**Table S1.** General steps in the downstream processing of split-virion inactivated influenza vaccines (IIVs).

| Step | Description |
|---|---|
| VCAF collection | Allantoic fluid is collected from infected chicken embryos after secondary incubation. |
| Virus inactivation | VCAF is inactivated with virus-inactivation agent (formalin or BPL). |
| Clarification | VCAF is subject to centrifugation, microfiltration, and ultrafiltration (with a cut-off threshold of at least 300 kDa) for cell debris removal. |
| Concentration of virions and dilution of viral concentrate | Clarified VCAF is centrifuged through a sucrose gradient, and the virus-enriched fraction is selected. The resulting viral concentrate is diluted with saline buffer to achieve the required concentration of the total protein. |
| Virion cleavage* | VCAF is incubated with detergent (octyl glycoside, Triton X 100, etc.) that destroys the virion lipid membrane and transfers virion coat proteins (HA, NA, M2, NP) into the solution [21]. |
| Ultracentrifugation and ultrafiltration* | After treatment with the detergent, VCAF is subject to ultracentrifugation for virion-core sedimentation and subsequent ultrafiltration through a 100 kDa filter[21]. |
| Sterilizing filtration and MPH blending | MPH is produced as a result of the sterilizing filtration of the VCAF. MPHs are blended to produce triple and quadruple vaccines. |

Notes: VCAF, virus-containing allantoic fluid; CVV, candidate vaccine virus; BPL, beta-propiolactone; VCF, virus-containing fluid; MPH, monovalent pooled harvest; HA, hemagglutinin; NA, neuraminidase; M2, matrix protein; NP, nucleoprotein; *, steps ensuring changes in the ratio of virion proteins in split vaccines compared with native virion. Virion treatment with detergent and subsequent ultracentrifugation and ultrafiltration made it possible to split the virion into core structures and lipid-membrane proteins by mass. After virion cleavage, heavy core parts of virions consisting of viral RNA and internal proteins (M1, NP, PA, PB1, PB2) were settled during ultracentrifugation and retained by ultrafiltration through 100 kDa filter. Lightweight extrinsic virion proteins (HA, NA, and M2) and/or their aggregates remained in the supernatant volume after ultracentrifugation and were passed through a 100 kDa filter.

**Table S2.** Composition of LC–MS quality control mix.

| Control protein – UniProt accession number | Corresponding SIL peptide* | SIL peptide m/z (2+) | Native peptide m/z (2+) |
|---|---|---|---|
| Human apolipoprotein A1 (APOA)—P02647 | LLDNWDSVTSTFS**K** | 810.9034 | 806.8963 |
| Human apolipoprotein B (APOB)—P04114 | TEVIPPLIEN**R** | 645.8682 | 640.8641 |
| Human fibrinogen beta chain (FIBB)—P02675 | AHYGGFTVQNEAN**K** | 515.2508 | 512.5794 |
| Human fibrinogen gamma chain (FIBG) – P02679 | YEASILTHDSSI**R** | 751.3797 | 746.3755 |

Note: Bold, amino acids with incorporated stable isotopes R($^{13}C_6$, $^{15}N_4$), K($^{13}C_6$, $^{15}N_2$).

SIL, stable isotope labeled.

**Table S3.**
The densitometry results of the protein bands in the gel image for the vaccine preparations without a deglycosylation step. The bands numbering corresponds to that in the gel image in Figure S1A.

| Band | Spot ID | X Coordinates | Y Coordinate | MW, kDa | Intensity | Area | Vol | %Vol |
|---|---|---|---|---|---|---|---|---|
| 1-1 | 1 | 713 | 665 | 79 | 0.508673 | 7.74538 | 0.161586 | 0.361814 |
| 1-2 | 8 | 713 | 818 | 62 | 0.161054 | 7.30632 | 0.374341 | 0.838202 |
| 1-3 | 2 | 704 | 933 | 53 | 0.530191 | 6.79916 | 1.73982 | 3.8957 |
| 1-4 | 3 | 699 | 955 | 51 | 0.508229 | 6.71135 | 1.16255 | 2.60311 |
| 1-5 | 4 | 681 | 1 487 | 27 | 0.134433 | 11.6073 | 0.39286 | 0.87967 |
| 1-6 | 5 | 661 | 1 671 | 20 | 0.245019 | 13.5123 | 0.932379 | 2.08773 |
| 1-7 | 33 | 635 | 2 039 | 10 | 0.14009 | 10.4335 | 0.155381 | 0.34792 |
| 2-1 | 39 | 964 | 374 | 292 | 0.0554593 | 5.97122 | 0.119288 | 0.138327 |
| 2-2 | 9 | 957 | 678 | 75 | 0.501352 | 9.5303 | 0.315507 | 0.706465 |
| 2-3 | 10 | 937 | 828 | 61 | 0.271418 | 8.97654 | 0.638307 | 1.42926 |
| 2-4 | 12 | 947 | 915 | 54 | 0.91286 | 6.58232 | 1.88483 | 4.2204 |
| 2-5 | 13 | 939 | 948 | 51 | 0.786524 | 6.91565 | 2.38234 | 5.3344 |
| 2-6 | 14 | 940 | 982 | 49 | 0.443453 | 8.04824 | 1.39464 | 3.1228 |
| 2-7 | 15 | 933 | 1 021 | 47 | 0.17503 | 7.30453 | 0.260164 | 0.582544 |
| 2-8 | 16 | 911 | 1 502 | 27 | 0.321997 | 16.2721 | 1.74641 | 3.91047 |
| 2-9 | 17 | 914 | 1 685 | 19 | 0.279737 | 13.4335 | 1.67017 | 3.73974 |
| 2-10 | 34 | 917 | 2 041 | 10 | 0.0740936 | 16.1037 | 0.356249 | 0.797692 |
| 3-1 | 40 | 1 167 | 380 | 281 | 0.663071 | 7.54646 | 3.25951 | 3.77973 |
| 3-2 | 18 | 1 154 | 949 | 51 | 0.390433 | 10.8744 | 2.97584 | 6.66332 |
| 3-3 | 19 | 1 152 | 1 691 | 19 | 0.216291 | 14.6252 | 1.0492 | 2.34931 |
| 3-4 | 35 | 1 161 | 2 039 | 10 | 0.167154 | 15.6521 | 1.38876 | 3.10962 |
| 4-1 | 41 | 1 392 | 383 | 275 | 0.642773 | 8.56974 | 3.70634 | 4.29788 |
| 4-2 | 20 | 1 396 | 848 | 59 | 0.765893 | 9.99624 | 4.02262 | 9.0072 |
| 4-3 | 21 | 1 386 | 949 | 51 | 0.664513 | 10.2041 | 5.13104 | 11.4891 |
| 4-4 | 22 | 1 400 | 1 511 | 27 | 0.299258 | 11.5303 | 1.66358 | 3.72498 |
| 4-5 | 23 | 1 382 | 1 698 | 19 | 0.344402 | 12.7112 | 3.52621 | 7.89568 |
| 4-6 | 36 | 1 394 | 2 048 | 10 | 0.201317 | 15.2506 | 1.69307 | 3.79101 |
| 5-1 | 42 | 1 610 | 462 | 165 | 3.51568 | 14.7184 | 33.5009 | 38.8476 |
| 5-2 | 24 | 1 615 | 934 | 52 | 0.159723 | 9.28837 | 0.623188 | 1.3954 |
| 5-3 | 25 | 1 622 | 1 492 | 27 | 0.0855182 | 10.2937 | 0.217365 | 0.486711 |
| 5-4 | 26 | 1 622 | 1 678 | 20 | 0.145525 | 9.69875 | 0.301124 | 0.67426 |
| 5-5 | 37 | 1 641 | 2 069 | 10 | 0.198766 | 12.0768 | 0.261822 | 0.586255 |
| 6-1 | 43 | 1 813 | 384 | 274 | 0.989061 | 9.26148 | 0.990554 | 1.14865 |
| 6-2 | 27 | 1 821 | 490 | 144 | 0.661851 | 9.70771 | 0.538223 | 1.20516 |
| 6-3 | 28 | 1 820 | 654 | 82 | 0.219508 | 10.9156 | 0.83594 | 1.87179 |
| 6-4 | 29 | 1 841 | 814 | 62 | 0.220395 | 8.67368 | 0.977296 | 2.1883 |
| 6-5 | 30 | 1 848 | 929 | 53 | 0.524201 | 11.4693 | 2.88792 | 6.46646 |
| 6-6 | 31 | 1 845 | 1 490 | 27 | 0.249789 | 9.40664 | 0.329864 | 0.738613 |
| 6-7 | 32 | 1 865 | 1 681 | 20 | 0.181463 | 9.78119 | 0.515525 | 1.15433 |
| 6-8 | 38 | 1 885 | 2 063 | 10 | 0.145303 | 9.95681 | 0.153884 | 0.344568 |

**Table S4.** The densitometry results of the protein bands in the gel image with preliminary deglycosylation step. The bands numbering corresponds to that in the gel image in Figure S1B.

| Band | Spot ID | X Coordinate | Y Coordinate | MW, kDa | Intensity | Area | Vol | %Vol |
|---|---|---|---|---|---|---|---|---|
| 1-1 | 1 | 676 | 622 | 64 | 0.0947245 | 8.78658 | 0.305122 | 0.553283 |
| 1-2 | 2 | 674 | 659 | 60 | 0.0703224 | 6.49092 | 0.13596 | 0.246538 |
| 1-3 | 3 | 666 | 688 | 58 | 0.0907314 | 6.8135 | 0.218948 | 0.397023 |
| 1-4 | 4 | 659 | 719 | 55 | 0.169705 | 6.95687 | 0.439735 | 0.79738 |
| 1-5 | 5 | 672 | 743 | 53 | 0.0743155 | 5.21675 | 0.164518 | 0.298323 |
| 1-6 | 6 | 671 | 768 | 51 | 0.241248 | 7.39055 | 0.200483 | 0.36354 |
| 1-7 | 7 | 666 | 887 | 44 | 0.098052 | 10.3726 | 0.394072 | 0.714577 |
| 1-8 | 9 | 654 | 935 | 41 | 0.173144 | 7.39772 | 0.484535 | 0.878615 |
| 1-9 | 10 | 656 | 962 | 40 | 0.221504 | 9.17726 | 0.937845 | 1.70061 |
| 1-10 | 11 | 655 | 1 019 | 37 | 0.242024 | 11.7596 | 1.23386 | 2.23738 |
| 1-11 | 12 | 647 | 1 191 | 30 | 0.0594524 | 6.72031 | 0.144259 | 0.261587 |
| 1-12 | 13 | 645 | 1 465 | 20 | 0.110475 | 11.679 | 0.439456 | 0.796873 |
| 1-13 | 14 | 624 | 1 610 | 15 | 0.117906 | 11.3726 | 0.621966 | 1.12782 |
| 1-14 | 15 | 627 | 1 836 | 10 | 0.213851 | 7.44252 | 0.16666 | 0.302208 |
| 2-1 | 16 | 932 | 493 | 82 | 0.220506 | 8.71669 | 0.123458 | 0.223869 |
| 2-2 | 17 | 924 | 620 | 64 | 0.151848 | 12.0625 | 0.52042 | 0.943686 |
| 2-3 | 18 | 920 | 699 | 57 | 0.264652 | 10.1145 | 1.27102 | 2.30476 |
| 2-4 | 19 | 918 | 734 | 54 | 0.313789 | 9.88334 | 1.59008 | 2.88333 |
| 2-5 | 20 | 923 | 782 | 50 | 0.238475 | 10.6127 | 0.356151 | 0.645815 |
| 2-6 | 21 | 920 | 888 | 44 | 0.0785304 | 11.0769 | 0.412927 | 0.748767 |
| 2-7 | 22 | 925 | 933 | 41 | 0.213629 | 9.69875 | 0.569905 | 1.03342 |
| 2-8 | 23 | 908 | 979 | 39 | 0.287168 | 13.4926 | 1.98148 | 3.59306 |
| 2-9 | 24 | 909 | 1 031 | 37 | 0.313234 | 12.5141 | 1.85415 | 3.36216 |
| 2-10 | 25 | 898 | 1 198 | 30 | 0.222614 | 6.9049 | 0.239715 | 0.434679 |
| 2-11 | 26 | 913 | 1 482 | 19 | 0.185123 | 14.5302 | 1.3572 | 2.46103 |
| 2-12 | 27 | 902 | 1 610 | 15 | 0.122121 | 13.3976 | 0.769343 | 1.39506 |
| 2-13 | 28 | 908 | 1 834 | 10 | 0.0525754 | 8.60558 | 0.147439 | 0.267354 |
| 3-1 | 73 | 1 165 | 295 | 175 | 0.190225 | 7.32604 | 0.679618 | 0.692481 |
| 3-2 | 29 | 1 161 | 708 | 56 | 0.158503 | 8.17548 | 0.381053 | 0.690969 |
| 3-3 | 30 | 1 167 | 739 | 53 | 0.175695 | 6.82963 | 0.964648 | 1.74921 |
| 3-4 | 31 | 1 166 | 785 | 50 | 0.226829 | 10.2973 | 1.39866 | 2.53621 |
| 3-5 | 32 | 1 173 | 892 | 43 | 0.18601 | 9.63603 | 0.488605 | 0.885996 |
| 3-6 | 33 | 1 157 | 934 | 41 | 0.202537 | 7.09306 | 0.817584 | 1.48254 |
| 3-7 | 34 | 1 162 | 976 | 39 | 0.165158 | 9.8009 | 1.11848 | 2.02815 |
| 3-8 | 35 | 1 166 | 1 029 | 37 | 0.181907 | 8.58587 | 0.602271 | 1.09211 |
| 3-9 | 53 | 1 164 | 1 205 | 30 | 0.212187 | 8.49447 | 0.659643 | 1.19614 |
| 3-10 | 36 | 1 170 | 1 482 | 19 | 0.152513 | 13.6001 | 1.22917 | 2.22887 |
| 3-11 | 37 | 1 173 | 1 611 | 15 | 0.242024 | 13.6897 | 1.5892 | 2.88172 |
| 3-12 | 69 | 1 170 | 1 824 | 11 | 0.181796 | 14.661 | 1.08121 | 1.96058 |
| 4-1 | 74 | 1 405 | 302 | 168 | 0.41295 | 9.04106 | 2.21829 | 2.26028 |
| 4-2 | 38 | 1 392 | 641 | 62 | 0.340631 | 6.45867 | 1.49051 | 2.70276 |
| 4-3 | 39 | 1 400 | 703 | 56 | 0.220506 | 8.63963 | 1.15871 | 2.10111 |
| 4-4 | 40 | 1 399 | 777 | 50 | 0.304693 | 9.25073 | 1.45611 | 2.64039 |
| 4-5 | 41 | 1 413 | 892 | 43 | 0.364811 | 8.68623 | 1.57454 | 2.85514 |
| 4-6 | 42 | 1 401 | 938 | 41 | 0.203425 | 7.64861 | 1.01117 | 1.83357 |
| 4-7 | 43 | 1 399 | 986 | 39 | 0.190447 | 8.75253 | 0.987141 | 1.79 |
| 4-8 | 44 | 1 402 | 1 035 | 36 | 0.304139 | 9.24715 | 1.75817 | 3.18812 |
| 4-9 | 54 | 1 413 | 1 202 | 30 | 0.23914 | 7.76151 | 1.26551 | 2.29477 |
| 4-10 | 45 | 1 426 | 1 470 | 20 | 0.319002 | 10.7991 | 1.51534 | 2.7478 |
| 4-11 | 46 | 1 422 | 1 610 | 15 | 0.303584 | 13.8188 | 2.12754 | 3.85791 |
| 4-12 | 70 | 1 429 | 1 819 | 11 | 0.183016 | 12.679 | 0.994798 | 1.80388 |
| 5-1 | 75 | 1 617 | 283 | 187 | 3.7252 | 14.6252 | 39.0364 | 39.7752 |
| 5-2 | 47 | 1 621 | 623 | 64 | 0.228492 | 8.23462 | 1.07061 | 1.94136 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 5-3 | 48 | 1 625 | 701 | 57 | 0.194773 | 8.44429 | 0.815255 | 1.47832 |
| 5-4 | 49 | 1 630 | 743 | 53 | 0.21252 | 8.53569 | 0.530533 | 0.962025 |
| 5-5 | 50 | 1 636 | 914 | 42 | 0.1219 | 6.05366 | 0.252993 | 0.458756 |
| 5-6 | 51 | 1 638 | 945 | 41 | 0.108922 | 6.23286 | 0.416165 | 0.754638 |
| 5-7 | 52 | 1 646 | 1 003 | 38 | 0.263432 | 9.32062 | 0.937748 | 1.70043 |
| 5-8 | 55 | 1 651 | 1 164 | 31 | 0.115134 | 9.39589 | 0.27639 | 0.501182 |
| 5-9 | 56 | 1 658 | 1 455 | 21 | 0.0932825 | 10.7633 | 0.214304 | 0.388602 |
| 5-10 | 57 | 1 658 | 1 591 | 15 | 0.149186 | 11.9908 | 0.224895 | 0.407807 |
| 5-11 | 71 | 1 692 | 1 821 | 11 | 0.170038 | 10.5249 | 0.347606 | 0.63032 |
| 6-1 | 76 | 1 807 | 282 | 188 | 0.334974 | 9.94427 | 1.06072 | 1.0808 |
| 6-2 | 58 | 1 873 | 616 | 65 | 0.220506 | 8.09305 | 0.845283 | 1.53277 |
| 6-3 | 59 | 1 882 | 674 | 59 | 0.215404 | 6.72569 | 0.297724 | 0.539867 |
| 6-4 | 60 | 1 886 | 712 | 56 | 0.424153 | 8.85468 | 1.54335 | 2.79858 |
| 6-5 | 61 | 1 881 | 752 | 52 | 0.300478 | 8.03391 | 0.518387 | 0.94 |
| 6-6 | 62 | 1 887 | 864 | 45 | 0.0911751 | 7.44252 | 0.249139 | 0.451768 |
| 6-7 | 63 | 1 883 | 909 | 43 | 0.121789 | 9.1665 | 0.485663 | 0.880661 |
| 6-8 | 64 | 1 883 | 954 | 40 | 0.17747 | 8.61633 | 0.687456 | 1.24658 |
| 6-9 | 65 | 1 899 | 1 014 | 37 | 0.319224 | 11.1916 | 1.44847 | 2.62654 |
| 6-10 | 66 | 1 900 | 1 164 | 31 | 0.206863 | 9.16292 | 0.238615 | 0.432684 |
| 6-11 | 67 | 1 928 | 1 444 | 21 | 0.0795286 | 10.4837 | 0.252931 | 0.458644 |
| 6-12 | 68 | 1 932 | 1 581 | 16 | 0.32122 | 10.0715 | 0.499075 | 0.90498 |
| 6-13 | 72 | 1 972 | 1 814 | 11 | 0.180021 | 14.9281 | 0.244204 | 0.44282 |

Notes: The bands used to calculate HA% are highlighted in color. For the calculation method see the Materials and Methods – 2.8. Data Processing.

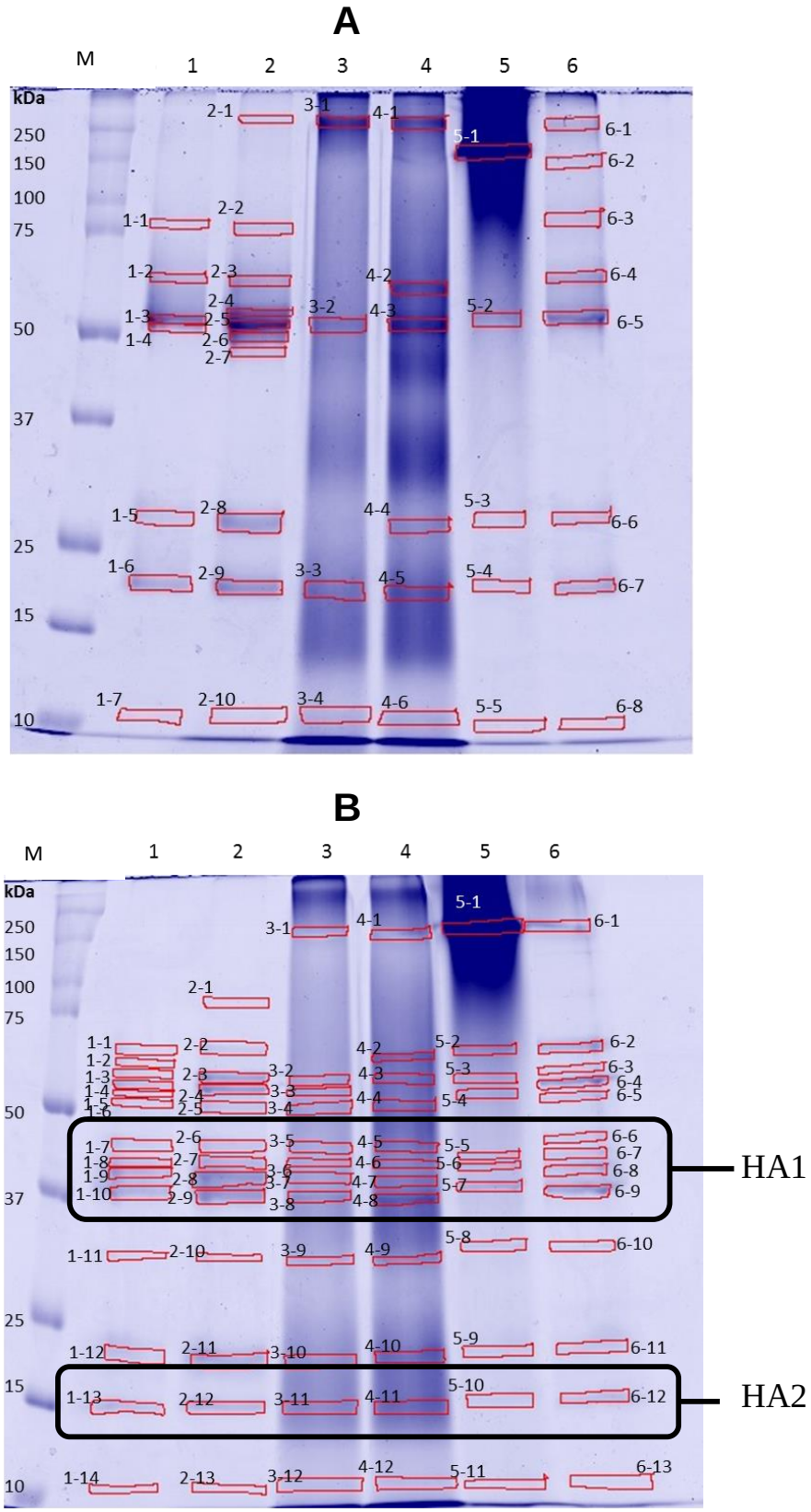



**Figure S1**. SDS-PAGE images for the vaccine preparations under reducing conditions without preliminary deglycosylation of proteins (**A**) and with preliminary deglycosylation of proteins (**B**). Numbering of the bands on the gels: 1 – U4; 2 – U3; 3 – SGU; 4 – SGF; 5 – GP; 6 – VG. 32.8 µl of the original vaccine were applied to each lane. Red rectangles indicate the protein bands subject to densitometry. The densitometry results are given in Supplementary Tables 2 and 3. HA1 and HA2 bands are outlined in black rectangles in the figure.

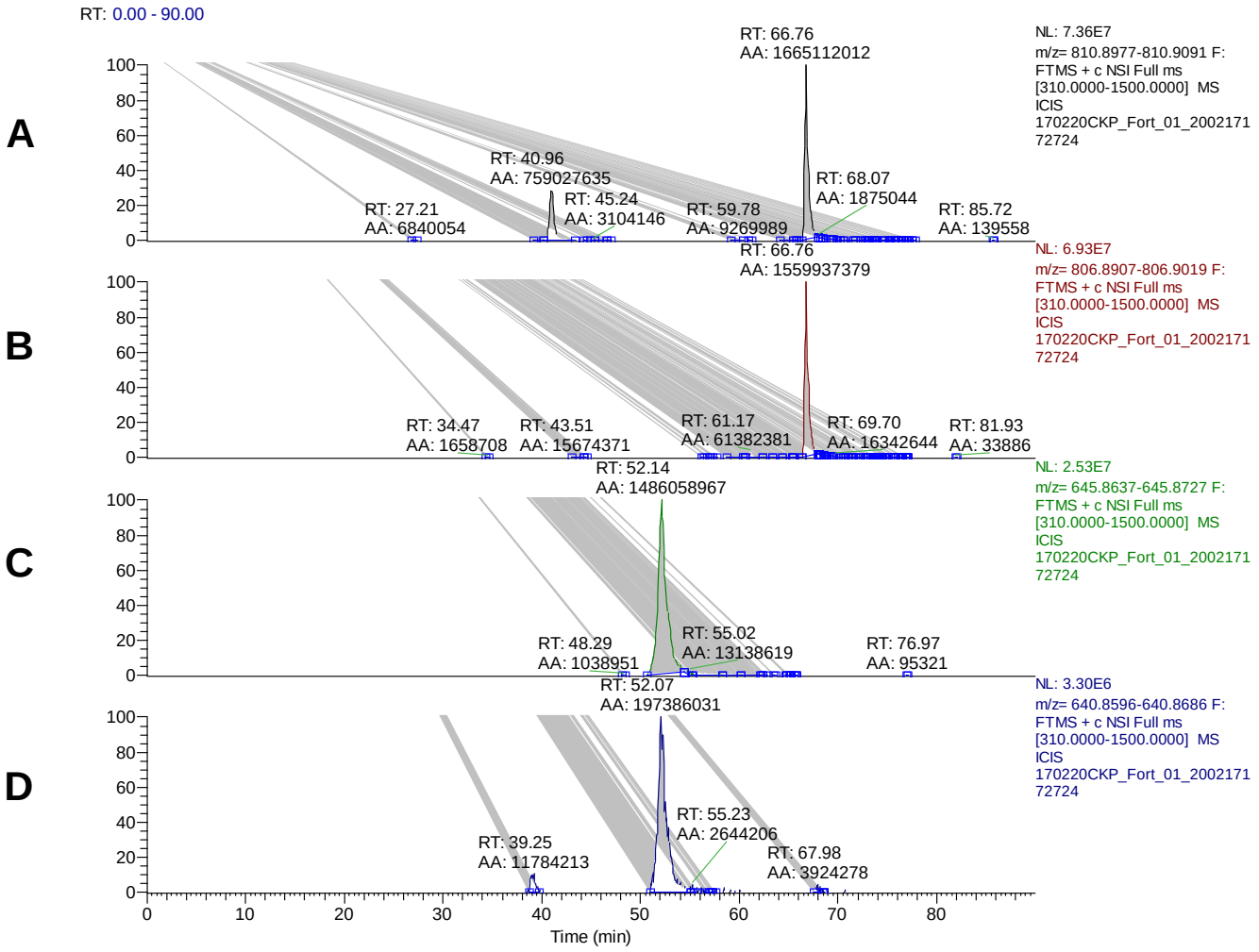



**Figure S2**. Sample U3. Peptide chromatograms of the extracted isotopically labeled ion (m/z 810.9034$^{2+}$) (**A**) and the natural ion (m/z 806.8963$^{2}$) (**B**) for the APOA1_HUMAN protein, and peptide chromatograms of the extracted isotopically labeled ion (m/z 645.8682$^{2+}$) (**C**) and the natural ion (m/z 640.8641$^{2+}$) (**D**) for the APOB_HUMAN protein.

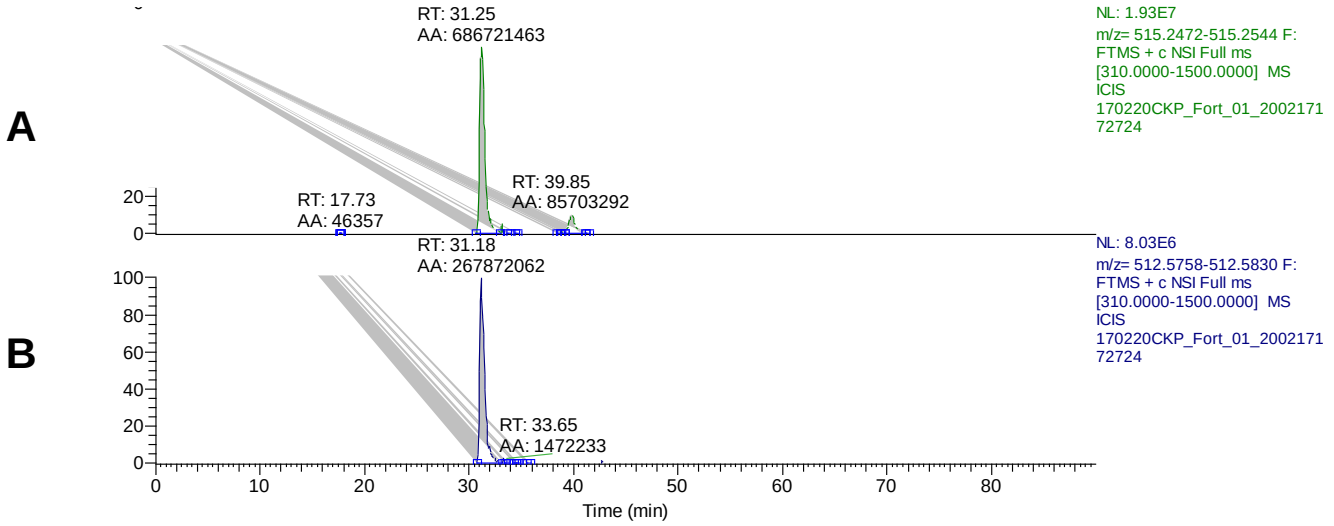



**Figure S3**. Sample U3. Peptide chromatograms of the extracted isotopically labeled ion (m/z 515.2508$^{3+}$) (**A**) and the natural ion (m/z 512.5794$^{3+}$) (**B**) for the FIBB_HUMAN protein.

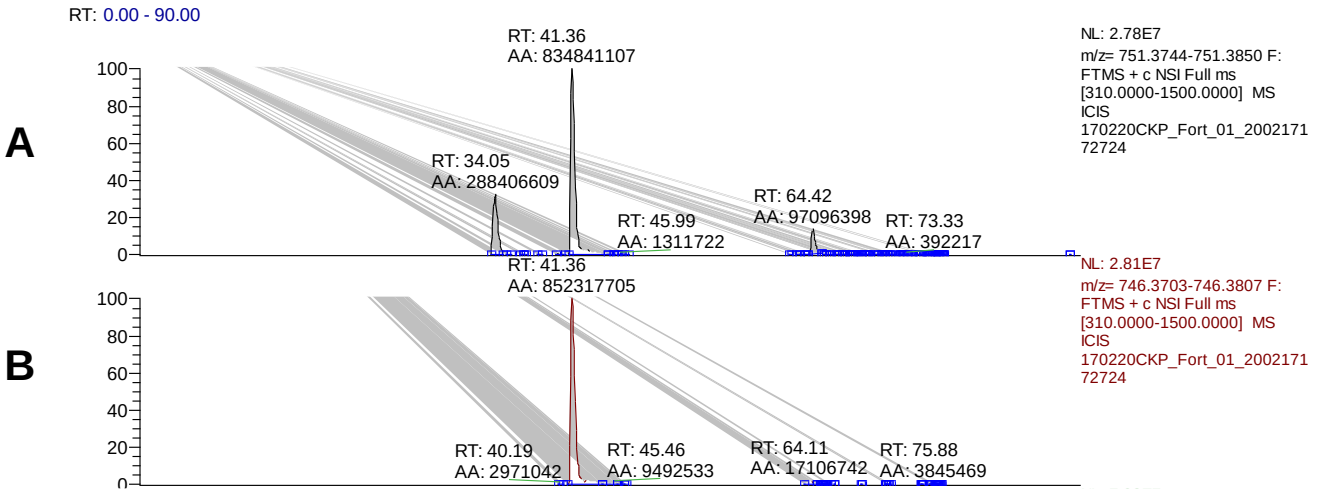



**Figure S4**. Sample U3. Peptide chromatograms of the extracted isotopically labeled ion (m/z $751.3797^{2+}$) (**A**) and the natural ion (m/z $746.3755^{2+}$) (**B**) for the FIBG_HUMAN protein.

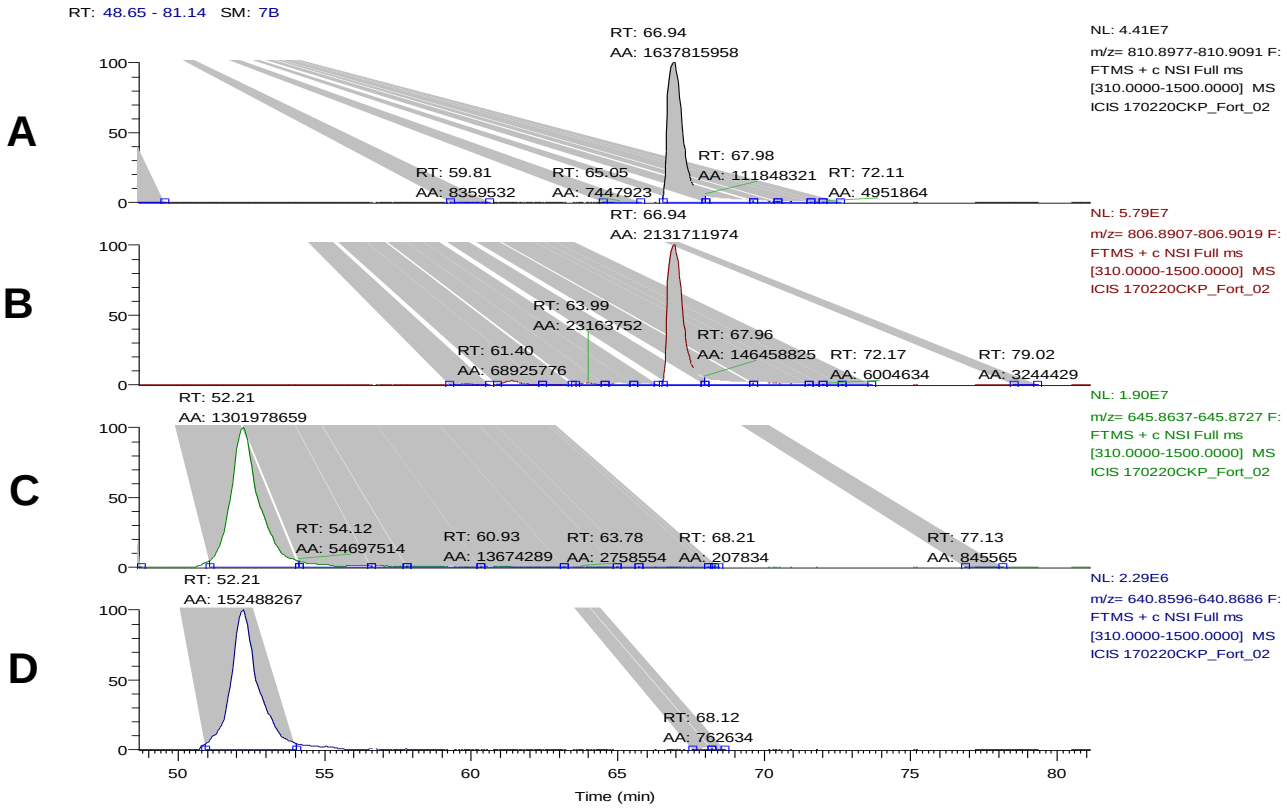



**Figure S5**. Sample U4. Peptide chromatograms of the extracted isotopically labeled ion (m/z $810.9034^{2+}$) (**A**) and the natural ion (m/z $806.8963^{2}$) (**B**) for the APOA1_HUMAN protein, and peptide chromatograms of the extracted isotopically labeled ion (m/z $645.8682^{2+}$) (**C**) and the natural ion (m/z $640.8641^{2+}$) (**D**) for the APOB_HUMAN protein.

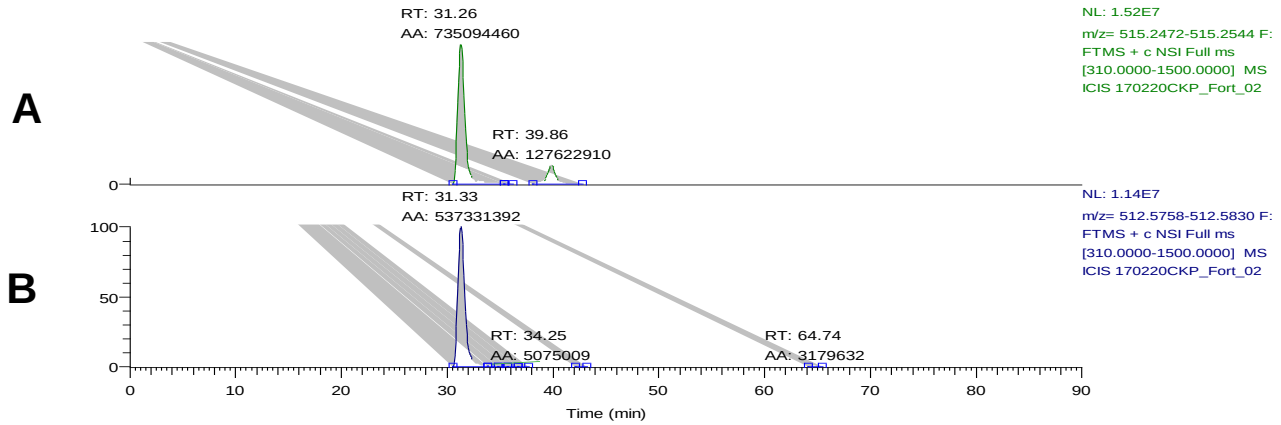



**Figure S6**. Sample U4. Peptide chromatograms of the extracted isotopically labeled ion (m/z 515.2508$^{3+}$) (**A**) and the natural ion (m/z 512.5794$^{3+}$) (**B**) for the FIBB_HUMAN protein.

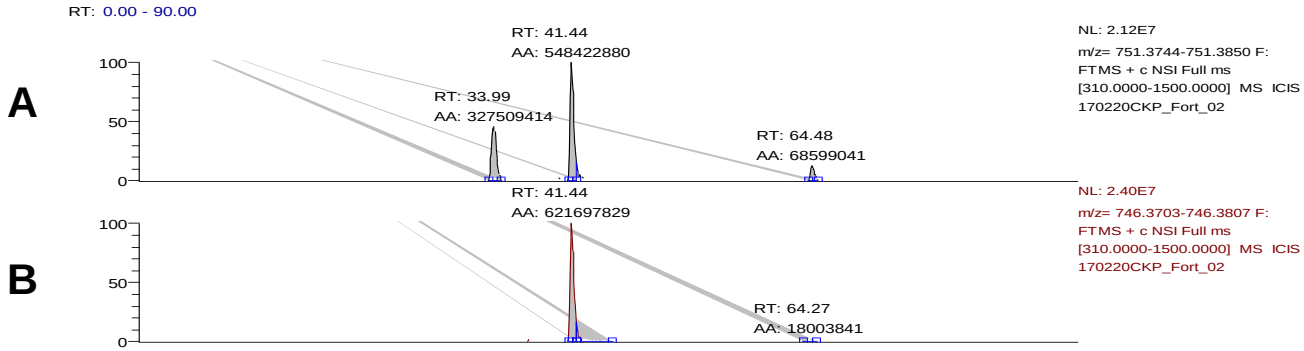



**Figure S7**. Sample U4. Peptide chromatograms of the extracted isotopically labeled ion (m/z 751.3797$^{2+}$) (**A**) and the natural ion (m/z 746.3755$^{2+}$) (**B**) for the FIBG_HUMAN protein.

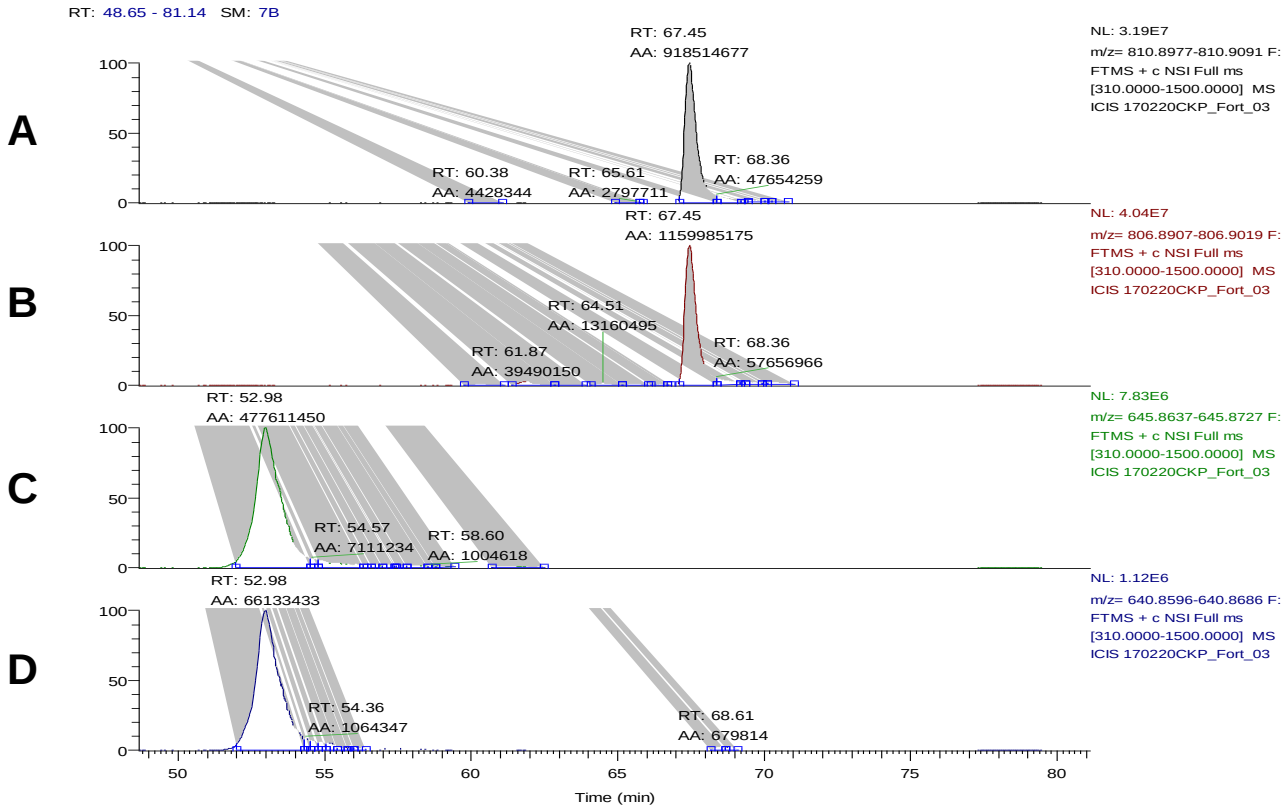



**Figure S8**. Sample SGU. Peptide chromatograms of the extracted isotopically labeled ion (m/z 810.9034$^{2+}$) (**A**) and the natural ion (m/z 806.8963$^{2}$) (**B**) for the APOA1_HUMAN protein, and peptide chromatograms of the extracted isotopically labeled ion (m/z 645.8682$^{2+}$) (**C**) and the natural ion (m/z 640.8641$^{2+}$) (**D**) for the APOB_HUMAN protein.

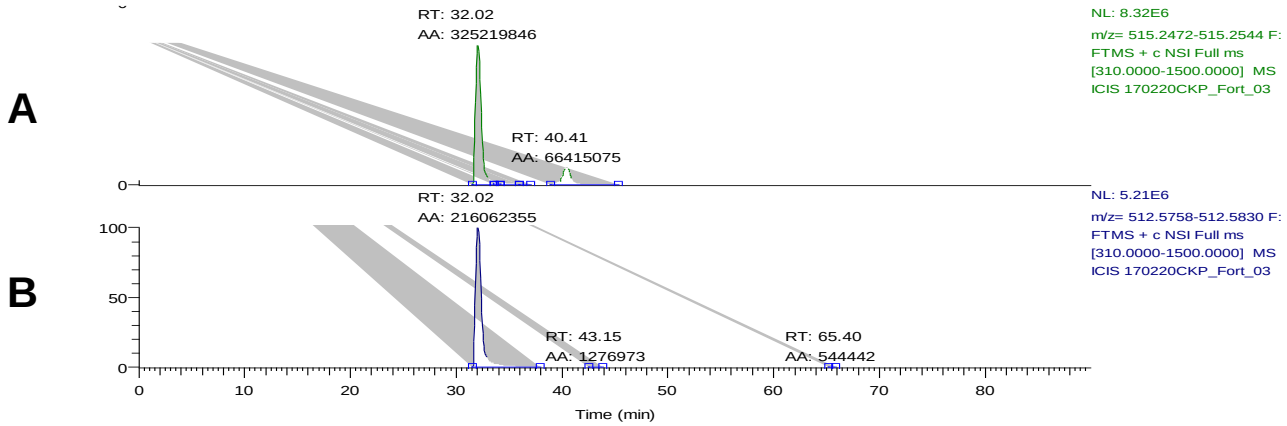



**Figure S9**. Sample SGU. Peptide chromatograms of the extracted isotopically labeled ion (m/z 515.2508$^{3+}$) (**A**) and the natural ion (m/z 512.5794$^{3+}$) (**B**) for the FIBB_HUMAN protein.

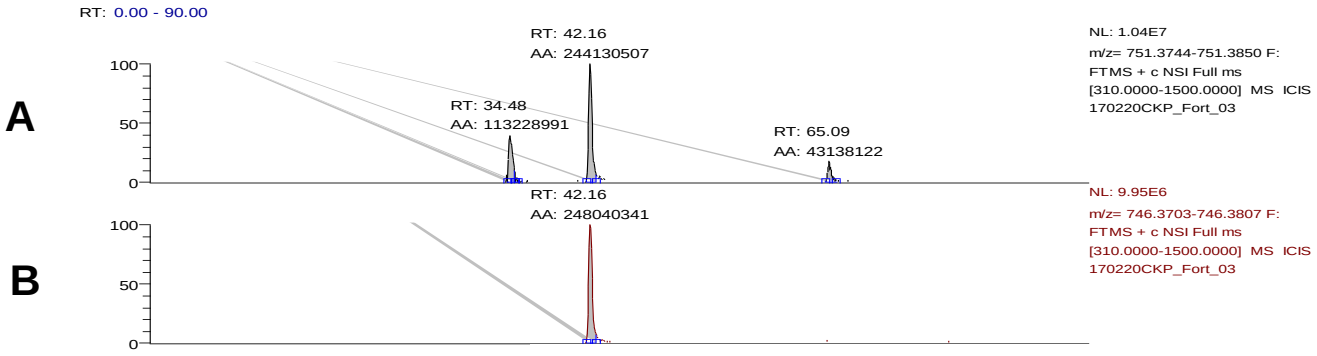



**Figure S10**. Sample SGU. Peptide chromatograms of the extracted isotopically labeled ion (m/z 751.3797$^{2+}$) (**A**) and the natural ion (m/z 746.3755$^{2+}$) (**B**) for the FIBG_HUMAN protein.

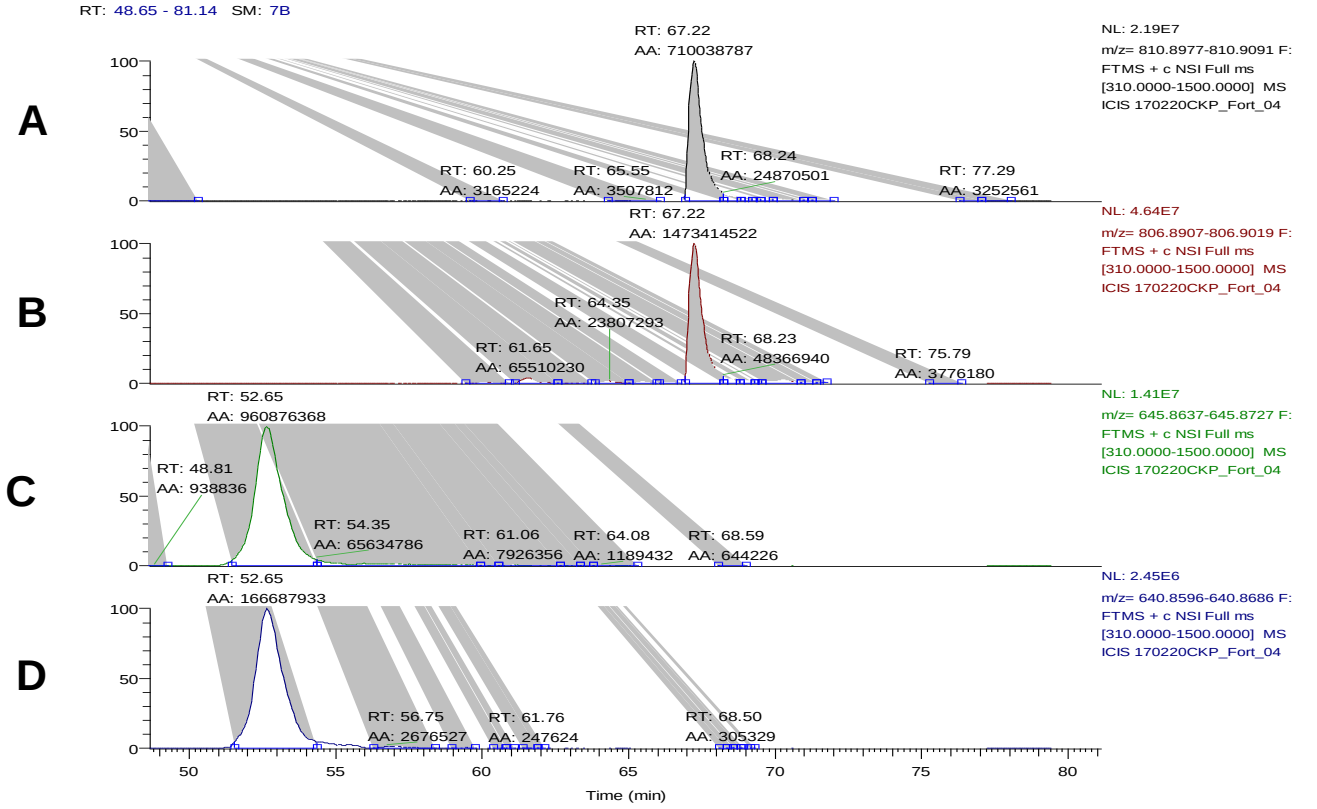



**Figure S11**. Sample SGF. Peptide chromatograms of the extracted isotopically labeled ion (m/z 810.9034$^{2+}$) (**A**) and the natural ion (m/z 806.8963$^{2}$) (**B**) for the APOA1_HUMAN protein, and peptide chromatograms of the extracted isotopically labeled ion (m/z 645.8682$^{2+}$) (C) and the natural ion (m/z 640.8641$^{2+}$) (D) for the APOB_HUMAN protein.

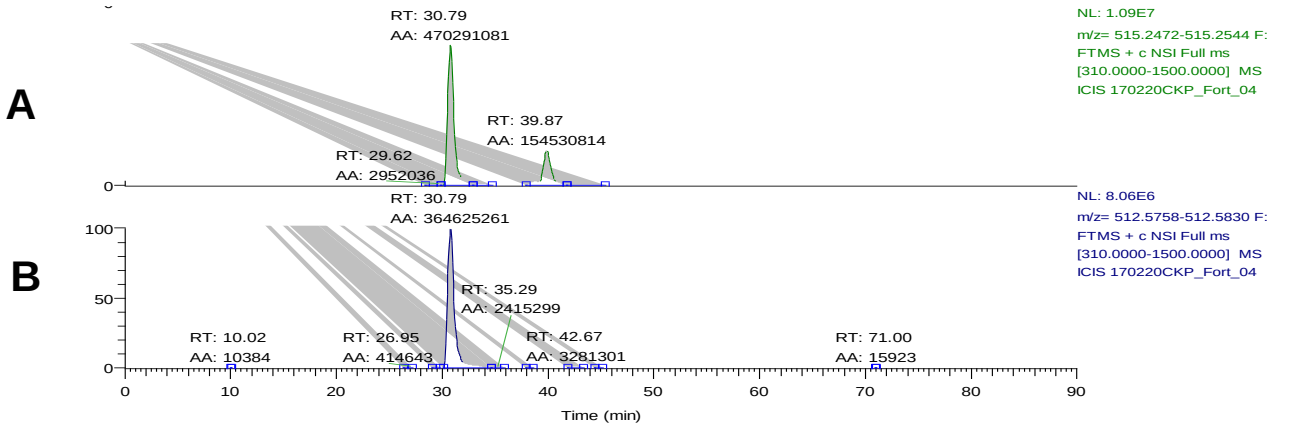



**Figure S12**. Sample SGF. Peptide chromatograms of the extracted isotopically labeled ion (m/z 515.2508$^{3+}$) (**A**) and the natural ion (m/z 512.5794$^{3+}$) (**B**) for the FIBB_HUMAN protein.

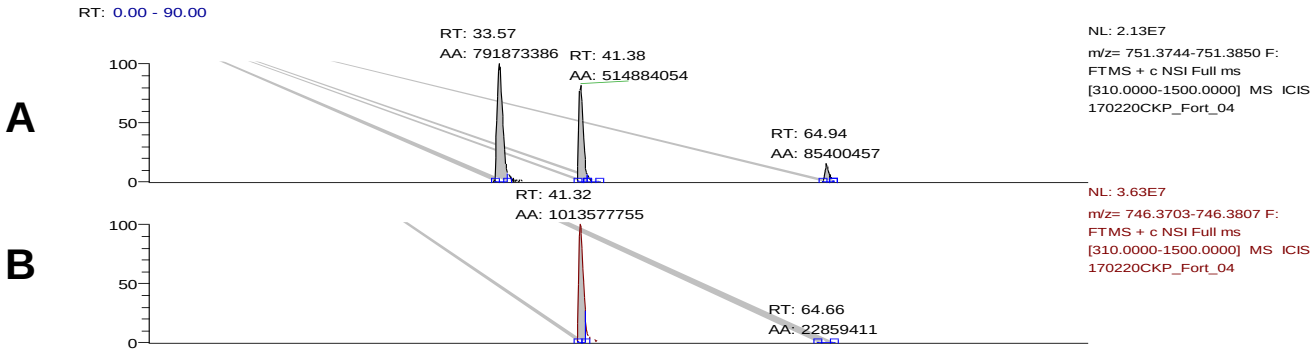



**Figure S13**. Sample SGF. Peptide chromatograms of the extracted isotopically labeled ion (m/z 751.3797$^{2+}$) (**A**) and the natural ion (m/z 746.3755$^{2+}$) (**B**) for the FIBG_HUMAN protein.

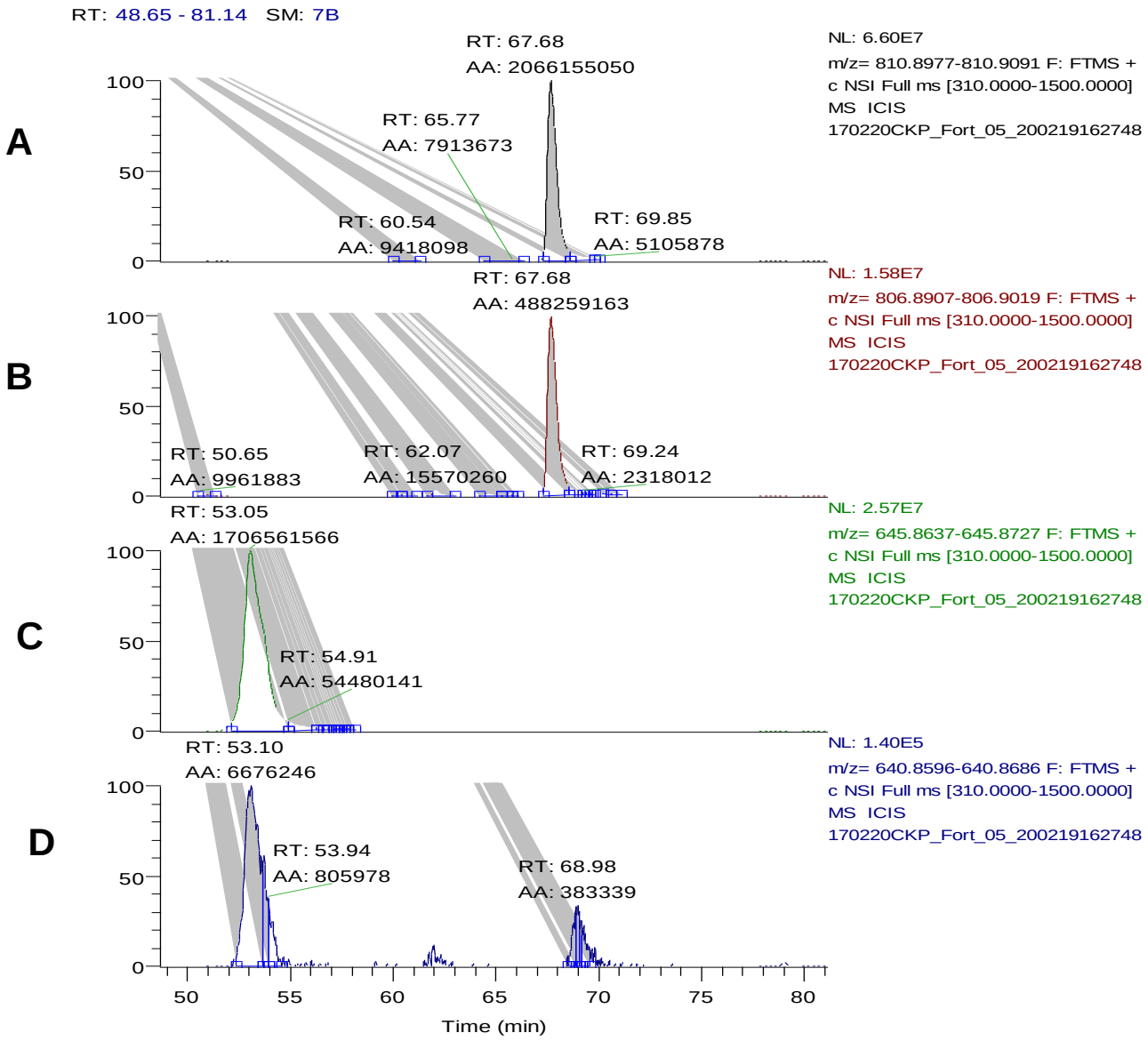



**Figure S14**. Sample GP. Peptide chromatograms of the extracted isotopically labeled ion (m/z $810.9034^{2+}$) (**A**) and the natural ion (m/z $806.8963^{2}$) (**B**) for the APOA1_HUMAN protein, and peptide chromatograms of the extracted isotopically labeled ion (m/z $645.8682^{2+}$) (**C**) and the natural ion (m/z $640.8641^{2+}$) (**D**) for the APOB_HUMAN protein.

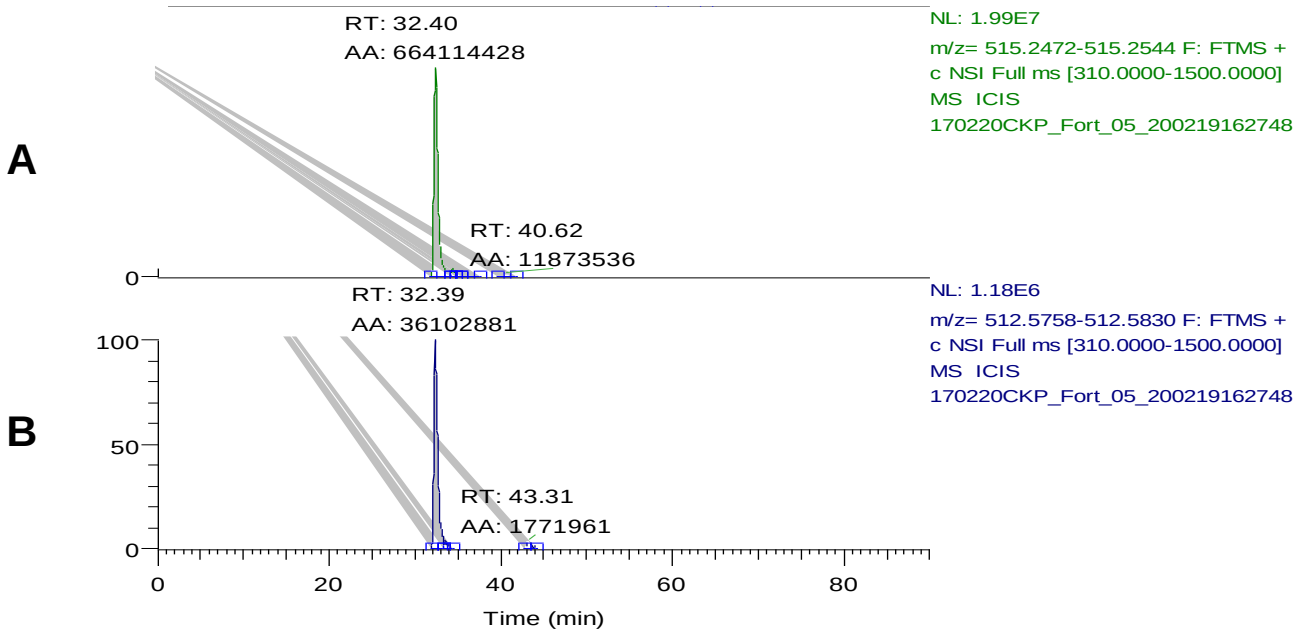



**Figure S15**. Sample GP. Peptide chromatograms of the extracted isotopically labeled ion (m/z 515.2508$^{3+}$) (**A**) and the natural ion (m/z 512.5794$^{3+}$) (**B**) for the FIBB_HUMAN protein.

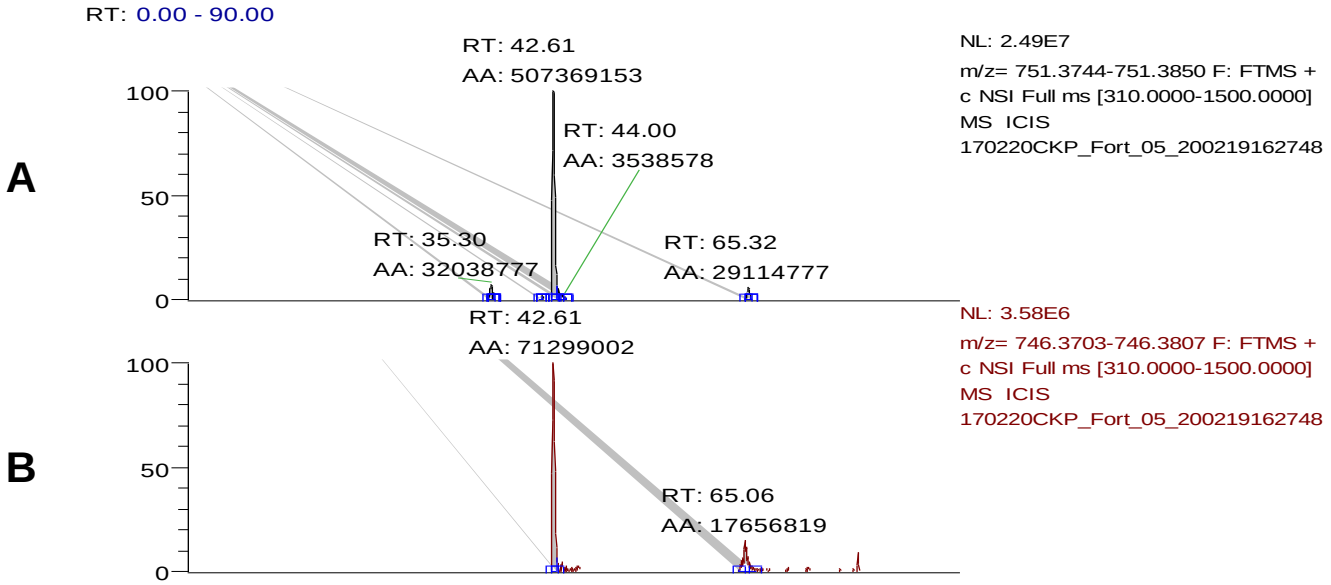



**Figure S16**. Sample GP. Peptide chromatograms of the extracted isotopically labeled ion (m/z 751.3797$^{2+}$) (**A**) and the natural ion (m/z 746.3755$^{2+}$) (**B**) for the FIBG_HUMAN protein.

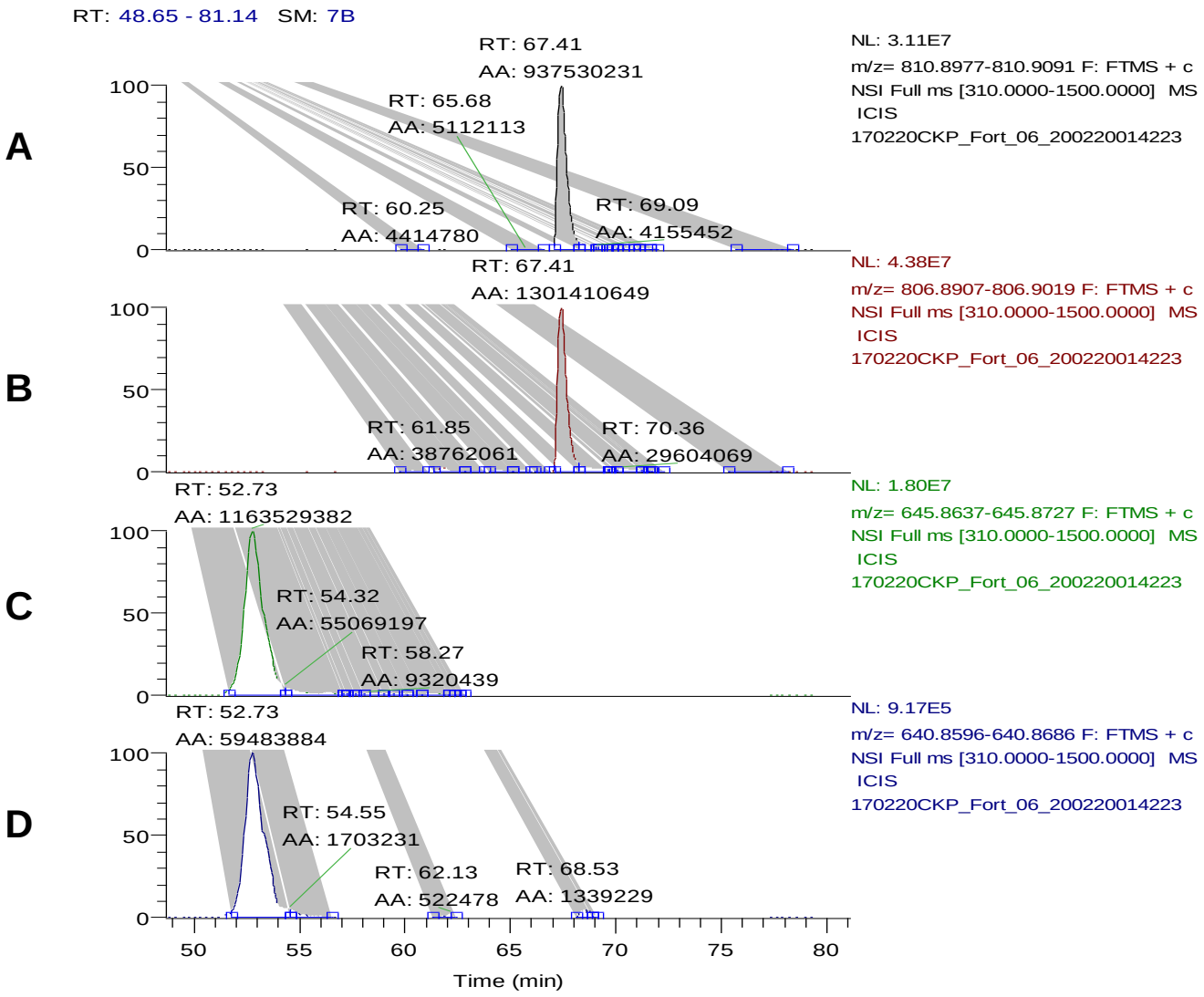



**Figure S17**. Sample VG. Peptide chromatograms of the extracted isotopically labeled ion (m/z 810.9034$^{2+}$) (**A**) and the natural ion (m/z 806.8963$^{2}$) (**B**) for the APOA1_HUMAN protein, and peptide chromatograms of the extracted isotopically labeled ion (m/z 645.8682$^{2+}$) (**C**) and the natural ion (m/z 640.8641$^{2+}$) (**D**) for the APOB_HUMAN protein.

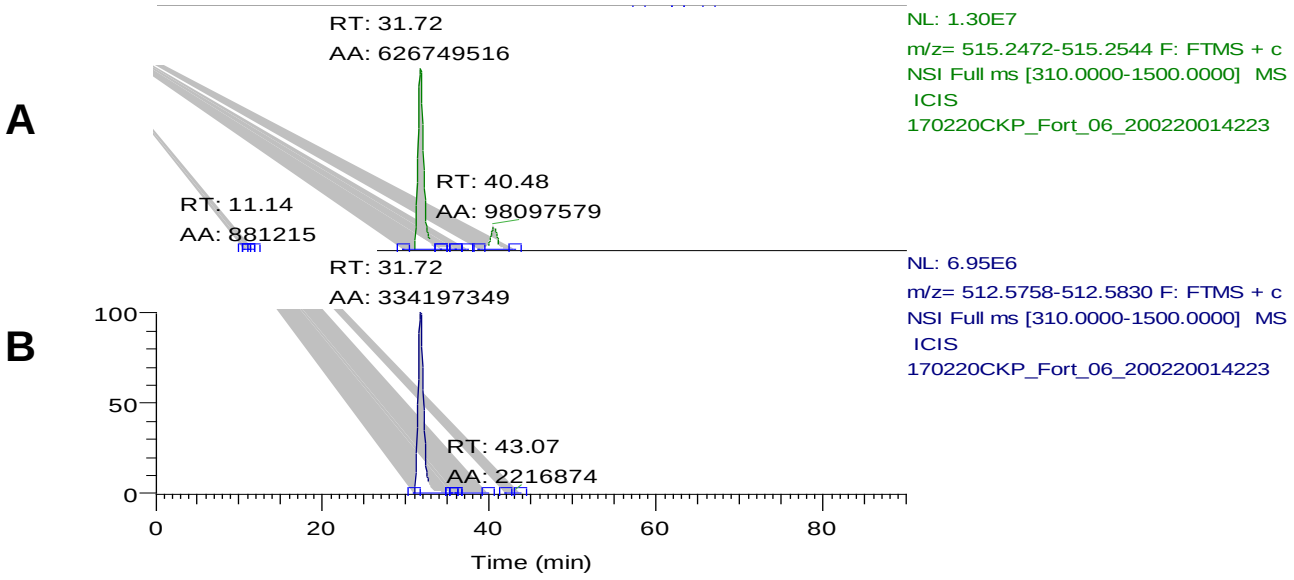



**Figure S18**. Sample VG. Peptide chromatograms of the extracted isotopically labeled ion (m/z 515.2508$^{3+}$) (**A**) and the natural ion (m/z 512.5794$^{3+}$) (**B**) for the FIBB_HUMAN protein.

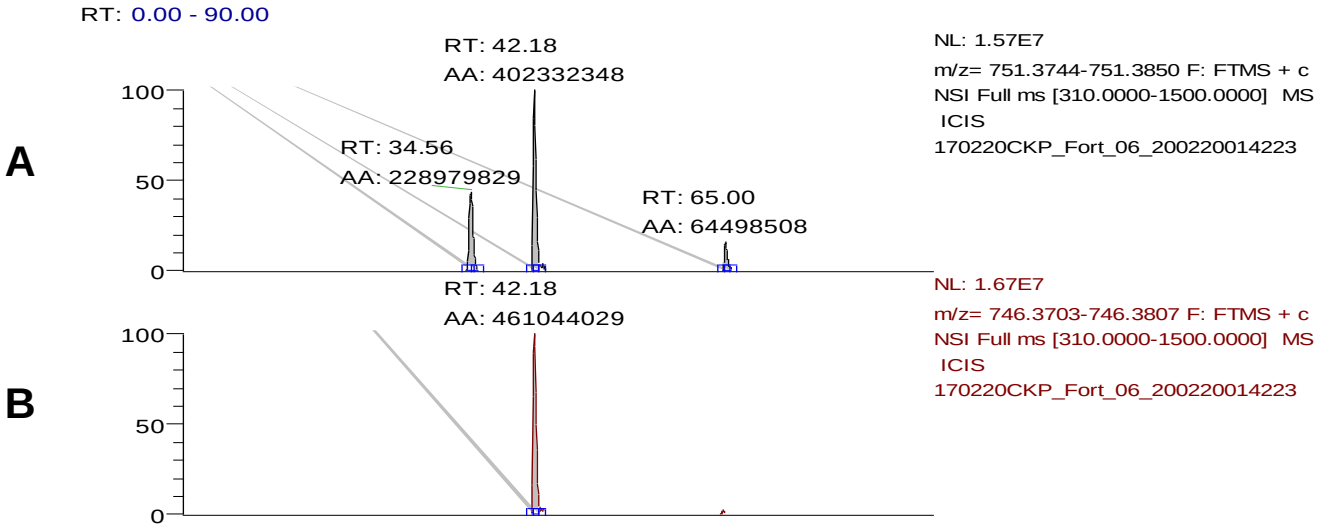



**Figure S19**. Sample VG. Peptide chromatograms of the extracted isotopically labeled ion (m/z 515.2508$^{3+}$) (**A**) and the natural ion (m/z 512.5794$^{3+}$) (**B**) for the FIBB_HUMAN protein.

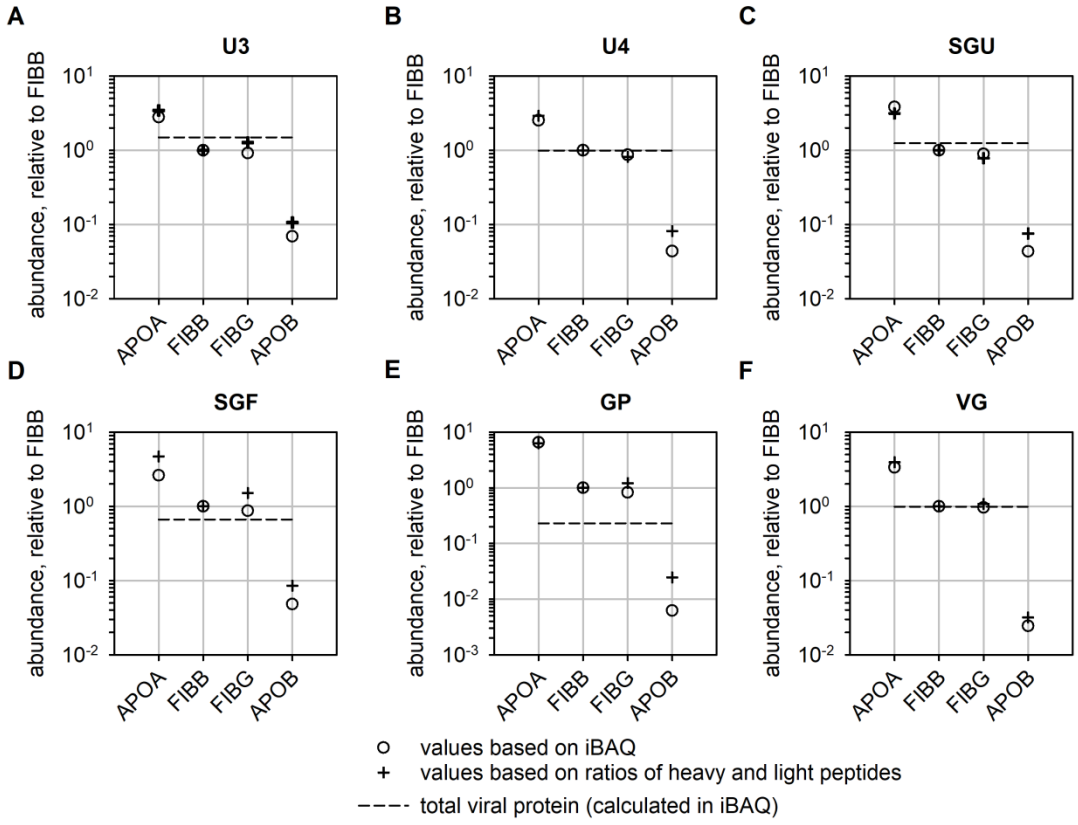



**Figure S20.** Verification of label-free LC–MS/MS-based quantitation of proteins in vaccine samples. The figures show the normalized concentrations of control proteins from the LC–MS QC mix included in vaccine samples (Figure 4). Crosses indicate concentrations measured by (HR) LC–MS using SIL peptides; circles show concentrations determined by results of label-free LC–MS/MS using the intensity-based absolute-quantitation (iBAQ) algorithm; dashed lines represent total concentrations of all viral proteins calculated using iBAQ. Samples: (**A**) U3, (**B**) U4, (**C**) SGU, (**D**) SGF, (**E**) GP, and (**F**) VG.

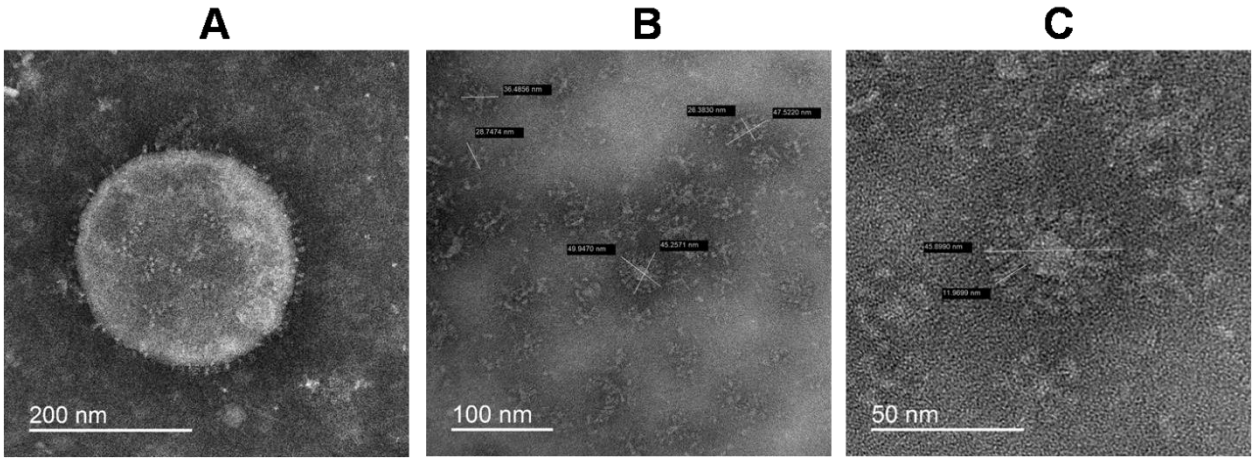



**Figure S21.** EM images of influenza virus virion from pooled harvest fluid (**A**), hemagglutinin aggregates in U4 sample (**B**), magnification of one hemagglutinin aggregate from B (**C**).