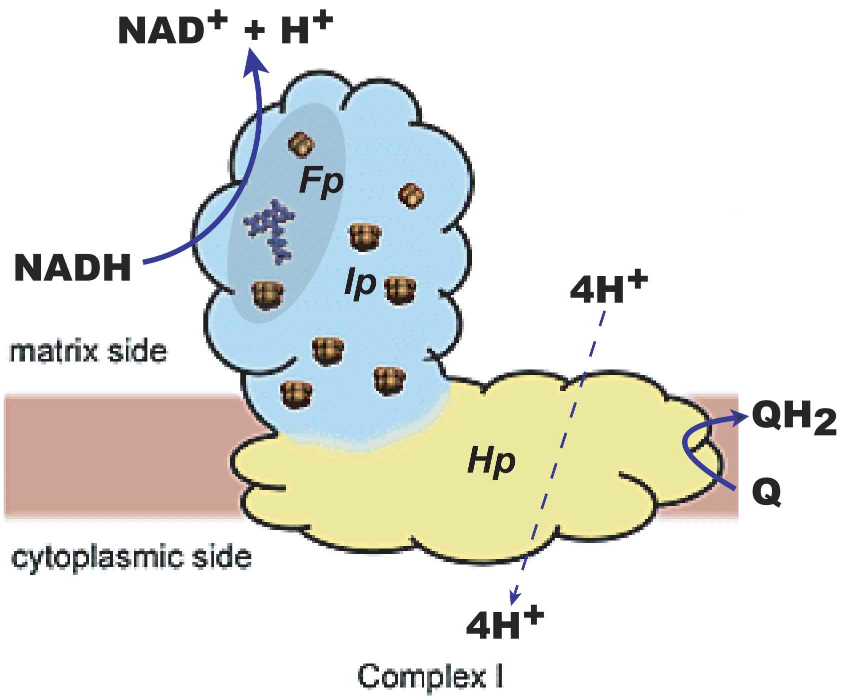
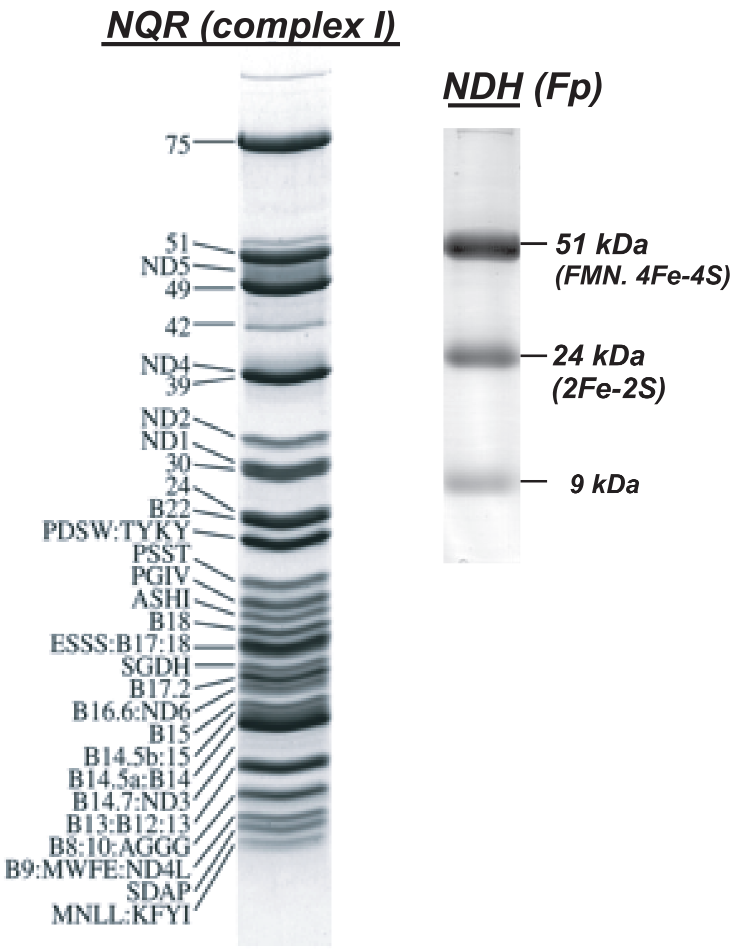
Supplemental Figure 1

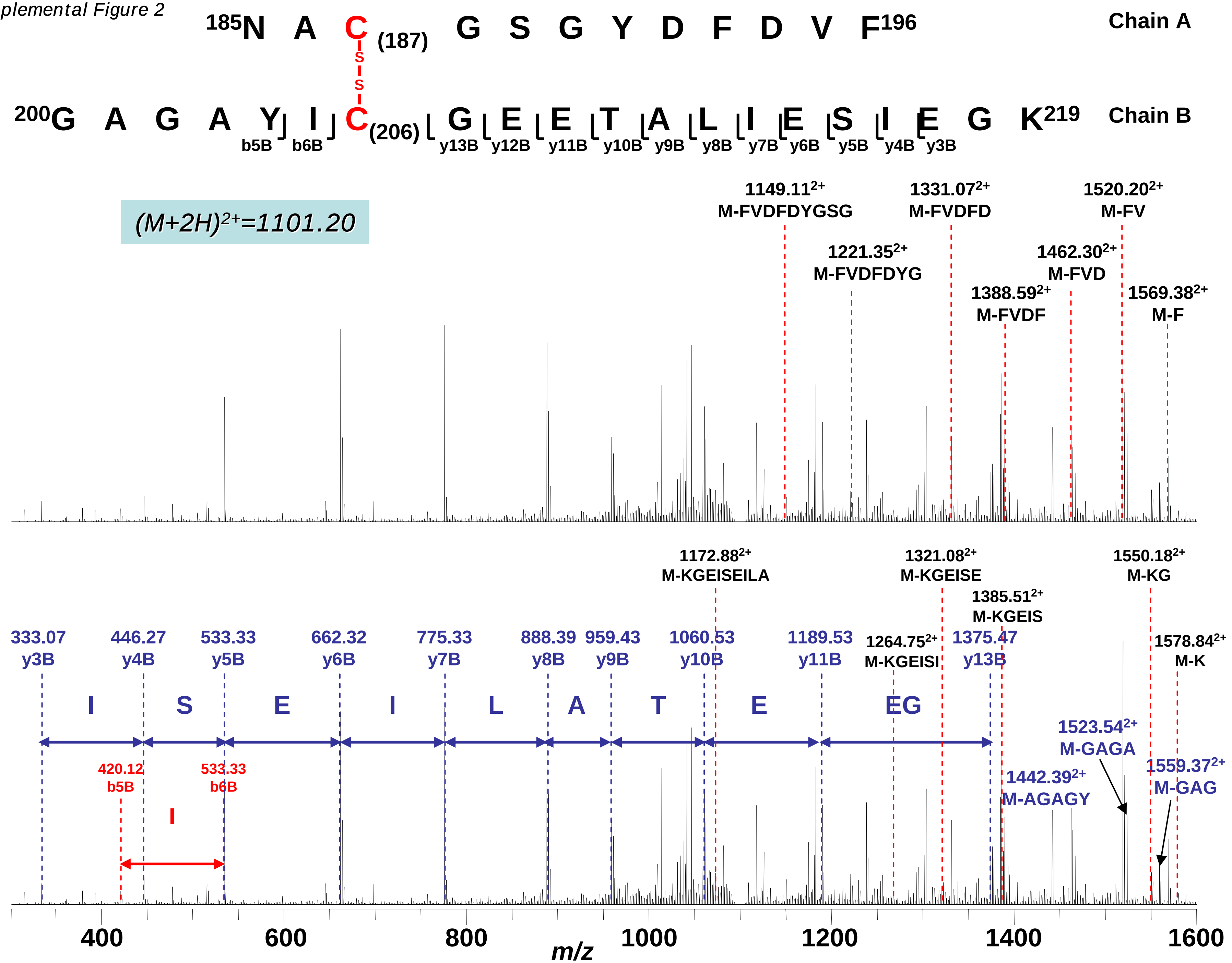


Schematic representation of structure and function of the respiratory complex I. Mammalian complex I has an L-shape structure based on EM analysis. The L-shape model contains two major segments, the peripheral domain and membrane domain. The enzyme complex catalyzes electron transfer from NADH to ubiquinone (Q) and proton translocation. The oval shadow indicates flavoprotein subcomplex (Fp). FMN and iron-sulfur clusters are drawn in dark blue and brown respectively.

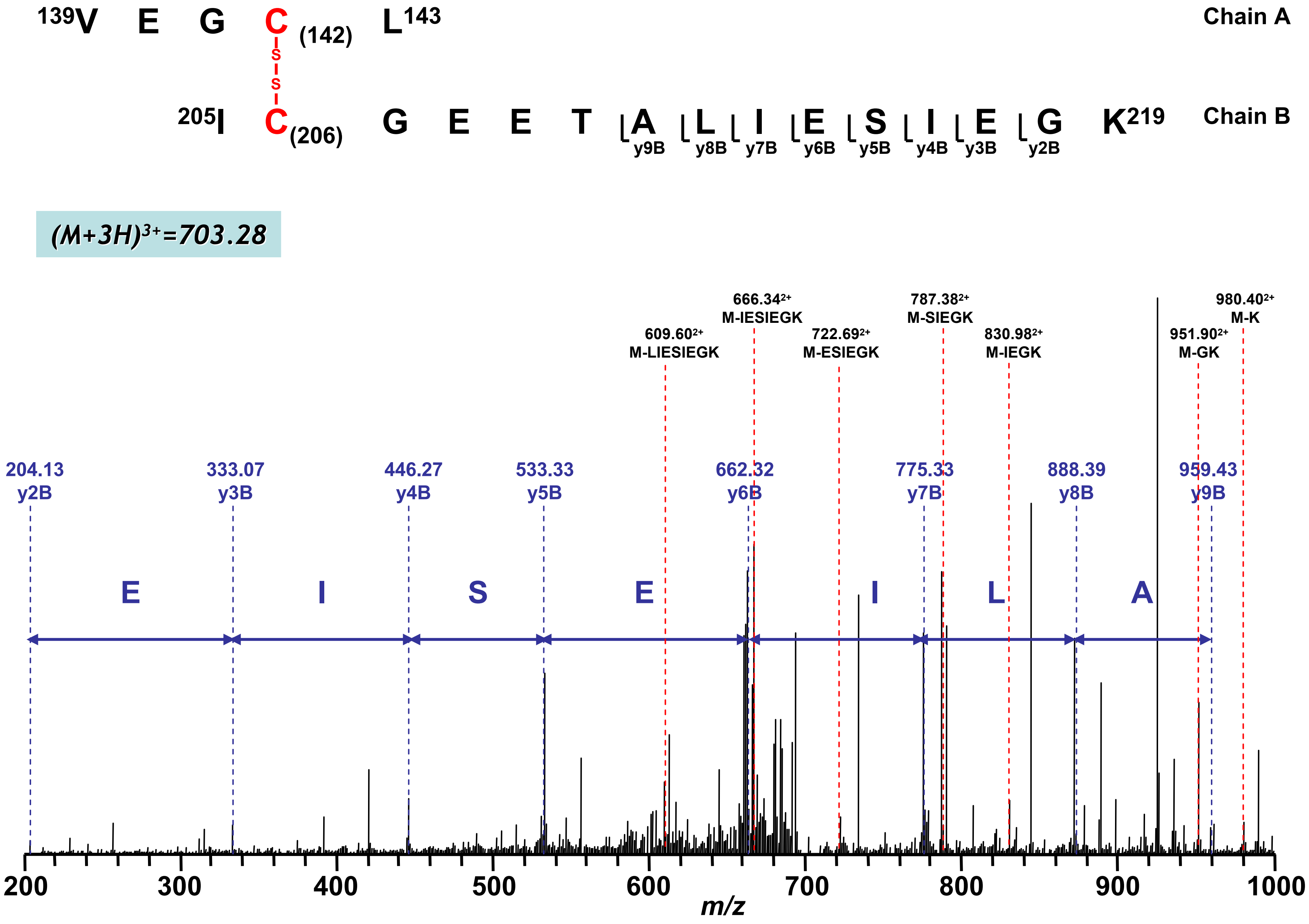


SDS-PAGE of intact bovine complex I (or NQR, ref. 2) hosting 45 subunits and Fp subcomplex (or NDH, ref. 15) hosting 3 subunit.
Fp subcomplex was extracted from intact complex I by 9% ethanol at 40 °C.

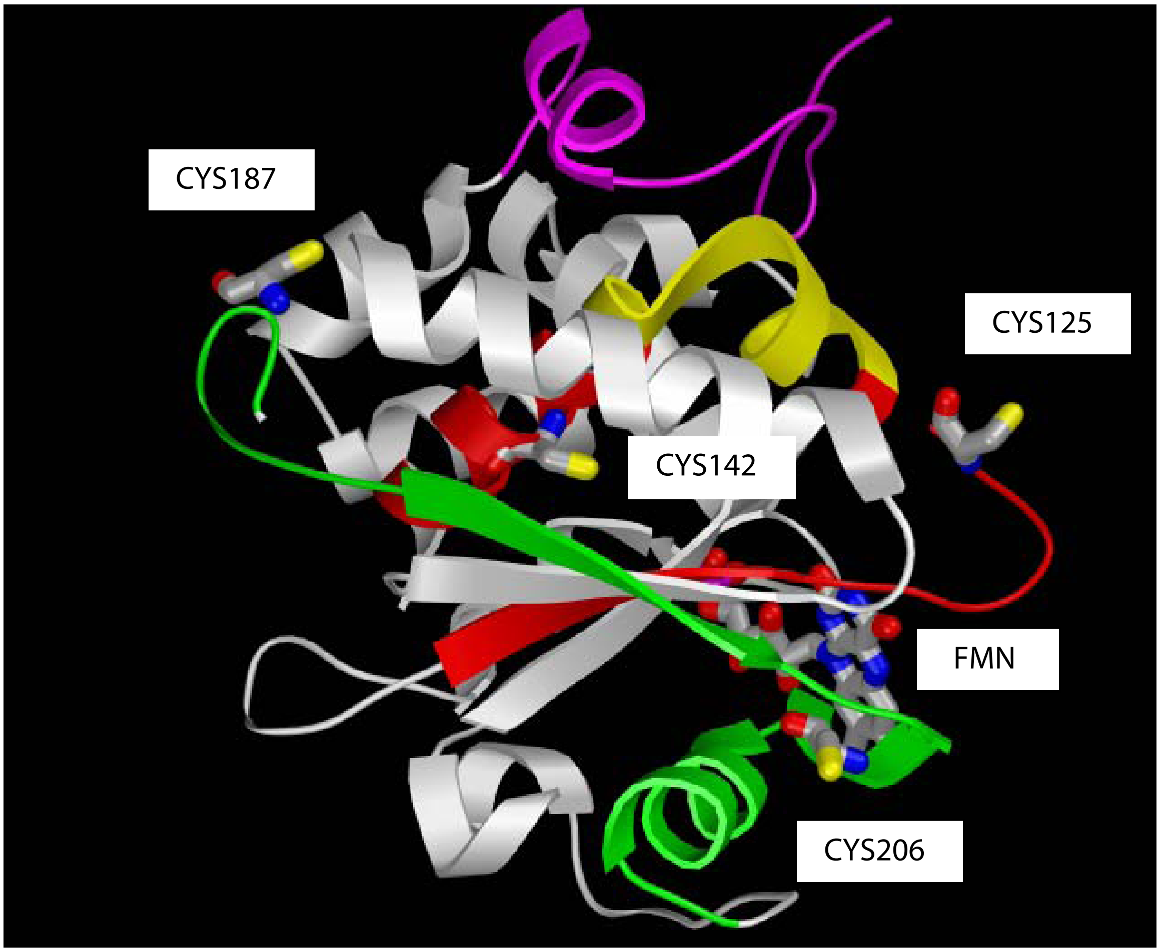
Supplemental Figure 2



Supplemental Figure 3



Supplemental Figure 4



***The ribbon representation of the FMN binding domain of Complex I**. C_{125}, C_{142}, C_{187}, C_{206} and FMN are shown in a ball-and-stick atomic model. Magenta ribbon: N-terminal (Glu38-Asp61); 1st β-strand red ribbon: Tyr112-Arg128; 1st α-helical red ribbon: Leu138-Arg147; 2nd β-strand green ribbon: Asn185-Arg199; 2nd α-helical green ribbon: Gly200-Lys219; yellow helix connecting 1st β-strand red ribbon and 1st α-helical red ribbon: ^{129}EIIRHDPHK^{137} (the kink is caused by Pro135).*

Supplemental Table 1 Details of the fragmentation ions for Peptide $_{112}$YLVVNADE GEPGTC^{125}KDR$_{128}$ (Chain A)/$_{138}$LVEGC^{142}LVGGR$_{147}$ (Chain B) (C_{125}-C_{142}). Observed *m/z* = 956.22^{3+}, theoretical *m/z* = 955.8005^{3+}.

Fragmentation Ion	Theoretical m/z	Observed m/z	Mass Error (Da)
b_{2A}	277.1547^{1+}	277.05^{1+}	-0.1047
b_{3A}	376.2231^{1+}	376.22^{1+}	-0.0031
b_{4A}	475.2915^{1+}	475.30^{1+}	0.0085
b_{5A}	589.3344^{1+}	589.28^{1+}	-0.0544
b_{6A}	660.3715^{1+}	660.41^{1+}	0.0385
b_{7A}	775.3985^{1+}	775.37^{1+}	-0.0285
y_{3B}	289.1619^{1+}	289.16^{1+}	-0.0019
y_{4B}	388.2303^{1+}	388.48^{1+}	0.2497
y_{5B}	501.3144^{1+}	501.22^{1+}	-0.0944
M-YLVVNADEGE	888.4482^{2+}	888.78^{2+}	0.6636
M-YLVVNADE	981.4802^{2+}	981.84^{2+}	0.7196
M-YLVVNAD	1046.0015^{2+}	1046.32^{2+}	0.637
M-YLVVNA	1103.5150^{2+}	1103.85^{2+}	0.67
M-YLVVN	1139.0335^{2+}	1139.25^{2+}	0.433
M-YLVV	1196.0550^{2+}	1196.36^{2+}	0.61
M-YLV	1245.5892^{2+}	1245.89^{2+}	0.6016
M-YL	1295.1234^{2+}	1295.44^{2+}	0.6332

Supplemental Table 2 Details of the fragmentation ions for Peptide $_{185}$NAC^{187}GSGYDFDVFVVR$_{199}$ (Chain A)/$_{200}$GAGAYIC^{206}GEETALIESIEGK$_{219}$ (Chain B) (C$_{187}$-C$_{206}$). Observed *m/z* = 1219.95^{3+}, theoretical *m/z* = 1219.5676^{3+}.

Fragmentation Ion	Theoretical m/z	Observed m/z	Mass Error (Da)
y_{3A}	373.2558^{1+}	373.38^{1+}	0.1242
y_{4A}	520.3242^{1+}	520.45^{1+}	0.1258
y_{5A}	619.3926^{1+}	619.50^{1+}	0.1074
y_{6A}	734.4196^{1+}	734.54^{1+}	0.1204
y_{7A}	881.4880^{1+}	881.58^{1+}	0.092
y_{8A}	996.5149^{1+}	996.54^{1+}	0.0251
y_{9A}	1159.5782^{1+}	1159.55^{1+}	-0.0282
y_{11A}	1303.6317^{1+}	1303.67^{1+}	0.0383
y_{12A}	1360.6532^{1+}	1360.68^{1+}	0.0268
b_{5B}	420.1878^{1+}	420.38^{1+}	0.1922
B_{6B}	533.2718^{1+}	533.42^{1+}	0.1482
y_{4B}	446.2609^{1+}	446.48^{1+}	0.2191
y_{5B}	533.2930^{1+}	533.42^{1+}	0.127
y_{6B}	662.3355^{1+}	662.43^{1+}	0.0945
y_{7B}	775.4196^{1+}	775.49^{1+}	0.0704
y_{8B}	888.5037^{1+}	888.48^{1+}	-0.0237
y_{9B}	959.5408^{1+}	959.55^{1+}	0.0092
y_{10B}	1060.5885^{1+}	1060.60^{1+}	0.0115
y_{11B}	1189.6311^{1+}	1189.62^{1+}	-0.0111
y_{12B}	1318.6737^{1+}	1318.62^{1+}	-0.0537
y_{13B}	1375.6951^{1+}	1375.66^{1+}	-0.0351
M-GSGYDFDVFVVR	2297.0424^{1+}	1149.27^{2+}	0.4898
M-SGYDFDVFVVR	2354.0639^{1+}	1177.56^{2+}	0.0483
M-DFDVFVVR	2661.1807^{1+}	1331.54^{2+}	0.8915
M-FDVFVVR	2776.2077^{1+}	1388.88^{2+}	0.5445
M-DVFVVR	2923.2761^{1+}	1462.38^{2+}	0.4761
M-VFVVR	3038.3030^{1+}	1519.98^{2+}	0.6492
M-FVVR	3137.3714^{1+}	1569.43^{2+}	0.4808
M-VVR	3284.4398^{1+}	1643.02^{2+}	0.5924
M-VR	3383.5083^{1+}	1692.55^{2+}	0.5839
M-R	3482.5767^{1+}	1742.29^{2+}	0.9955
M-GEETALIESIEGK	2282.0005^{1+}	1141.81^{2+}	0.6117
M-EETALIESIEGK	2339.0220^{1+}	1170.28^{2+}	0.5380
M-TALIESIEGK	2597.1071^{1+}	1299.24^{2+}	0.3252
M-ALIESIEGK	2698.1548^{1+}	1350.08^{2+}	0.9374
M-LIESIEGK	2769.1919^{1+}	1385.30^{2+}	0.4003
M-IESIEGK	2882.2760^{1+}	1441.91^{2+}	0.5362
M-ESIEGK	2995.3601^{1+}	1498.57^{2+}	0.7721
M-SIEGK	3124.4027^{1+}	1563.40^{2+}	1.3895
M-EGK	3324.5188^{1+}	1663.42^{2+}	1.3134
M-GK	3453.5613^{1+}	1727.73^{2+}	0.8909

Supplemental Table 3 Details of the fragmentation ions for Peptide $_{138}$LVEGC^{142}LVGG R$_{147}$ (Chain A) (Chain A)/$_{200}$GAGAYIC^{206}GEETALIESIEGK$_{219}$ (Chain B) (C$_{142}$-C$_{206}$). Observed *m/z* = 1004.50^{3+}, theoretical *m/z* = 1004.1669^{3+}.

Fragmentation Ion	Theoretical m/z	Observed m/z	Mass Error (Da)
y_{3A}	289.1619^{1+}	289.16^{1+}	-0.0019
y_{4A}	388.2303^{1+}	388.23^{1+}	-0.0003
y_{5A}	501.3144^{1+}	501.42^{1+}	0.1056
b_{5B}	420.1878^{1+}	420.38^{1+}	0.1922
B_{6B}	533.2718^{1+}	533.34^{1+}	0.0682
y_{3B}	333.1769^{1+}	333.28^{1+}	0.1031
y_{4B}	446.2609^{1+}	446.38^{1+}	0.1191
y_{5B}	533.2930^{1+}	533.34^{1+}	0.047
y_{6B}	662.3355^{1+}	662.34^{1+}	0.0045
y_{7B}	775.4196^{1+}	775.45^{1+}	0.0304
y_{8B}	888.5037^{1+}	888.53^{1+}	0.0263
y_{9B}	959.5408^{1+}	959.53^{1+}	-0.0108
y_{10B}	1060.5885^{1+}	1060.58^{1+}	-0.0085
y_{11B}	1189.6311^{1+}	1189.61^{1+}	-0.0211
y_{12B}	1318.6737^{1+}	1318.71^{1+}	0.0363
y_{13B}	1375.6951^{1+}	1375.64^{1+}	-0.0551
M-EETALIESIEGK	1692.8196^{1+}	847.12^{2+}	0.4126
M-ETALIESIEGK	1821.8622^{1+}	911.68^{2+}	0.49
M-TALIESIEGK	1950.9048^{1+}	976.21^{2+}	0.5074
M-IESIEGK	2236.0737^{1+}	1118.80^{2+}	0.5185
M-ESIEGK	2349.1577^{1+}	1175.38^{2+}	0.5945
M-SIEGK	2478.2003^{1+}	1239.89^{2+}	0.5719
M-EGK	2678.3164^{1+}	1339.84^{2+}	0.3558
M-GK	2807.3590^{1+}	1404.41^{2+}	0.4532
M-GAGAYI	2478.2215^{1+}	1239.89^{2+}	0.5507
M-GAGAY	2565.2324^{1+}	1296.43^{2+}	0.5467