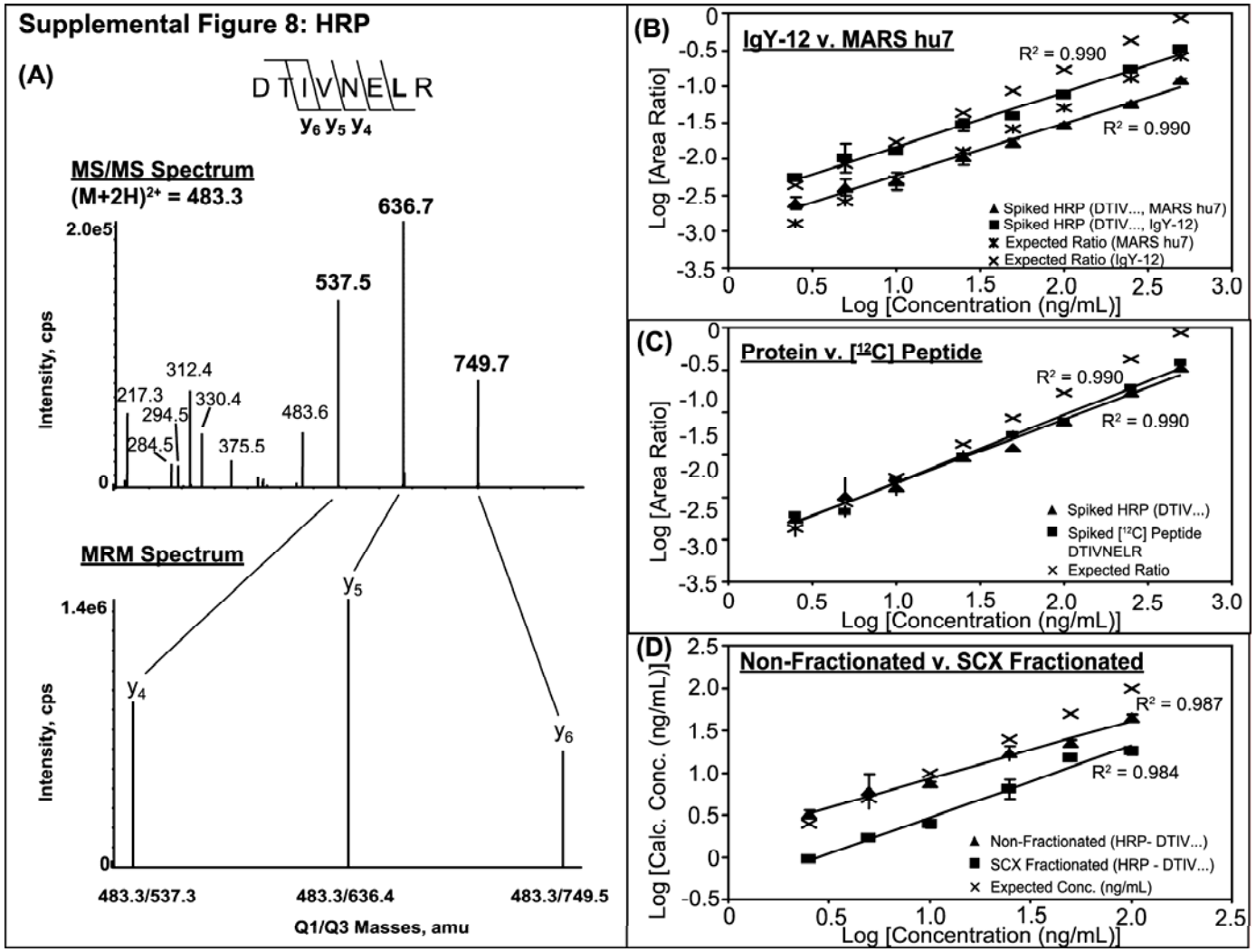
Supplemental Figure 5. **(A)** Full scan MS/MS (top) and optimized MRM transitions (bottom) of the [^{13}C] labeled YLASASTMDHAR peptide derived from MBP in buffer. Calibration curves obtained on the YLASASTMDHAR peptide **(B)** in IgY-12 and MARS hu7 matrices; **(C)** with intact protein added prior to digestion or [^{12}C] synthetic peptide added to digested plasma; and **(D)** in non-fractionated and SCX fractionated plasma.



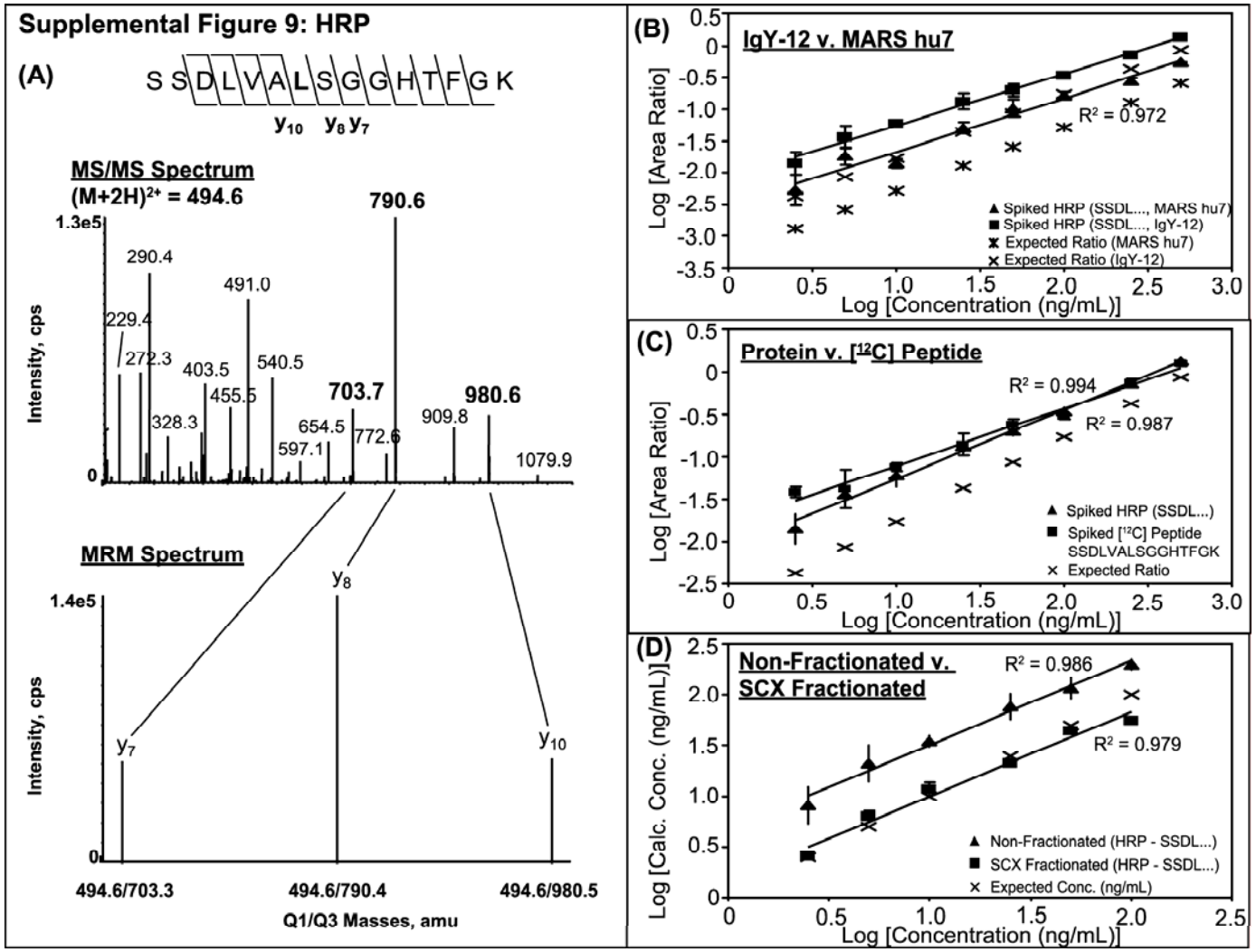
Supplemental Figure 6. **(A)** Full scan MS/MS (top) and optimized MRM transitions (bottom) of the [^{13}C] labeled IVGGWECamcEK peptide derived from PSA in buffer. Calibration curves obtained on the IVGGWECamcEK peptide **(B)** in IgY-12 and MARS hu7 matrices; **(C)** with intact protein added prior to digestion or [^{12}C] synthetic peptide added to digested plasma; and **(D)** in non-fractionated and SCX fractionated plasma.



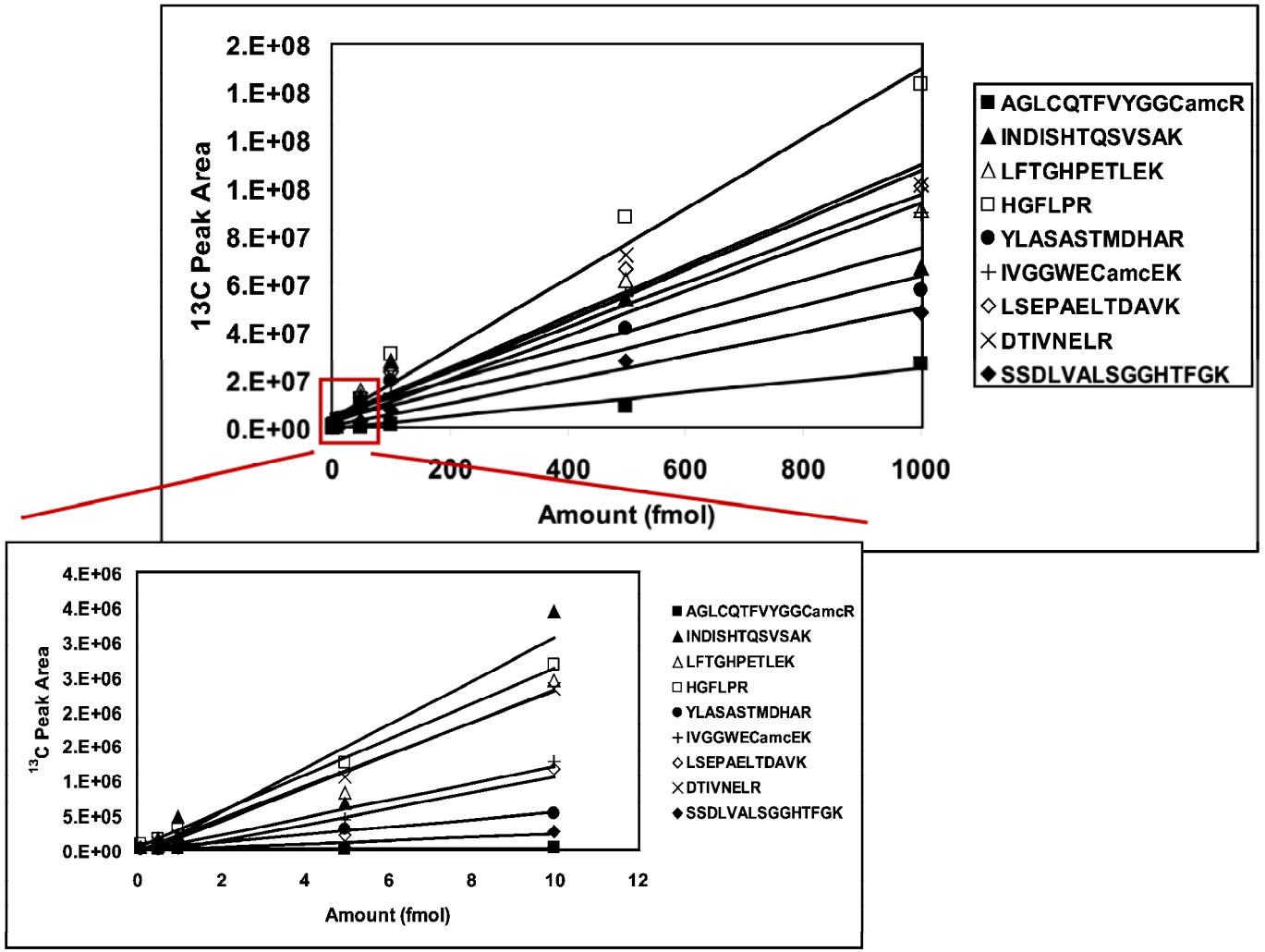
Supplemental Figure 7. **(A)** Full scan MS/MS (top) and optimized MRM transitions (bottom) of the [^{13}C] labeled LSEPAELTDAVK peptide derived from PSA in buffer. Calibration curves obtained on the LSEPAELTDAVK peptide **(B)** in IgY-12 and MARS hu7 matrices; **(C)** with intact protein added prior to digestion or [^{12}C] synthetic peptide added to digested plasma; and **(D)** in non-fractionated and SCX fractionated plasma.



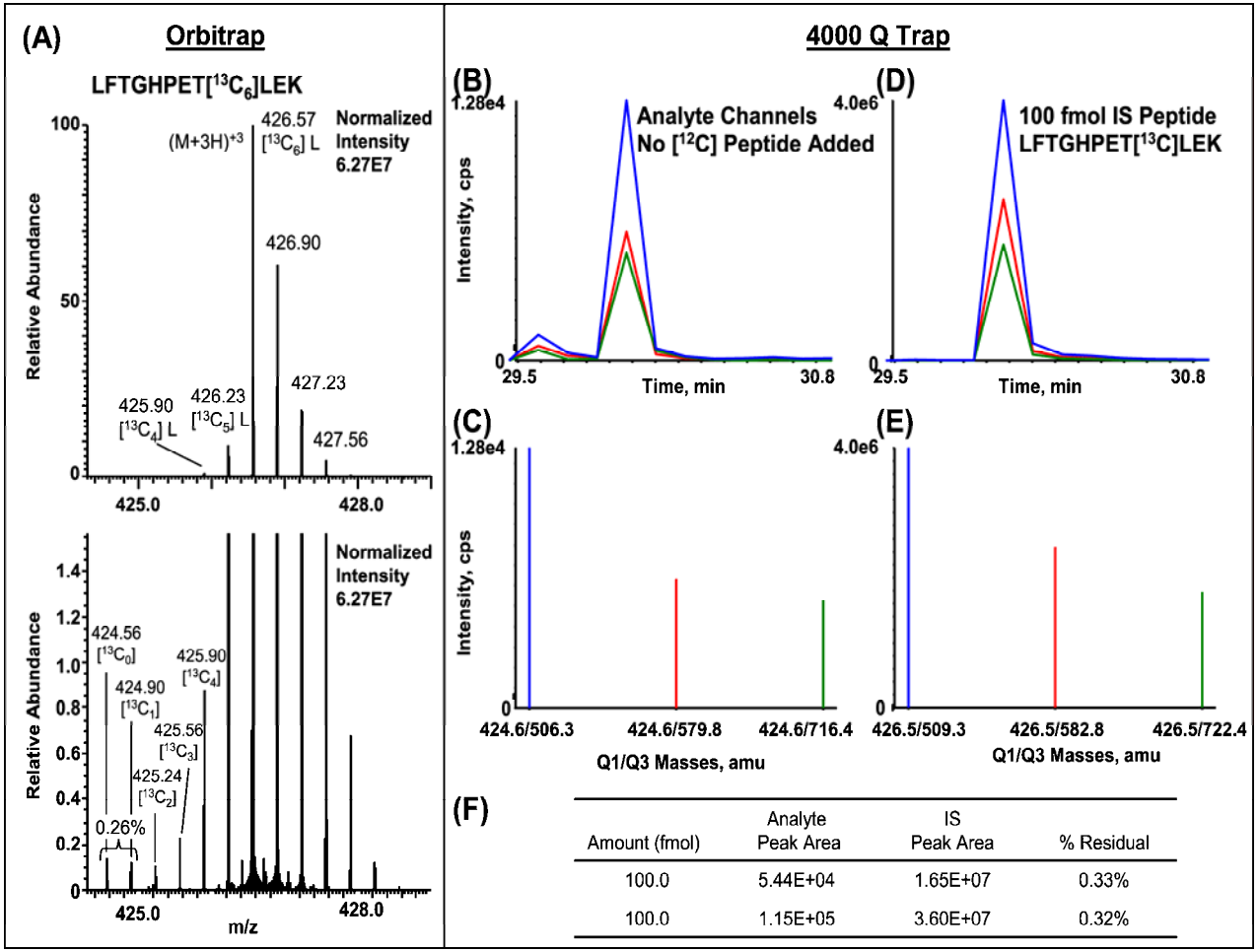
Supplemental Figure 8. **(A)** Full scan MS/MS (top) and optimized MRM transitions (bottom) of the [^{13}C] labeled DTIVNELR peptide derived from HRP in buffer. Calibration curves obtained on the DTIVNELR peptide **(B)** in IgY-12 and MARS hu7 matrices; **(C)** with intact protein added prior to digestion or [^{12}C] synthetic peptide added to digested plasma; and **(D)** in non-fractionated and SCX fractionated plasma.



Supplemental Figure 9. **(A)** Full scan MS/MS (top) and optimized MRM transitions (bottom) of the [^{13}C] labeled SSDLVALSGGHTFGK peptide derived from HRP in buffer. Calibration curves obtained on the SSDLVALSGGHTFGK peptide **(B)** in IgY-12 and MARS hu7 matrices; **(C)** with intact protein added prior to digestion or [^{12}C] synthetic peptide added to digested plasma; and **(D)** in non-fractionated and SCX fractionated plasma.

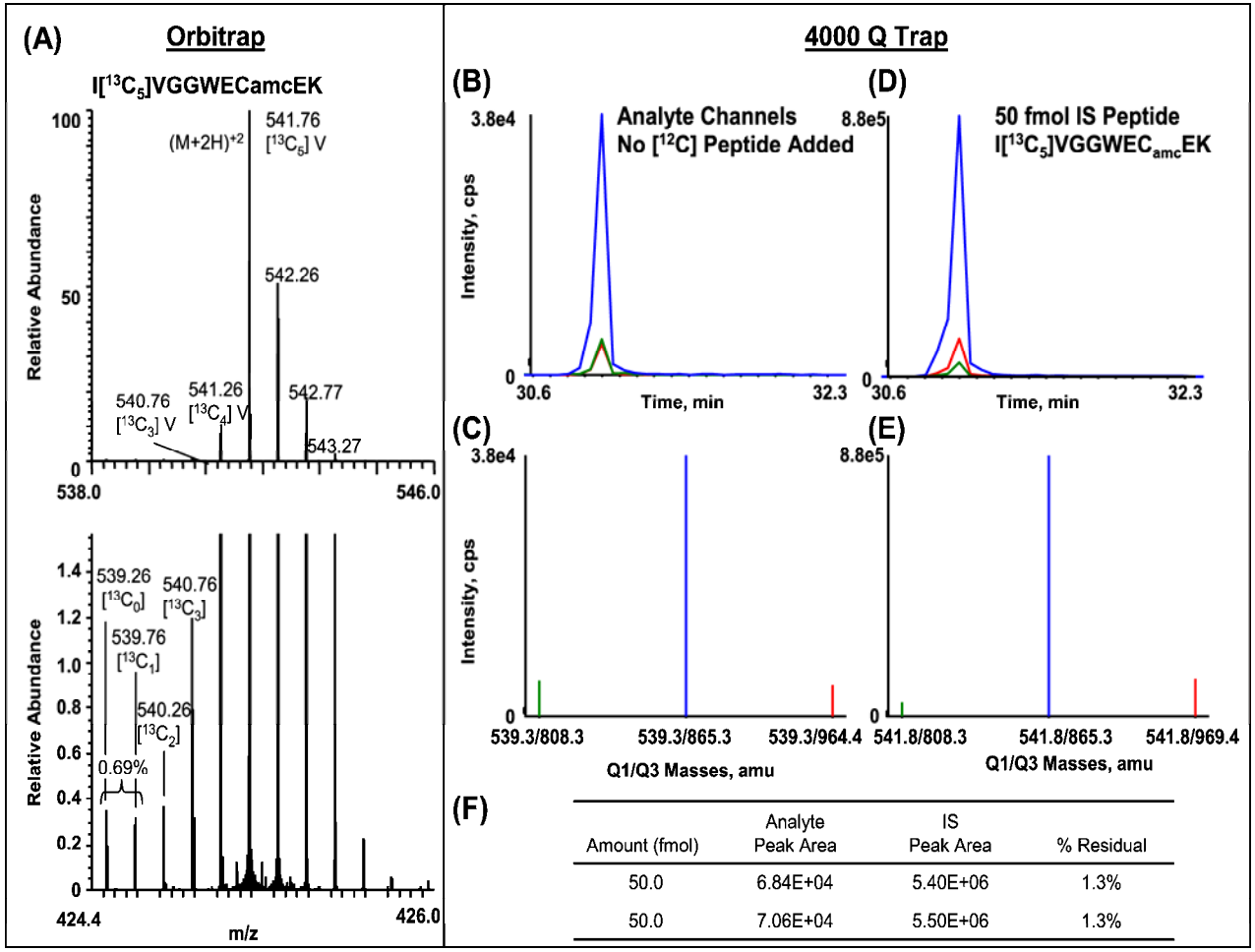


Supplemental Figure 10. Concentration curves for all nine [^{13}C] signature peptides in 0.1% formic acid over the range of 0.1 – 1000 fmol. Duplicate injections monitoring [^{12}C/^{13}C] transitions were performed to determine linear response for each peptide and percentage of isotopic impurity (Supplemental Figures 11 and 12). Variation in MS response for the same concentration of different peptides illustrates the need for multiple peptides per protein for a targeted MRM assay.



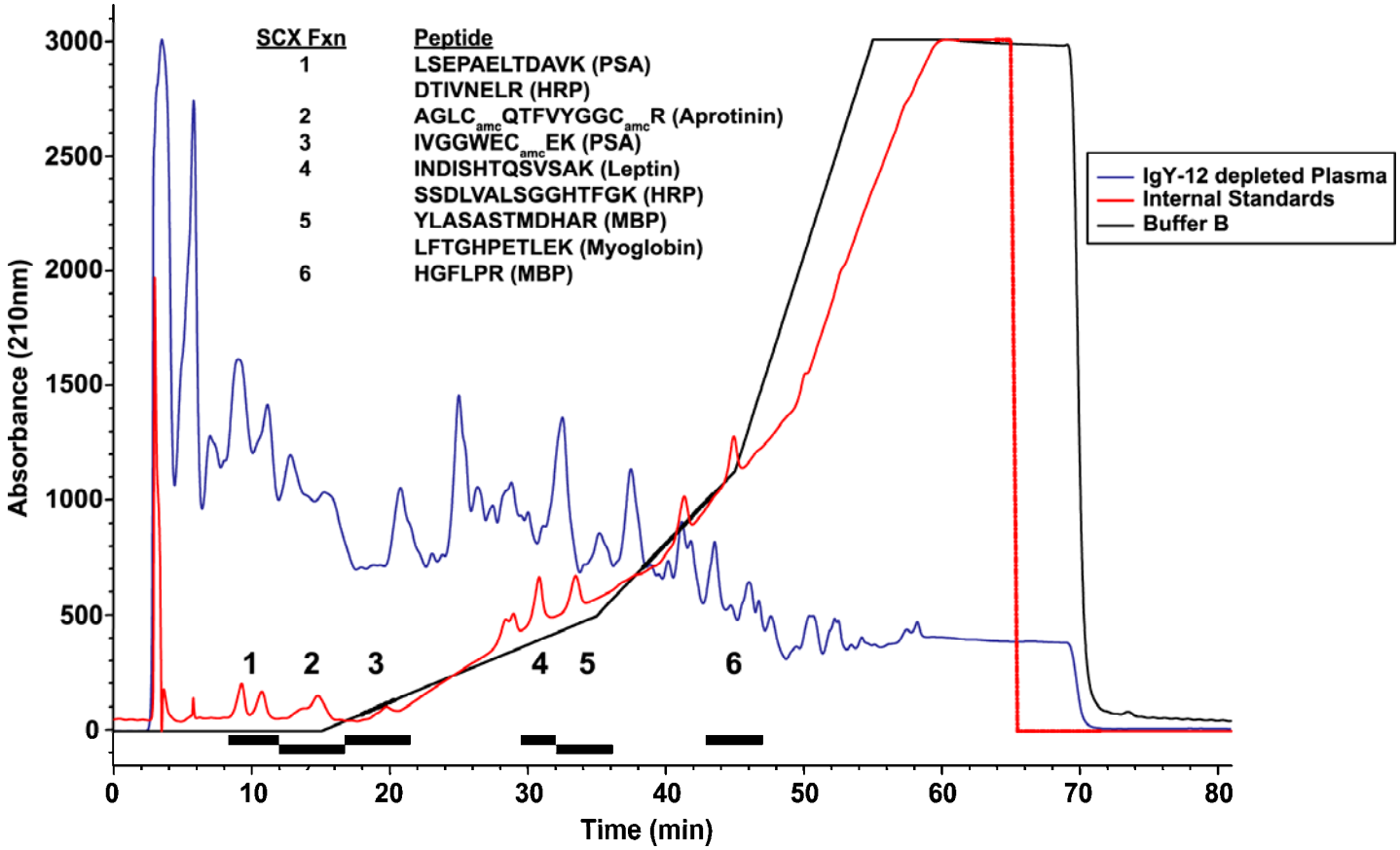
Amount (fmol)	Analyte Peak Area	IS Peak Area	% Residual
100.0	5.44E+04	1.65E+07	0.33%
100.0	1.15E+05	3.60E+07	0.32%

Supplemental Figure 11. **(A)** MS spectrum of [$^{13}C_6$] Leu containing peptide derived from myoglobin generated by infusion on the Orbitrap mass spectrometer at 60,000 resolution. The span of isotopic impurity (i.e. $^{13}C_0$ to $^{13}C_5$) of the heavy amino acid can be observed (bottom panel). Extracted ion chromatograms (**B, D**) and MRM spectra (**C, E**) of transitions monitored for the internal standard peptide derived from myoglobin in buffer and in the absence of added analyte. The amount of residual observed in the analyte channels was calculated from the area ratios and determined to be <1% (**F**).



Amount (fmol)	Analyte Peak Area	IS Peak Area	% Residual
50.0	6.84E+04	5.40E+06	1.3%
50.0	7.06E+04	5.50E+06	1.3%

Supplemental Figure 12. **(A)** MS spectrum of [$^{13}C_5$] Val containing peptide derived from PSA generated by infusion on the Orbitrap mass spectrometer at 60,000 resolution. The span of isotopic impurity (i.e. $^{13}C_0$ to $^{13}C_4$) of the heavy amino acid can be observed (bottom panel). Extracted ion chromatograms (**B, D**) and MRM spectra (**C, E**) of transitions monitored for the internal standard peptide derived from myoglobin in buffer and in the absence of added analyte. The amount of residual observed in the analyte channels was calculated from the area ratios and determined to be ca. 1% (**F**).



Supplemental Figure 13. SCX elution profiles of IgY-12 depleted plasma (blue trace; ca. 250 μg total protein) and the [^{13}C] internal standards (red trace). Six pools of SCX fractions from the separation of IgY-12 depleted plasma were generated for LC-MRM/MS based upon the elution profile of the internal standards. Location of the internal standards is indicated in the inset.

Supplemental Table 1. Percent recovery of target proteins added to plasma pre and post depletion.

Protein	Signature Peptide	Q1/Q3		Non-Fractionated			SCX Fractionated		
			Target ng/mL	250[a]	500[a]	5000[b]	2.5[c]	10[c]	50[c]
Leptin	INDISHTQSVSAK	467.2/643.8	% Recovery	100	100	100	100	100	100
Myo	LFTGHPETLEK	424.6/579.7	% Recovery	100	100	89.6	100	86.1	100
MBP	HGFLPR	363.7/589.3	% Recovery	ND[d]	ND[d]	ND[d]	ND[d]	ND[d]	ND[d]
PSA	IVGGWECEK	539.3/865.3	% Recovery	25.3	25.4	41.2	29.7	25.3	19.3
HRP	DTIVNELR	480.3/630.3	% Recovery	100	100	92.8	100	100	82.8

[a] Target proteins were added to plasma pre and post depletion at 50 and 500 ng/mL, and subsequently analyzed after digestion via LC-MRM/MS. % Recovery = Calculated concentration of proteins added pre-depletion (ng/mL) / calculated concentration of proteins added post depletion (ng/mL) x 100%

[b] Target proteins were added to plasma prior to depletion at 5000 ng/mL. LC-MRM/MS was performed on aliquots of digested plasma pre and post depletion. % Recovery = Calculated concentration of proteins in depleted, digested plasma (ng/mL) / calculated concentration of proteins in non-depleted, digested plasma (ng/mL) x 100%

[c] Target proteins were added to plasma pre and post depletion and the respective plasma digests were separated by SCX prior to LC-MRM/MS. % Recovery = Calculated concentration of proteins added pre-depletion and fractionated by SCX (ng/mL) / calculated concentration of proteins added post depletion and fractionated by SCX (ng/mL) x 100%

[d] MBP was not detected in any experiment..