

# Supporting Information

Figure S1

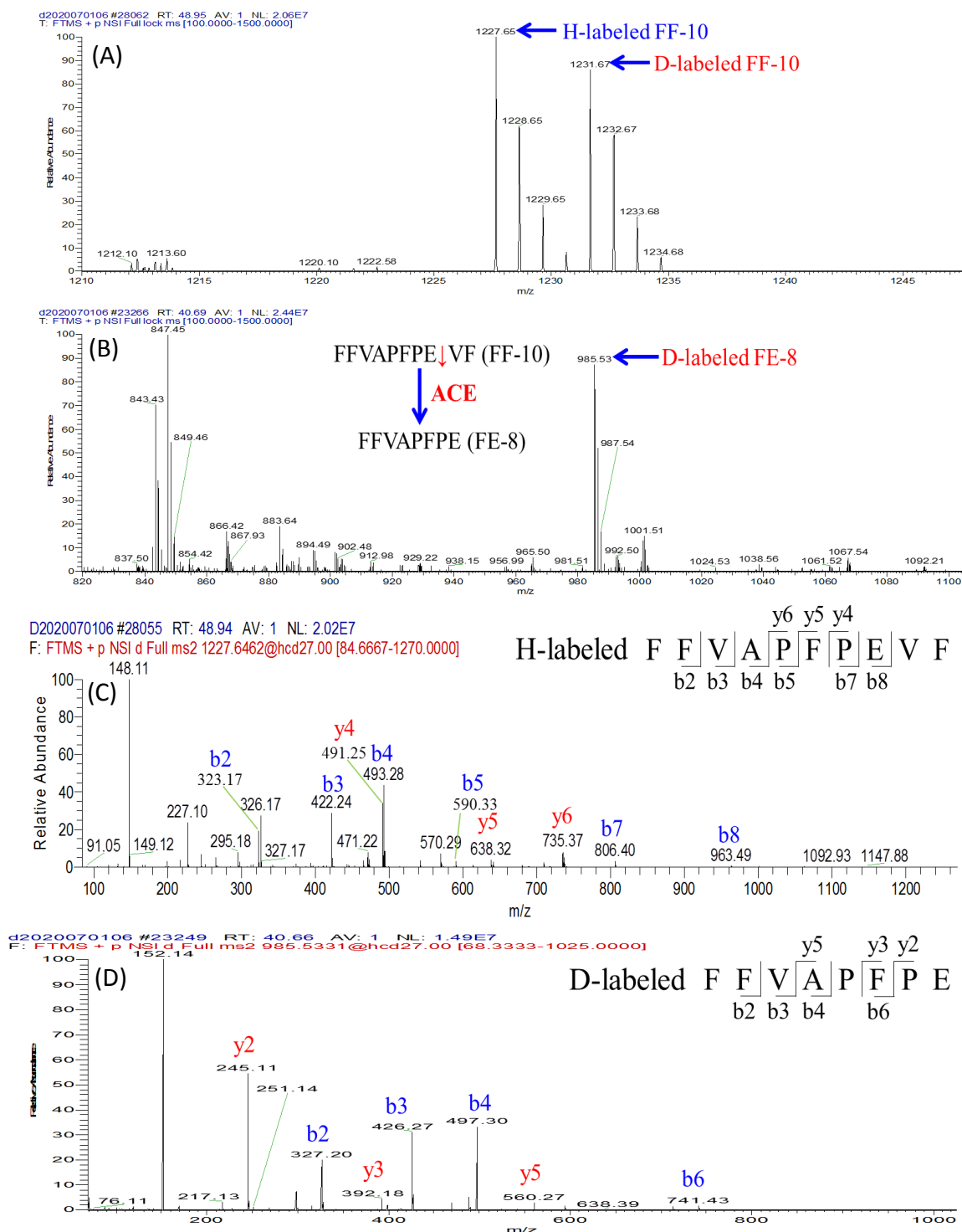
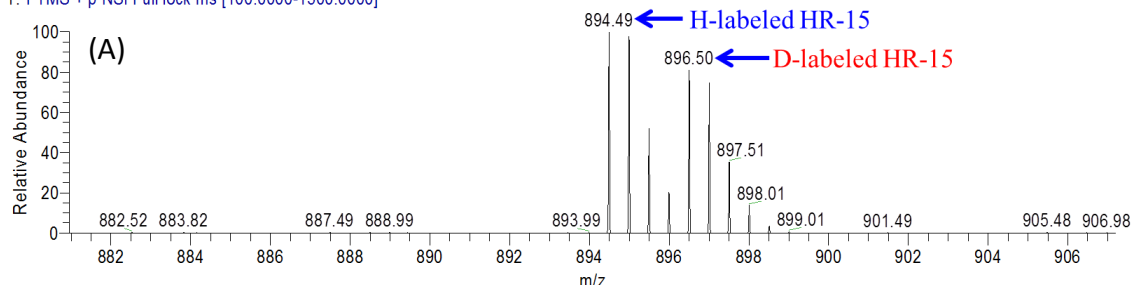


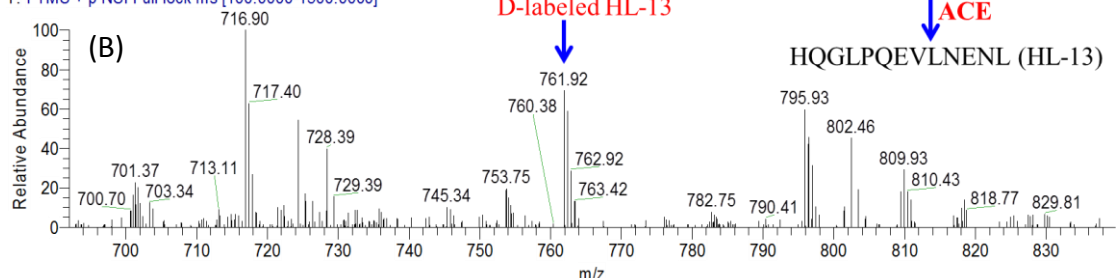
Figure S1. LC-MS/MS identification of ACE exogenous substrate FFVAPFPEVF (FF-10) and its product. (A) MS spectrum of D- and H-labeled FF-10; (B) MS spectrum of the product FFVAPFPE (FE-8); (C) MS/MS spectrum of FF-10 (m/z 1227.6); (D) MS/MS of FE-8 (m/z 985.5).

**Figure S2.**

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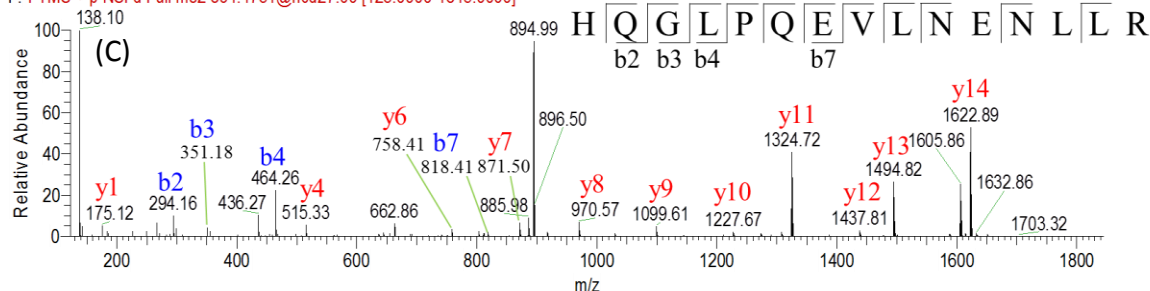


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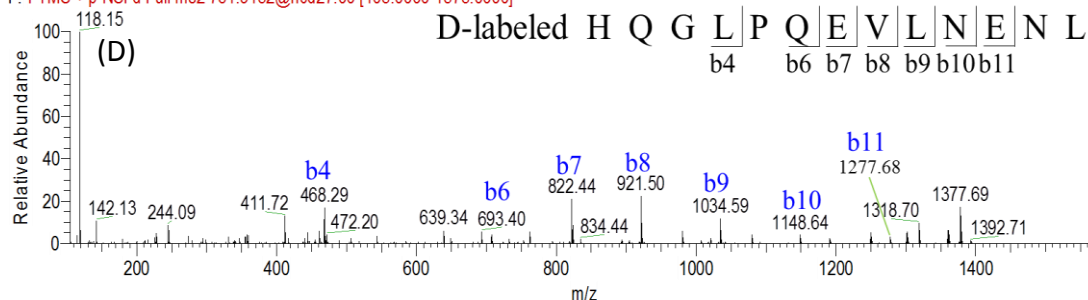
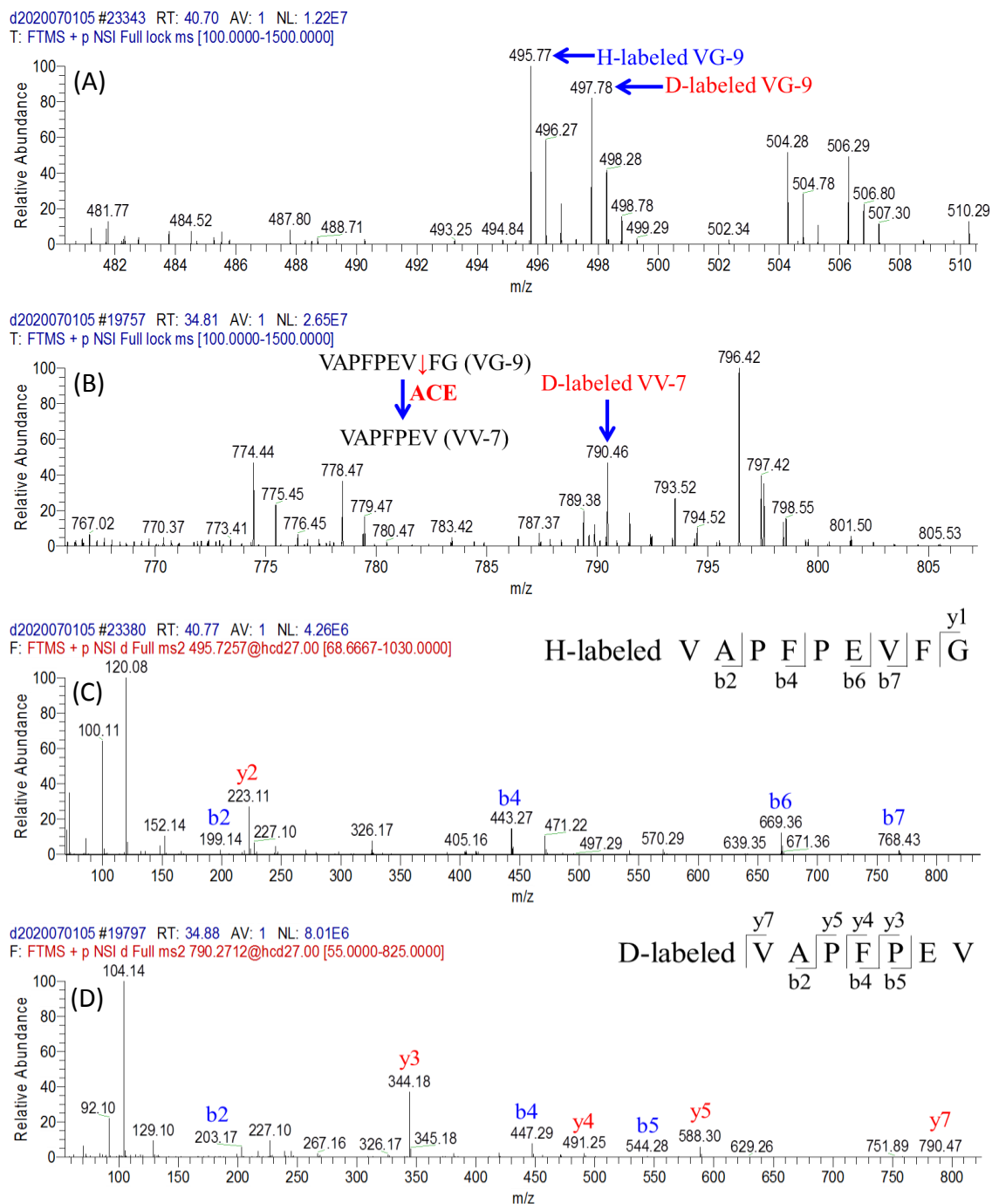


Figure S2. LC-MS/MS identification of ACE exogenous substrate HQGLPQEVLENLLR (HR-15) and its product. (A) MS spectrum of D- and H-labeled HR-15; (B) MS spectrum of the product HQGLPQEVLENL (HL-13); (C) MS/MS spectrum of HR-15 (m/z 894.4); (D) MS/MS of HL-13 (m/z 761.9).

**Figure S3**



**Figure S3.** LC-MS/MS identification of ACE exogenous substrate VAPFPEVFG (VG-9) and its product. (A) MS spectrum of D- and H-labeled VG-9; (B) MS spectrum of the product VAPFPEV (VV-7); (C) MS/MS spectrum of VG-9 (m/z 495.7, +2); (D) MS/MS of VV-7 (m/z 790.2).

**Figure S4**

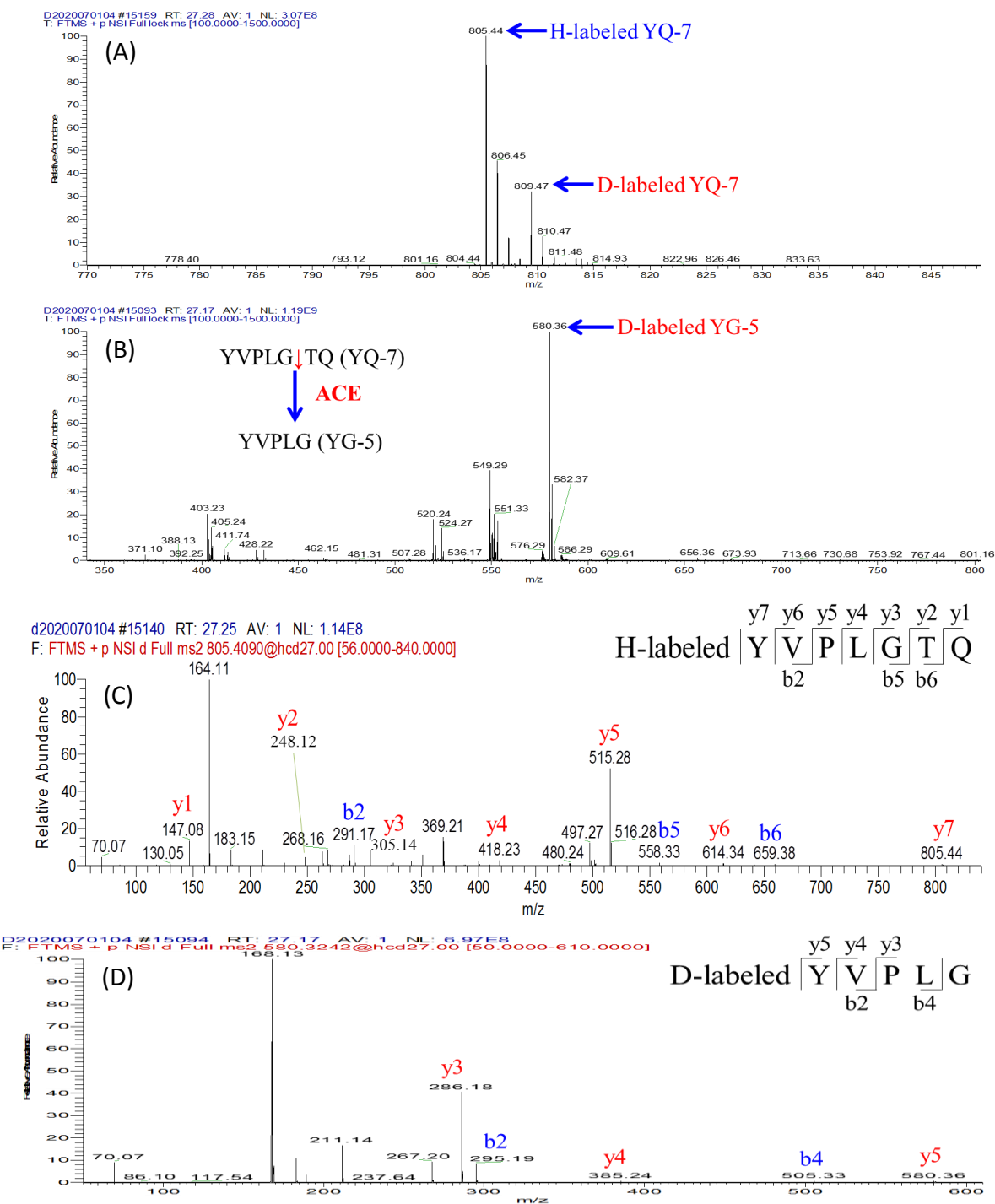
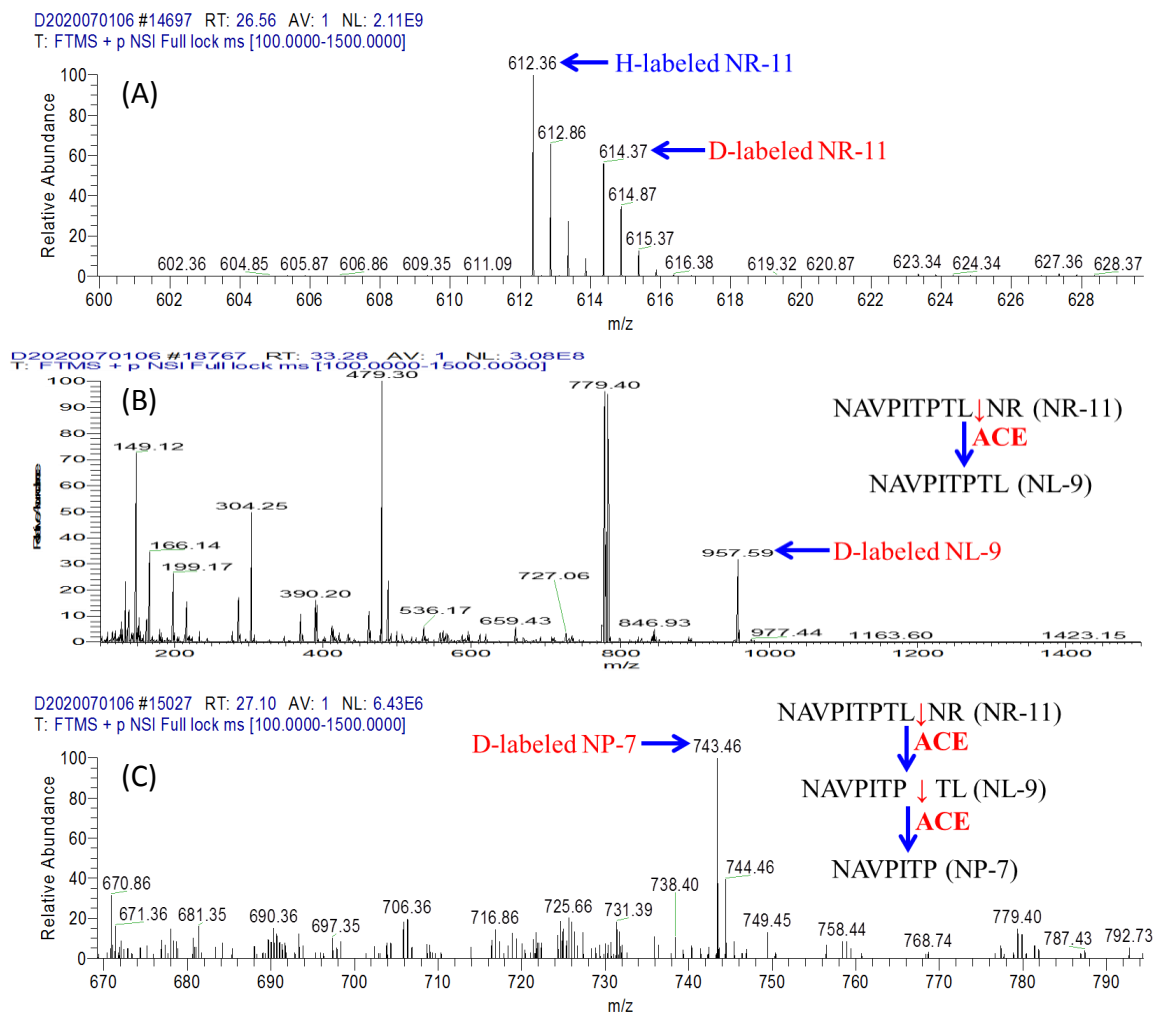


Figure S4. LC-MS/MS identification of ACE exogenous substrate YVPLGTQ (YQ-7) and its product. (A) MS spectrum of D- and H-labeled YQ-7; (B) MS spectrum of the product YVPLG (YG-5); (C) MS/MS spectrum of YQ-7 (m/z 805.4); (D) MS/MS of YG-5 (m/z 580.3).

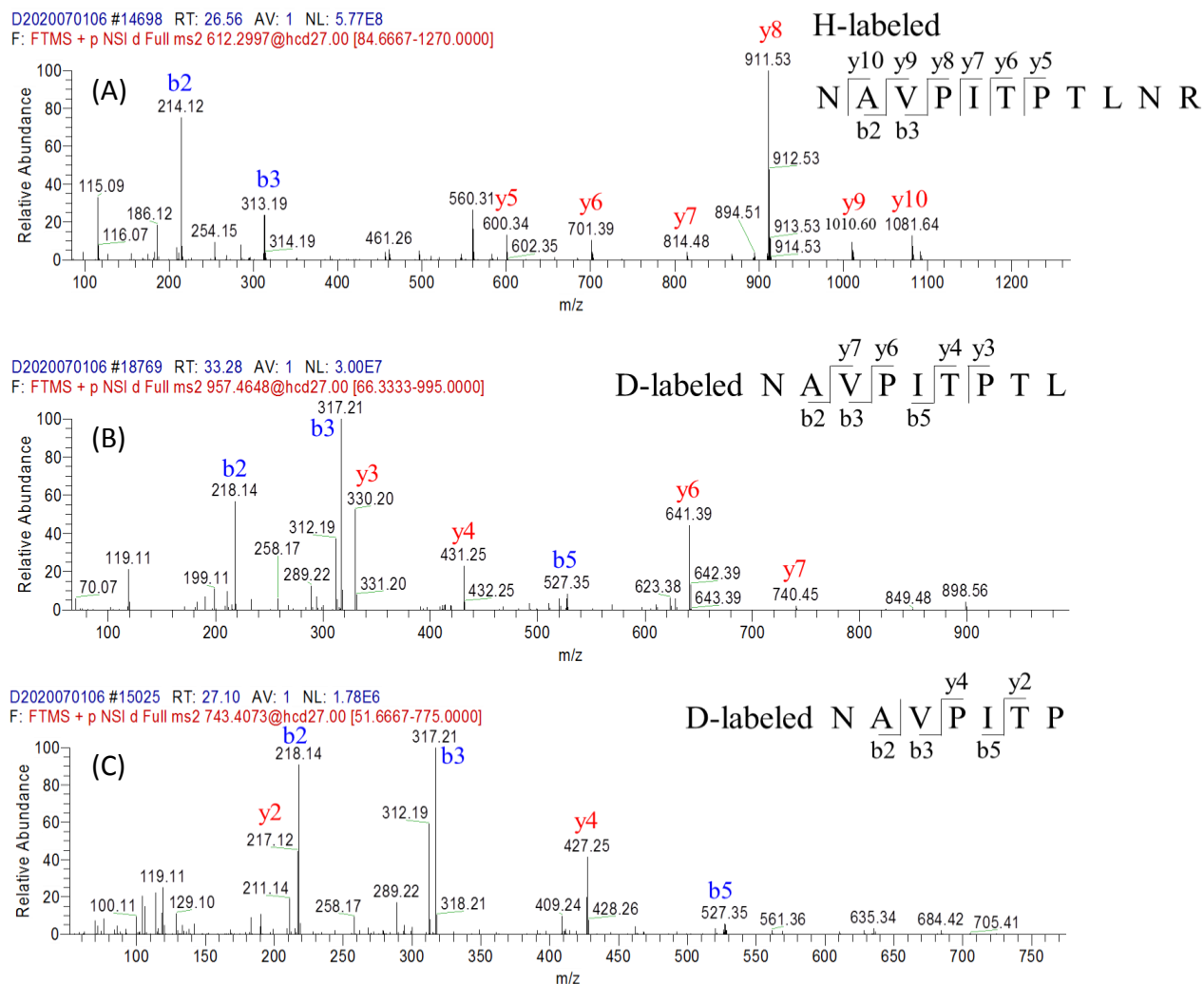


**Figure S5-1**



**Figure S5-1.** LC-MS/MS identification of ACE exogenous substrate NAVPITPTLNR (NR-11) and its product. (A) MS spectrum of D- and H-labeled NR-11; (B) MS spectrum of the product NAVPITPTL (NL-9); (C) MS spectrum of NAVPITP (NP-7).

**Figure S5-2**



**Figure S5-2.** LC-MS/MS identification of ACE exogenous substrate NAVPITPTLNR (NR-11) and its product. (A) MS/MS spectrum of H-labeled NR-11 (m/z 612.3); (B) MS/MS spectrum of the product NL-9 (m/z 957.5); (C) MS/MS spectrum of NP-7 (m/z 743.4).

**Figure S6**

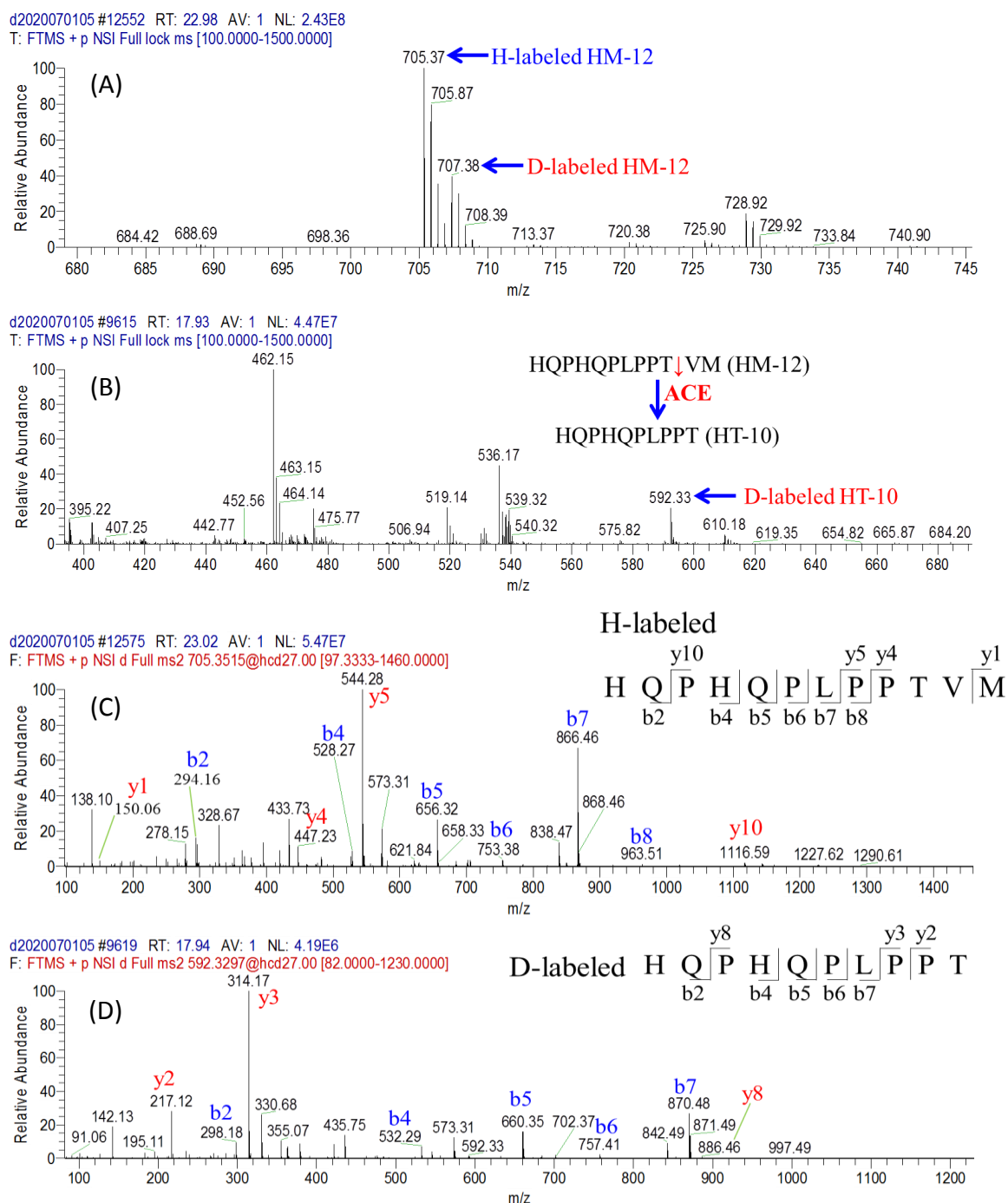


Figure S6. LC-MS/MS identification of ACE exogenous substrate HQPHQPLPPTVM (HM-12) and its product. (A) MS spectrum of D- and H-labeled HM-12; (B) MS spectrum of the product HQPHQPLPPT (HT-10); (C) MS/MS spectrum of HM-12 (m/z 705.3); (D) MS/MS of HT-10 (m/z 592.3).

**Figure S7**

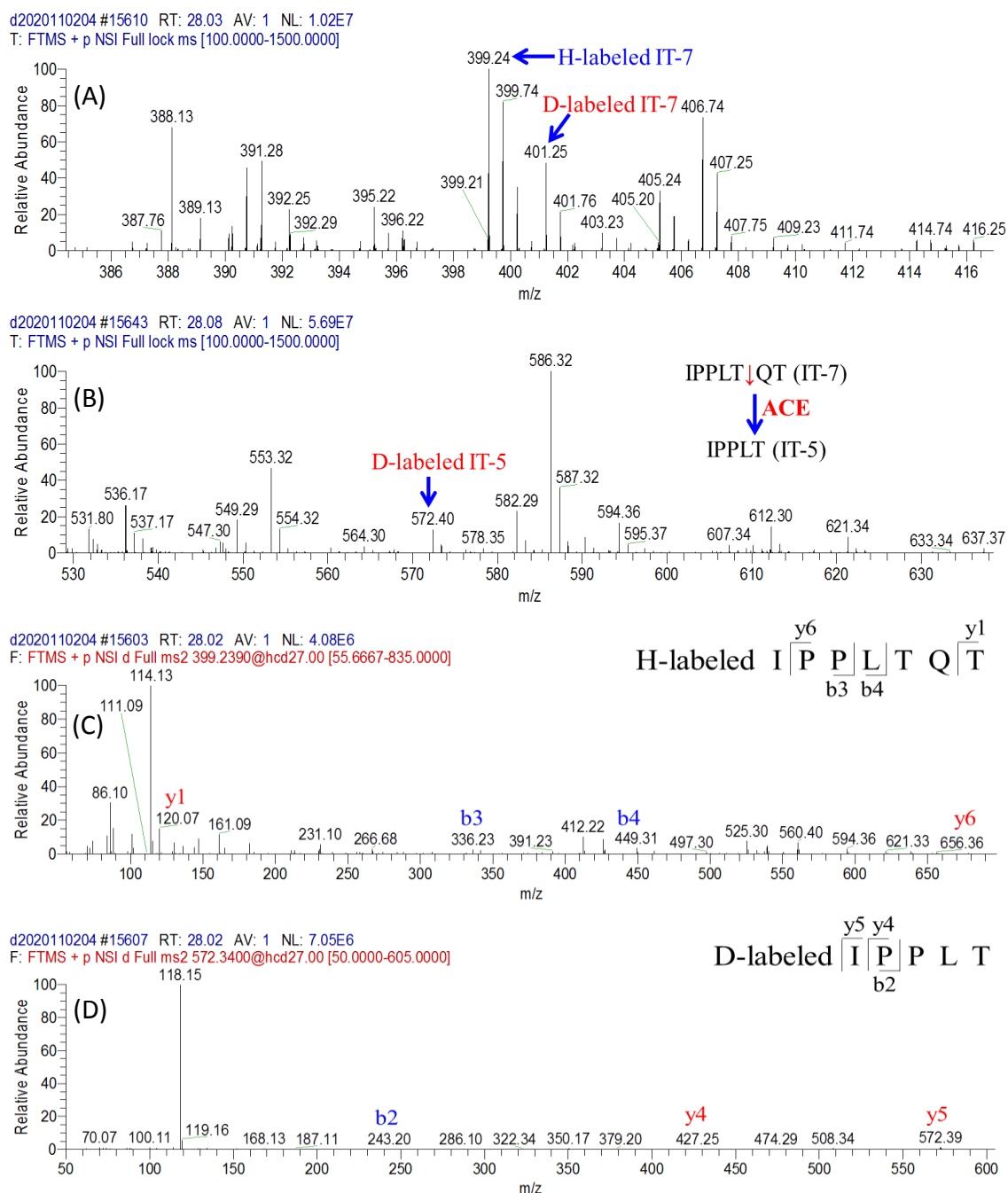


Figure S7. LC-MS/MS identification of ACE exogenous substrate IPPLTQT (IT-7) and its product. (A) MS spectrum of D- and H-labeled IT-7; (B) MS spectrum of the product IPPLT (IT-5); (C) MS/MS spectrum of IT-7 (m/z 399.2); (D) MS/MS of HT-5 (m/z 572.3).

**Figure S8**

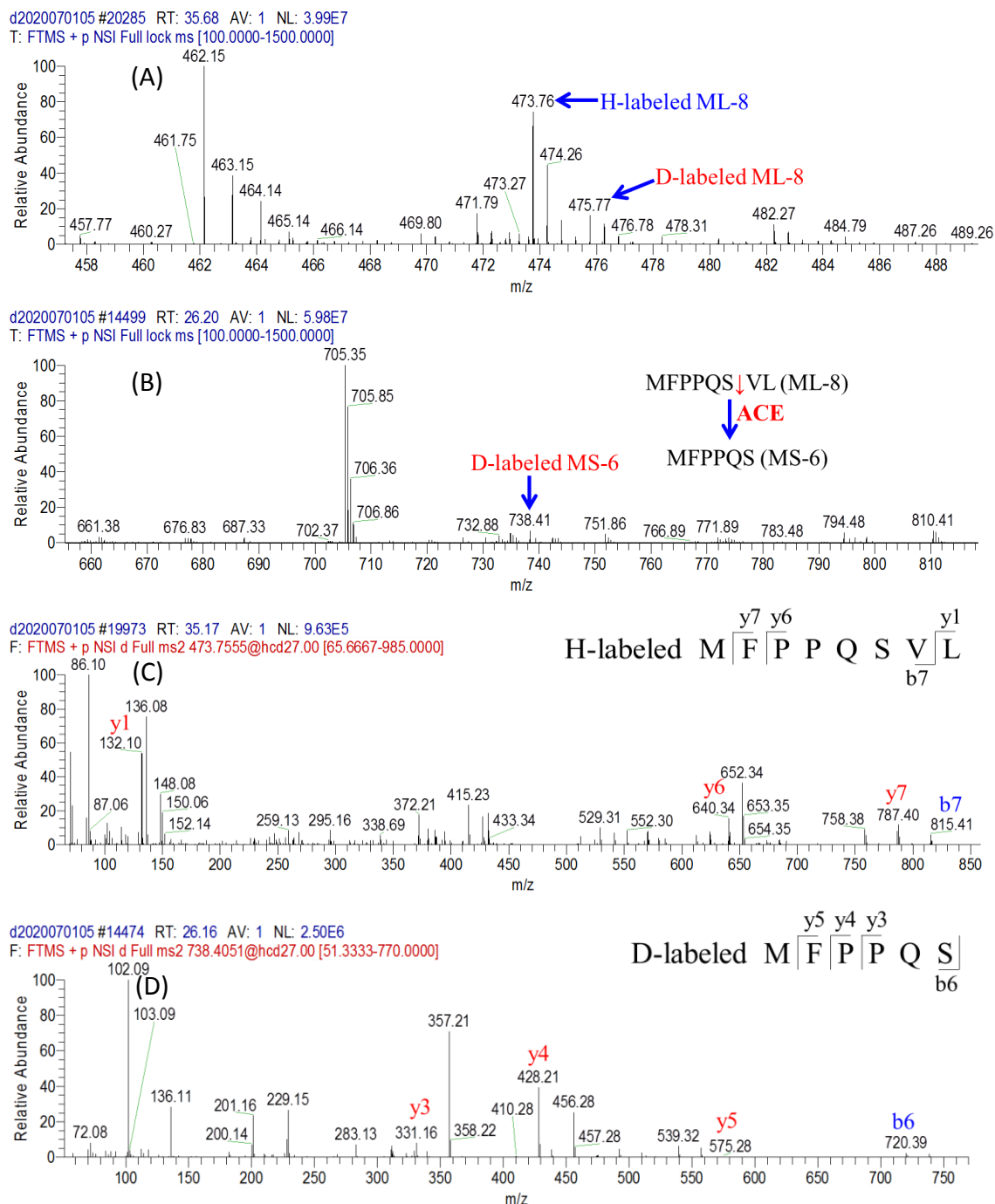


Figure S8. LC-MS/MS identification of ACE exogenous substrate MFPPQSVL (ML-8) and its product. (A) MS spectrum of D- and H-labeled ML-8; (B) MS spectrum of the product MFPPQS (MS-6); (C) MS/MS spectrum of ML-8 (m/z 473.7, +2); (D) MS/MS of MS-6 (m/z 738.4).

**Figure S9**

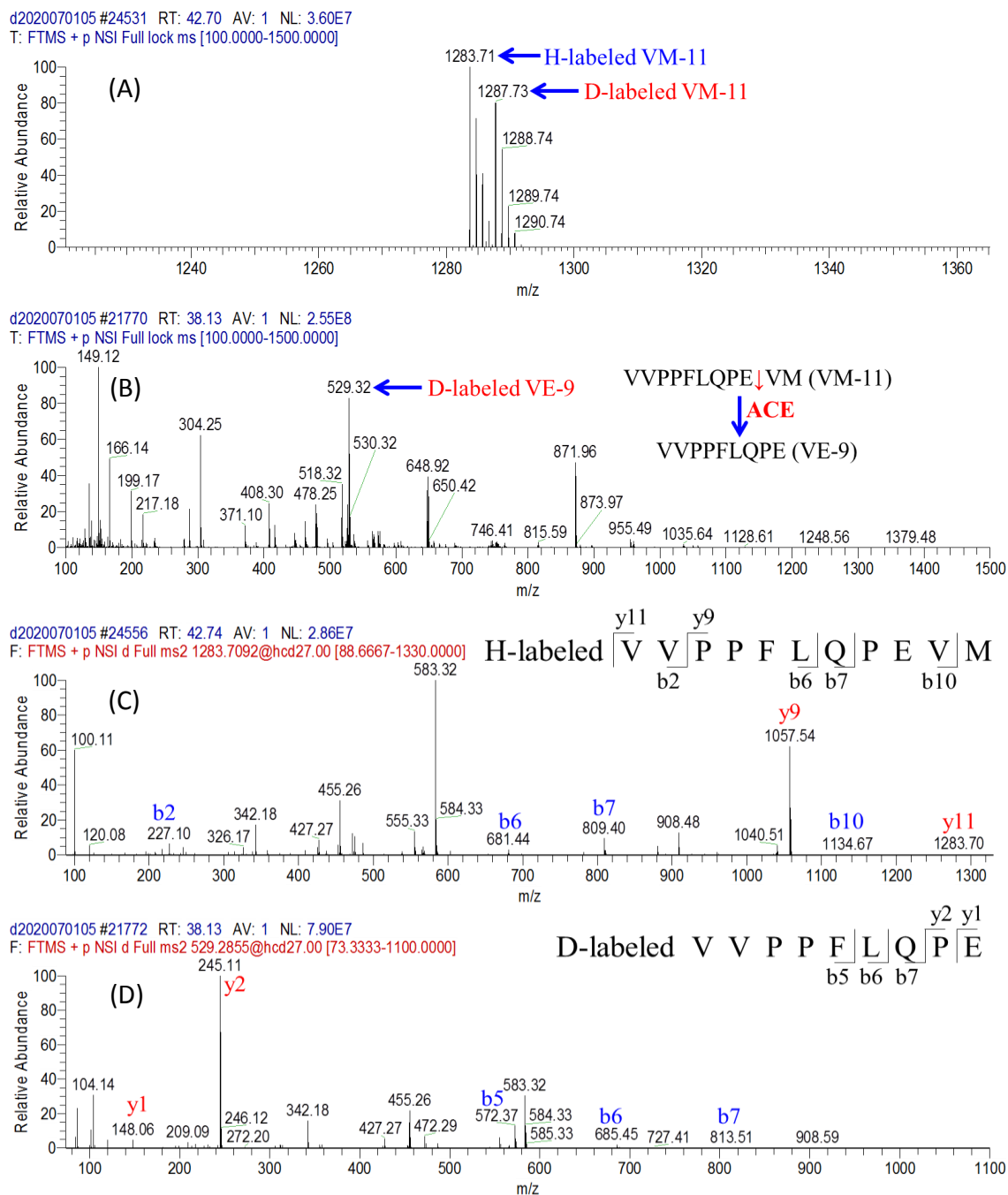


Figure S9. LC-MS/MS identification of ACE exogenous substrate VVPPFLQPEVM (VM-11) and its product. (A) MS spectrum of D- and H-labeled VM-11; (B) MS spectrum of the product VVPPFLQPE (VE-9); (C) MS/MS spectrum of VM-11 (m/z 1283.7); (D) MS/MS of VE-9 (m/z 529.2).

**Figure S10-1**

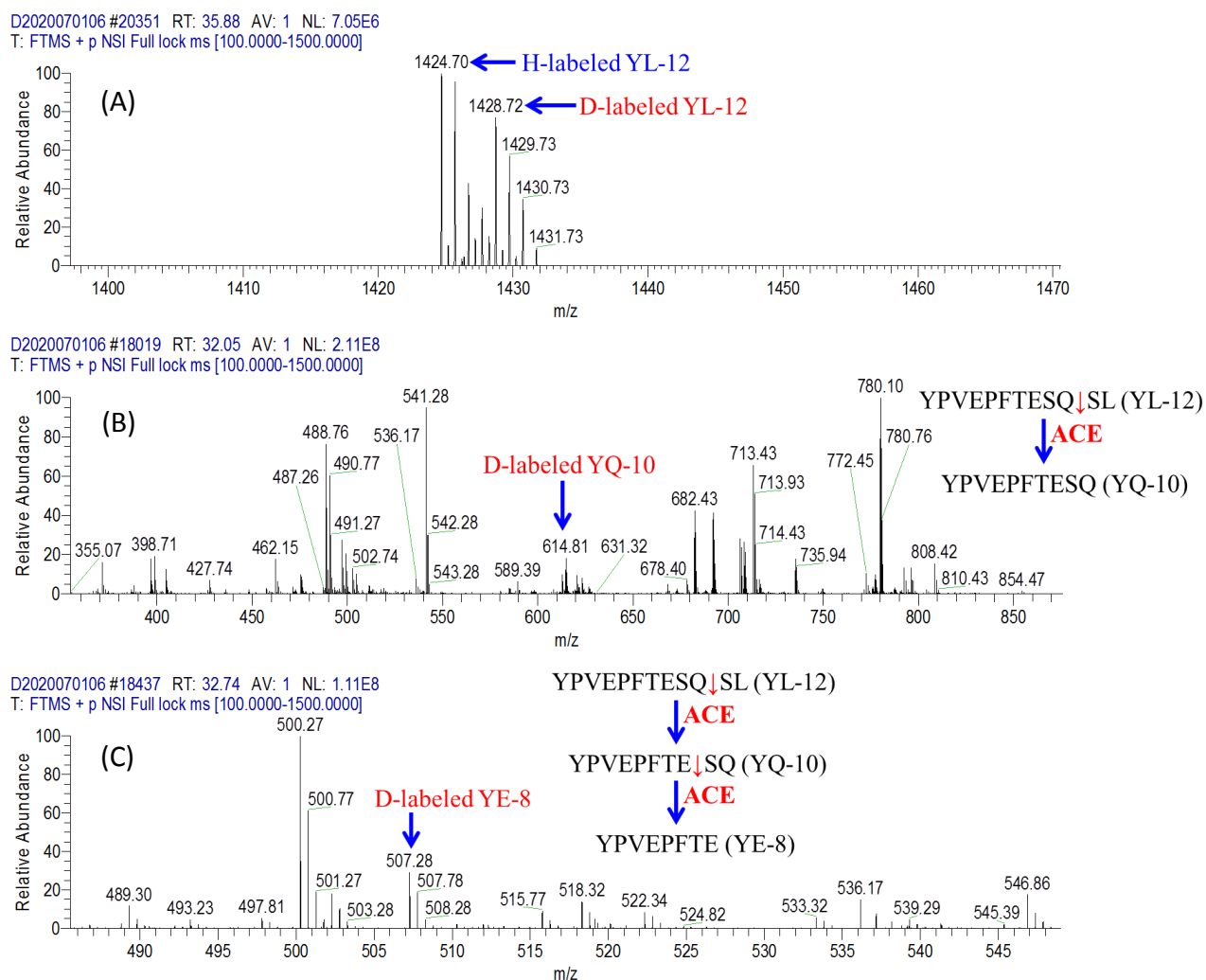


Figure S10-1. LC-MS/MS identification of ACE exogenous substrate YPVEPFOTESQSL (YL-12) and its product. (A) MS spectrum of D- and H-labeled YL-12; (B) MS spectrum of the product YPVEPFOTESQ (YQ-10); (C) MS spectrum of YPVEPFTE (YE-8).

**Figure 10S-2**

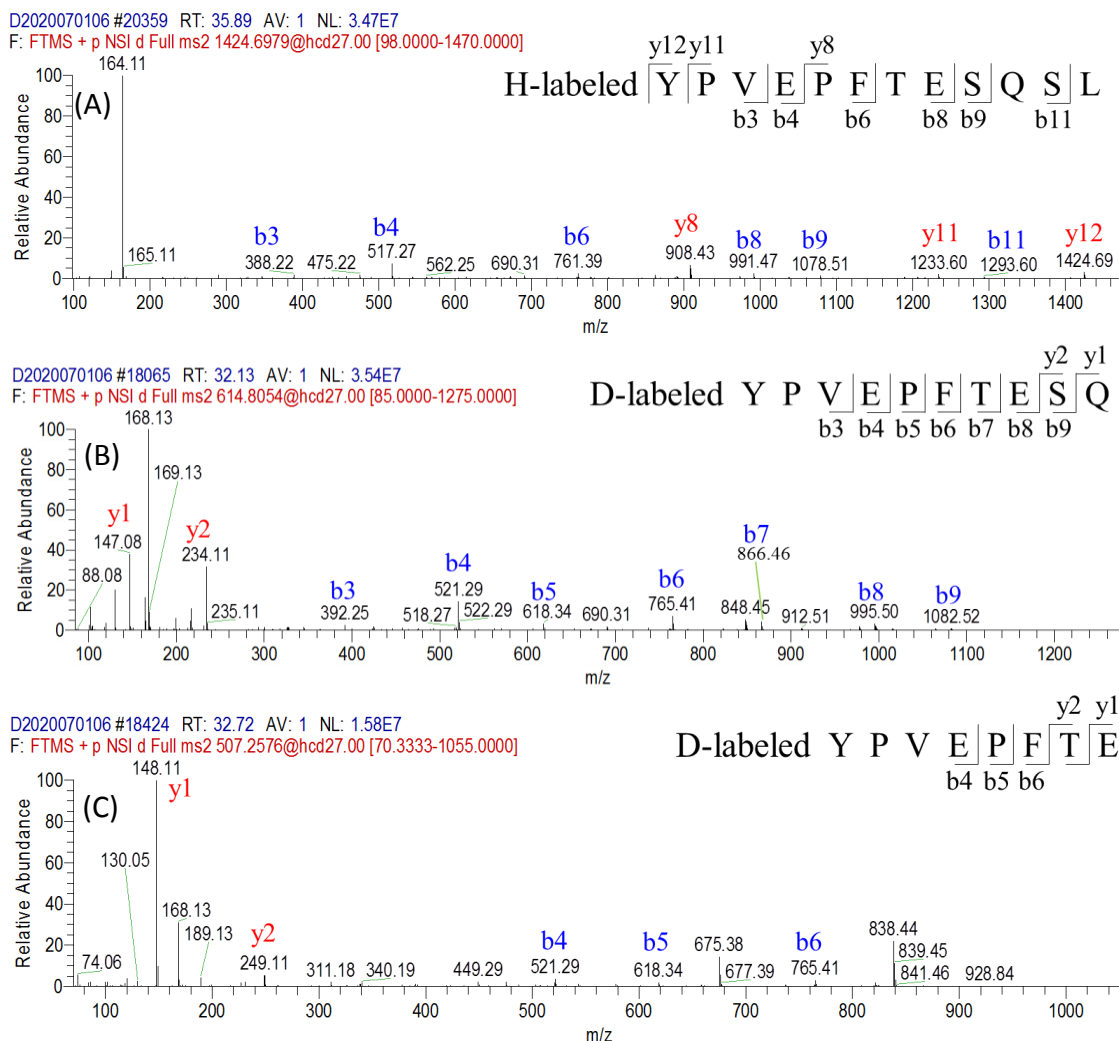
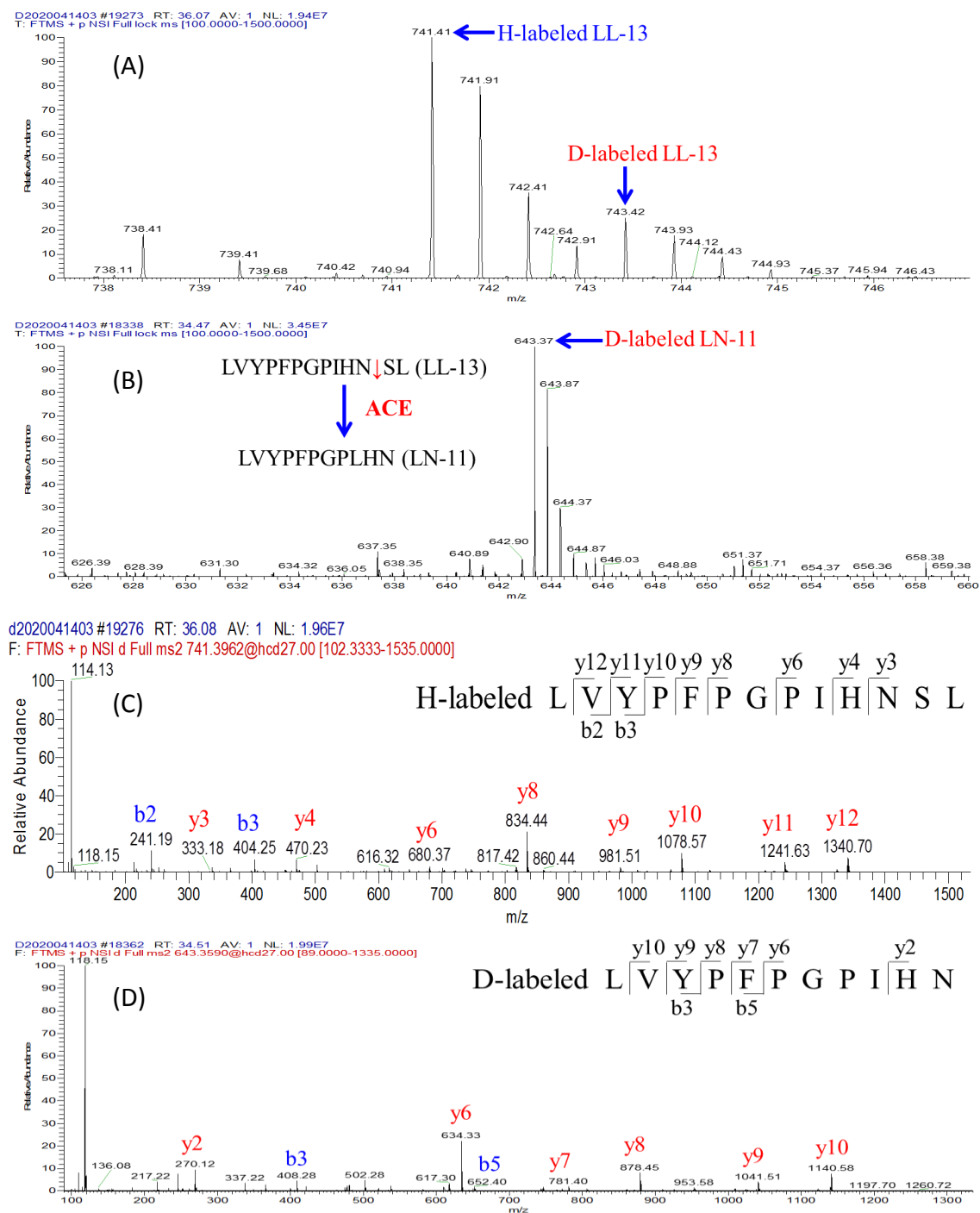


Figure S10-2. LC-MS/MS identification of ACE exogenous substrate YPVEPFTESQSL (YL-12) and its product. (A) MS/MS spectrum of YL-12 (m/z 1424.7); (B) MS/MS spectrum of the product YQ-10 (m/z 614.8); (C) MS/MS spectrum of YE-8 (m/z 507.2).

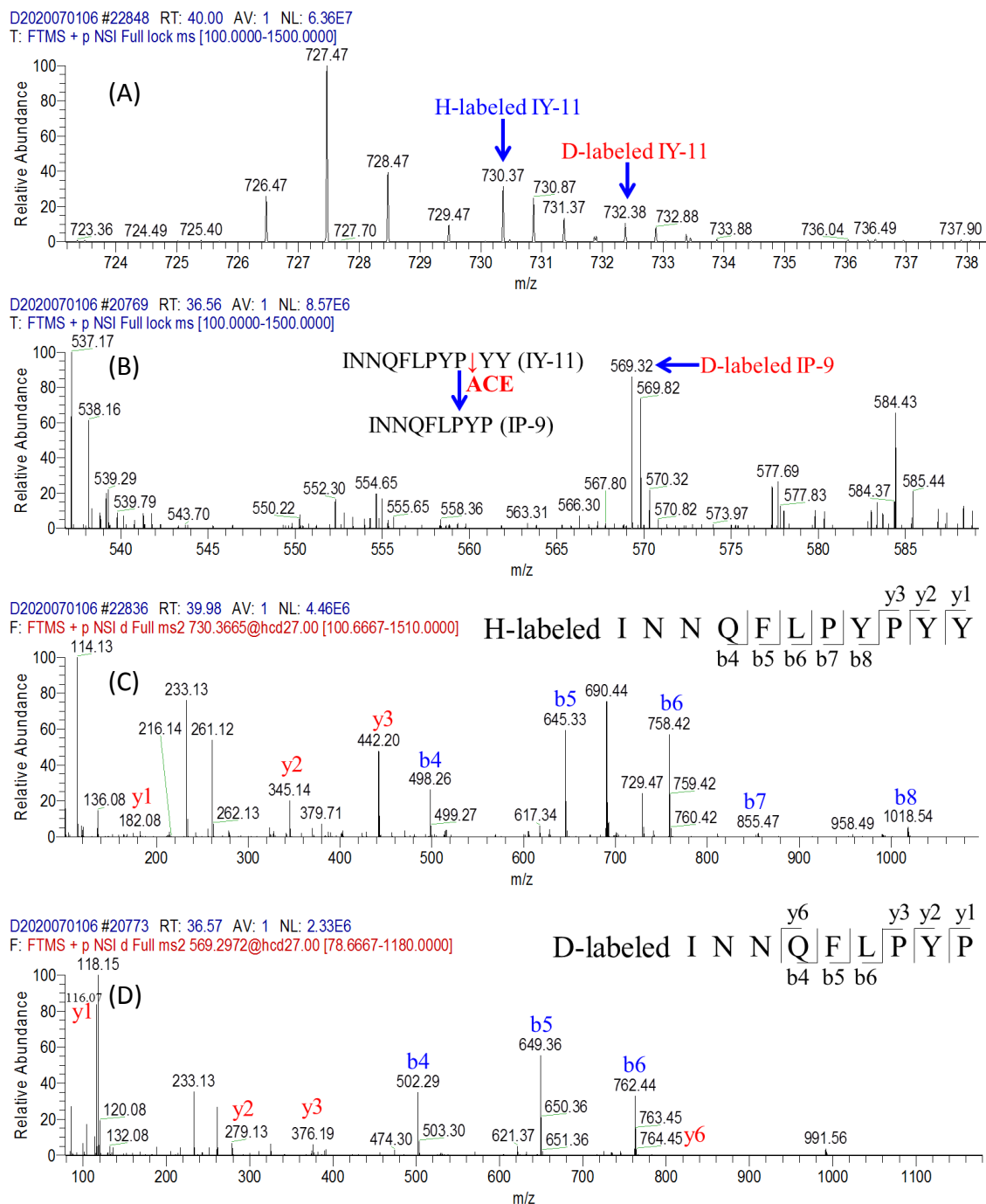


**Figure S11**



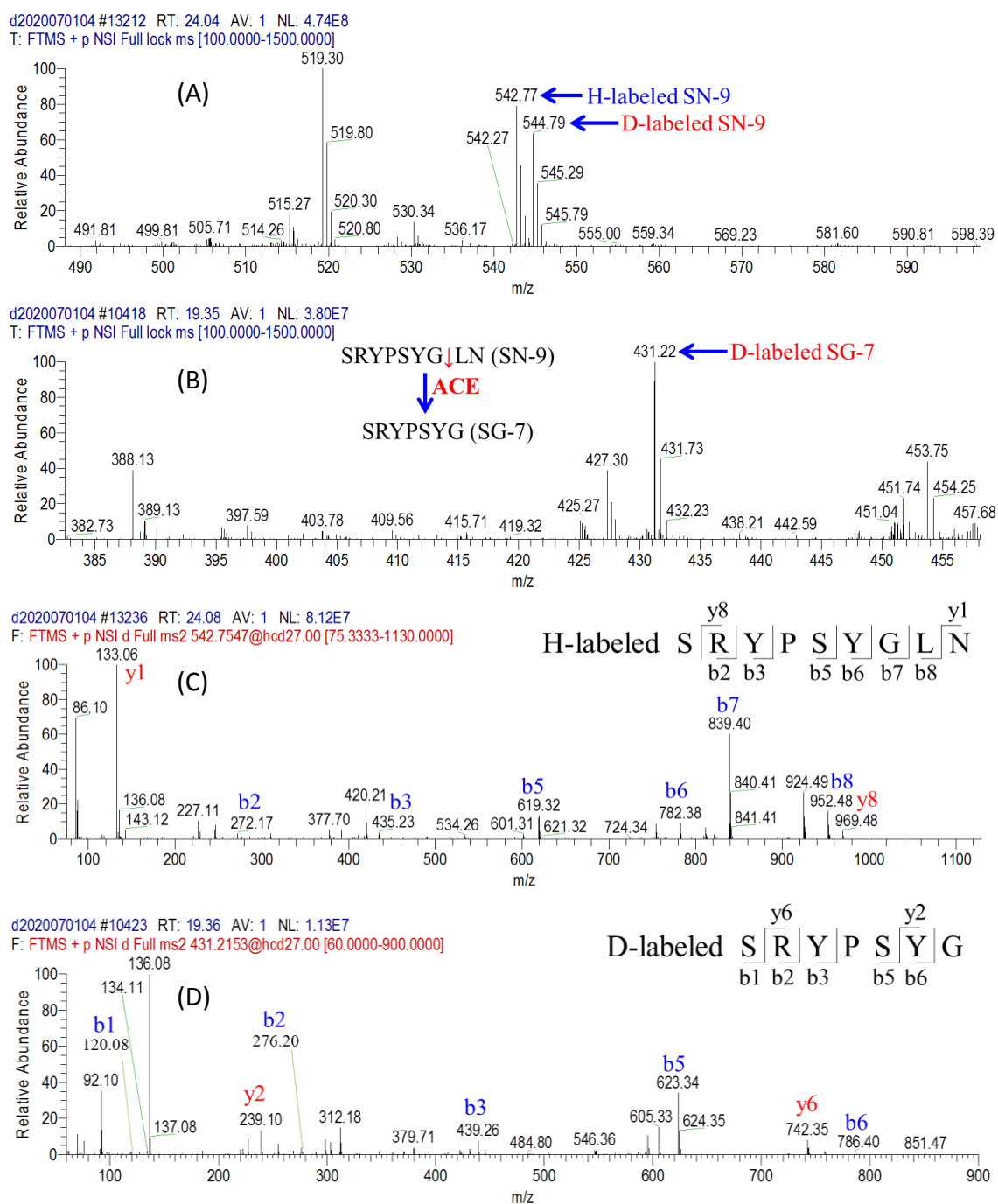
**Figure S11.** LC-MS/MS identification of ACE exogenous substrate LVYFPFGPIHNSL (LL-13) and its product. (A) MS spectrum of D- and H-labeled LL-13; (B) MS spectrum of the product LVYFPFGPIHN (LN-11); (C) MS/MS spectrum of LL-13 (m/z 741.4); (D) MS/MS of LN-11 (m/z 643.3).

**Figure S12**



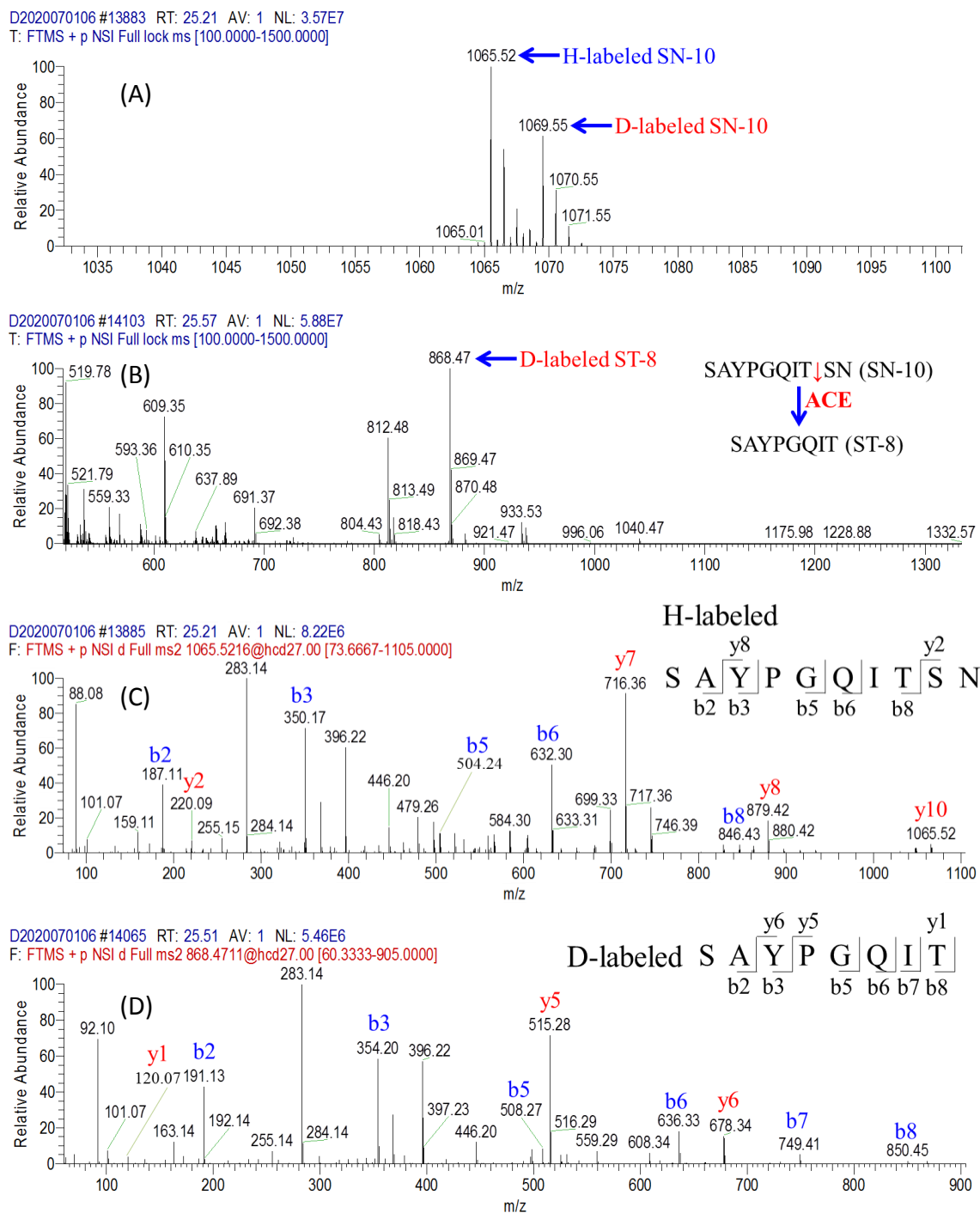
**Figure S12.** LC-MS/MS identification of ACE exogenous substrate INNQFLPYPYY (IY-11) and its product. (A) MS spectrum of D- and H-labeled IY-11; (B) MS spectrum of the product INNQFLPYP (IP-9); (C) MS/MS spectrum of IY-11 (m/z 730.3); (D) MS/MS of IP-9 (m/z 569.3).

**Figure S13**



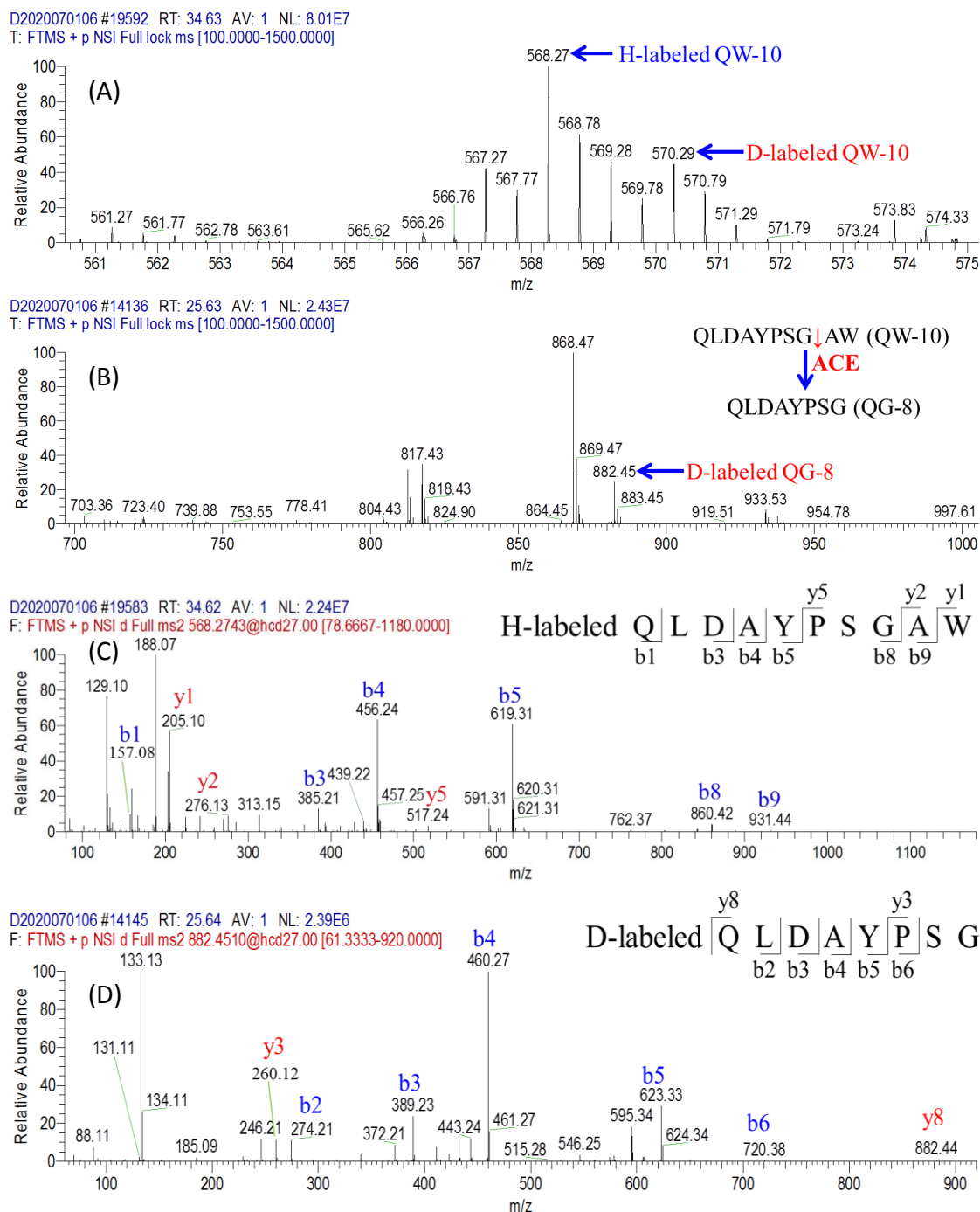
**Figure S13.** LC-MS/MS identification of ACE exogenous substrate SRYPsyGLN (SN-9) and its product. (A) MS spectrum of D- and H-labeled SN-9; (B) MS spectrum of the product SRYPsyG (SG-7); (C) MS/MS spectrum of SN-9 (m/z 542.7); (D) MS/MS of SG-7 (m/z 431.2).

**Figure S14**



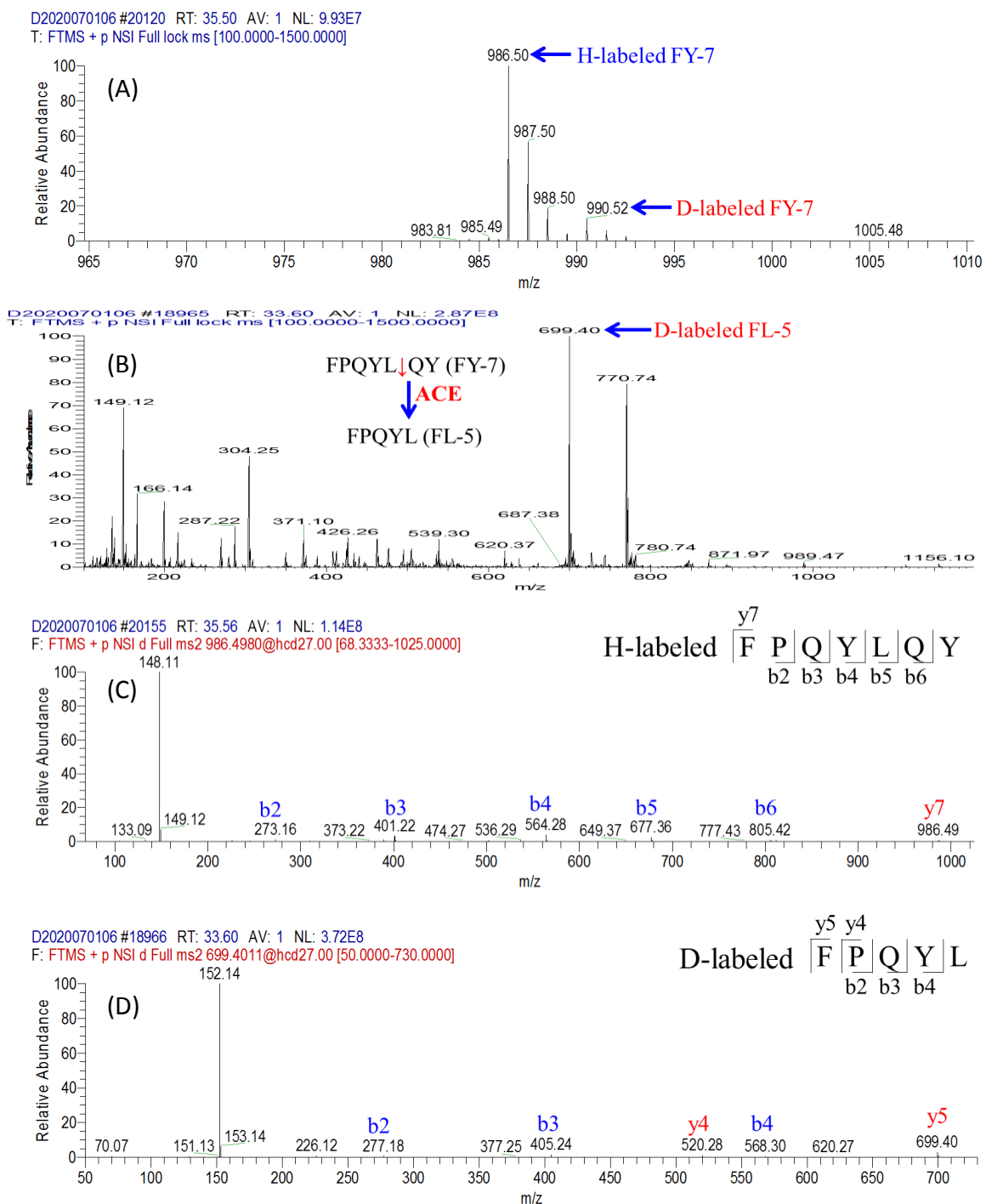
**Figure S14.** LC-MS/MS identification of ACE exogenous substrate SAYPGQITSN (SN-10) and its product. (A) MS spectrum of D- and H-labeled SN-10; (B) MS spectrum of the product SAYPGQIT (ST-8); (C) MS/MS spectrum of SN-10 (m/z 1065.5); (D) MS/MS of ST-8 (m/z 868.4).

**Figure S15**



**Figure S15.** LC-MS/MS identification of ACE exogenous substrate QLDAYPSGAW (QW-10) and its product. (A) MS spectrum of D- and H-labeled QW-10; (B) MS spectrum of the product QLDAYPSG (QG-8); (C) MS/MS spectrum of QW-10 (m/z 568.2); (D) MS/MS of QG-8 (m/z 882.4).

**Figure S16**



**Figure S16.** LC-MS/MS identification of ACE exogenous substrate FPQYLQY (FY-7) and its product. (A) MS spectrum of D- and H-labeled FY-7; (B) MS spectrum of the product FPQYL (FL-5); (C) MS/MS spectrum of FY-7 (m/z 986.4); (D) MS/MS of FL-5 (m/z 699.4).

**Figure S17**

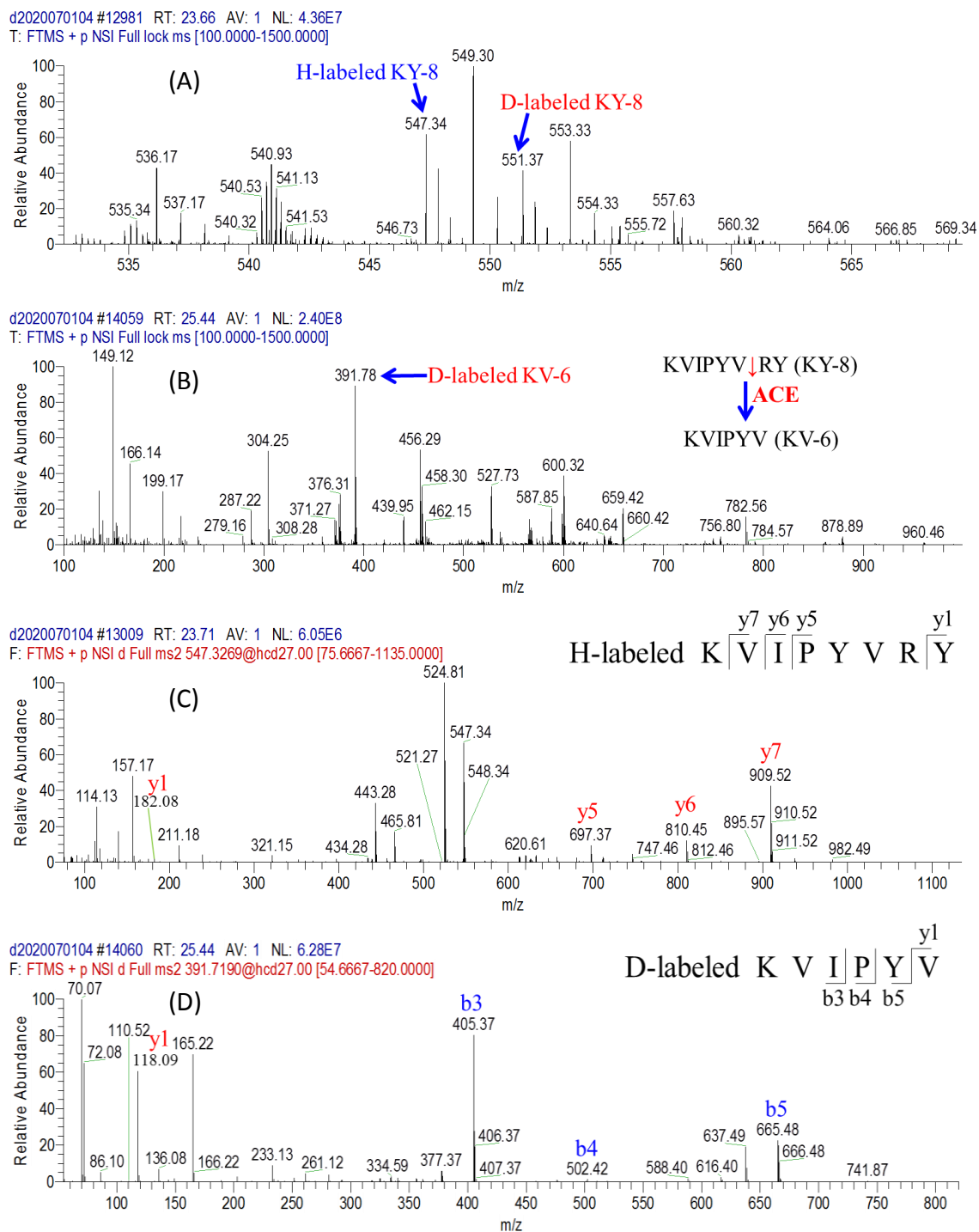


Figure S17. LC-MS/MS identification of ACE exogenous substrate KVIPYVRY (KY-8) and its product. (A) MS spectrum of D- and H-labeled KY-8; (B) MS spectrum of the product KVIPYV (KV-6); (C) MS/MS spectrum of KY-8 (m/z 547.3); (D) MS/MS of KV-6 (m/z 391.7).

**Figure S18**

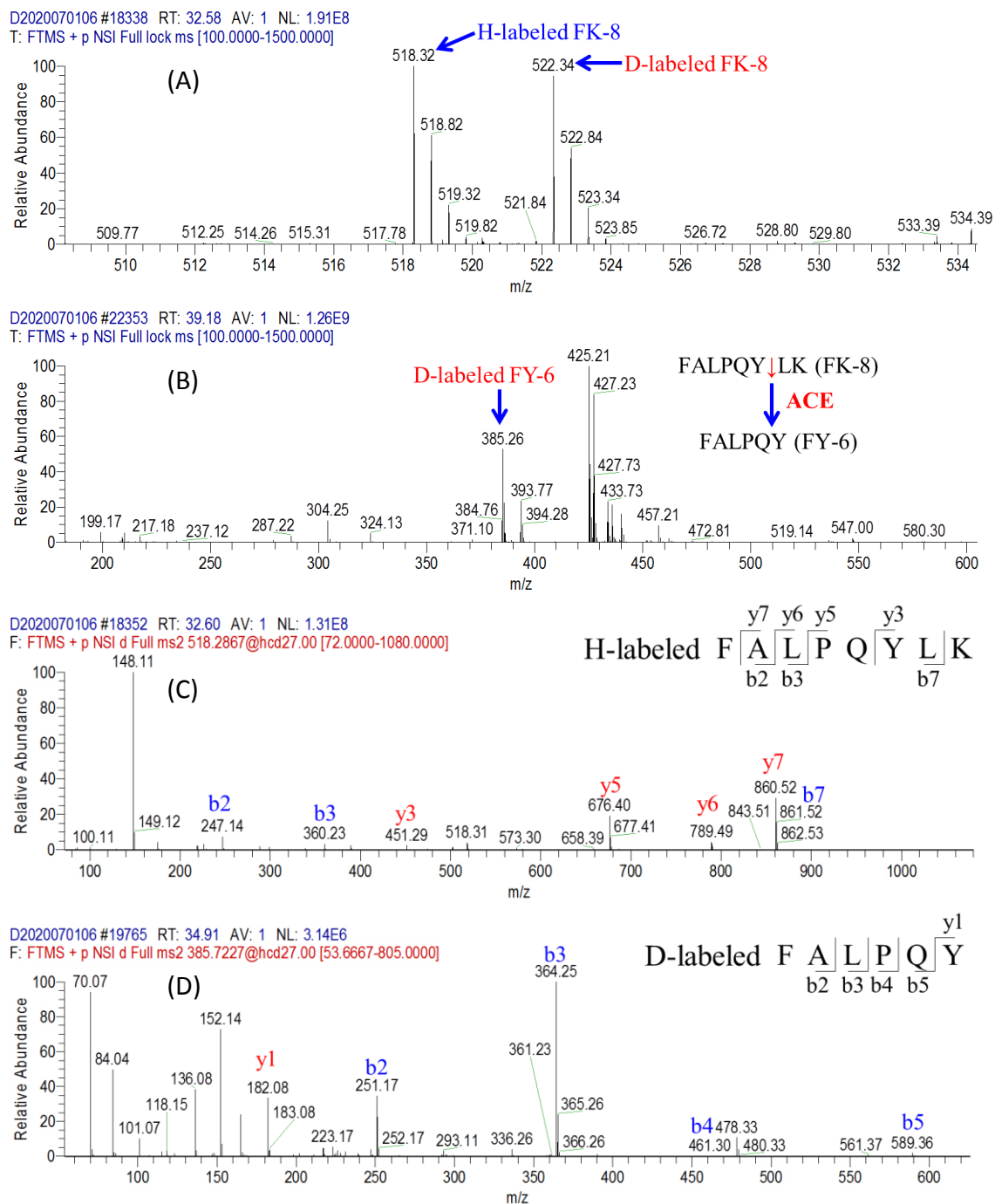


Figure S18. LC-MS/MS identification of ACE exogenous substrate FALPQYLK (FK-8) and its product. (A) MS spectrum of D- and H-labeled FK-8; (B) MS spectrum of the product FALPQY (FY-6); (C) MS/MS spectrum of FK-8 (m/z 518.2); (D) MS/MS of FY-6 (m/z 385.7).



**Figure S19-1**

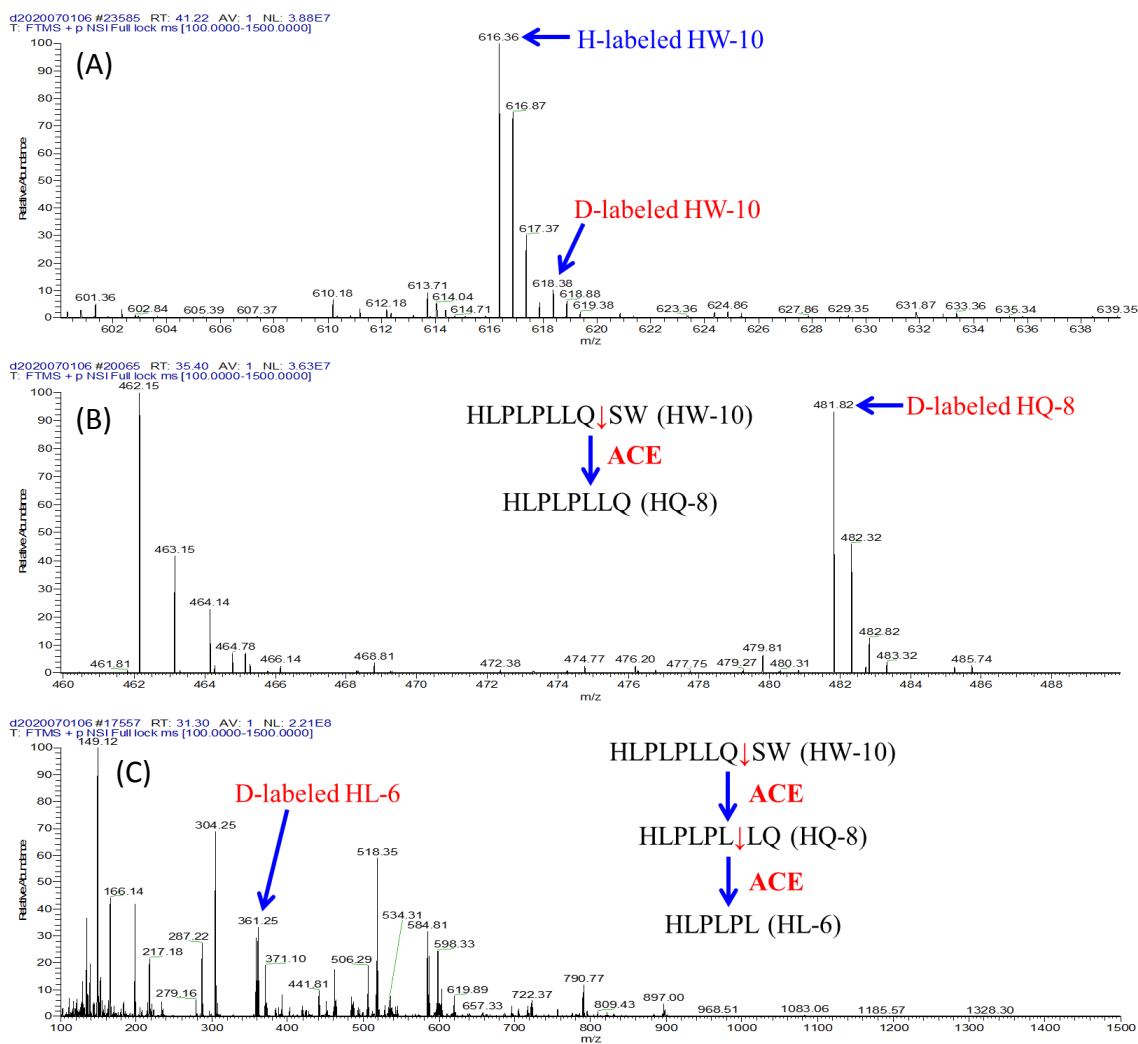


Figure S19-1. LC-MS/MS identification of ACE exogenous substrate HLPLPLLQSW (HW-10) and its product. (A) MS spectrum of D- and H-labeled HW-10; (B) MS spectrum of the product HLPLPLLQ (HQ-8); (C) MS spectrum of HLPLPL (HL-6).

**Figure S19-2**

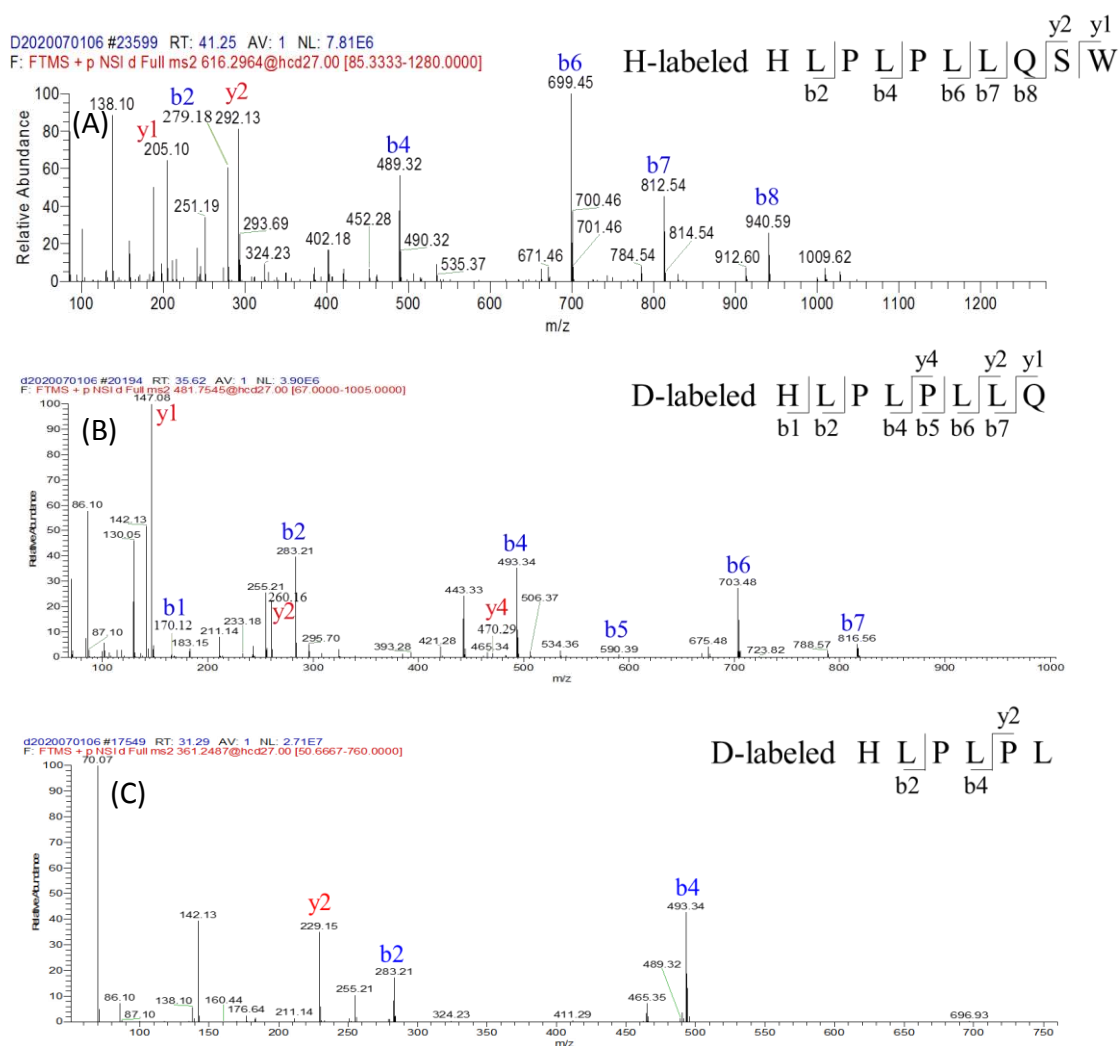


Figure S19-2. LC-MS/MS identification of ACE exogenous substrate HLPLLLQSW (HW-10) and its products. (A) MS spectrum of H-labeled HW-10 (m/z 616.2); (B) MS/MS spectrum of the product HQ-8 (m/z 481.7); (C) MS/MS spectrum of HL-6 (m/z 361.2).

**Figure S20**

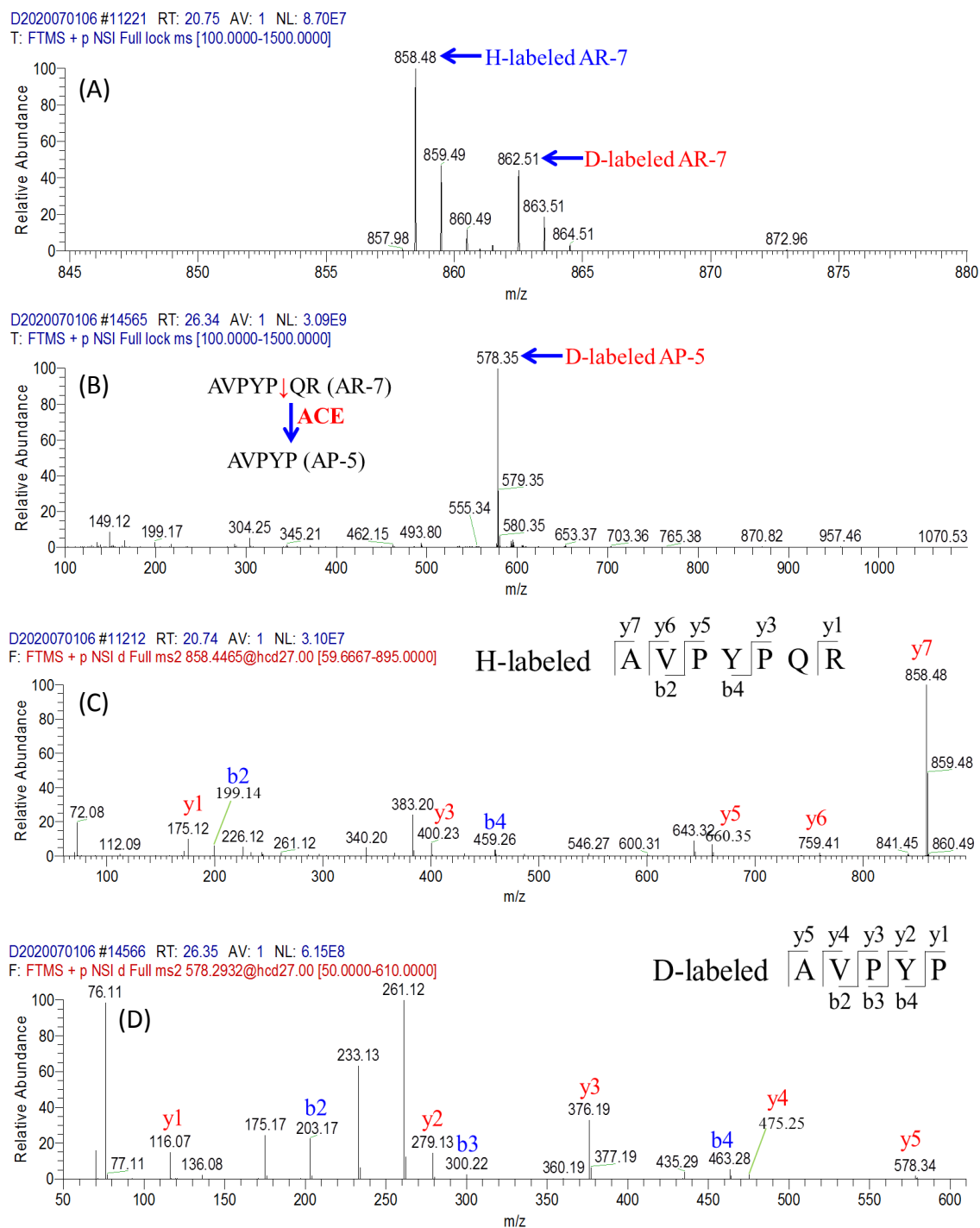


Figure S20. LC-MS/MS identification of ACE exogenous substrate AVPYPQR (AR-7) and its product. (A) MS spectrum of D- and H-labeled AR-7; (B) MS spectrum of the product AVPYP (AP-5); (C) MS/MS spectrum of AR-7 (m/z 858.4); (D) MS/MS of AP-5 (m/z 578.2).

**Figure S21**

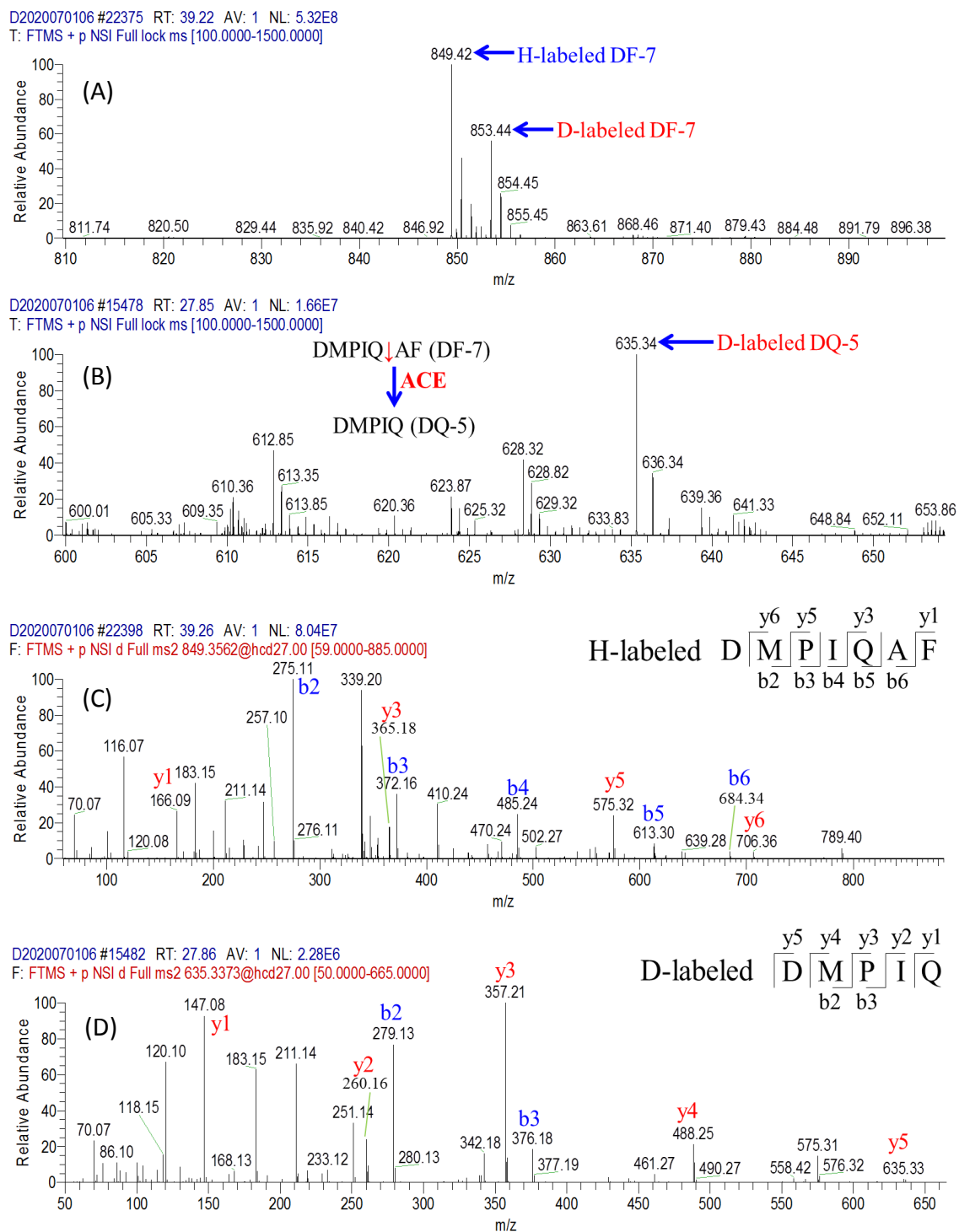
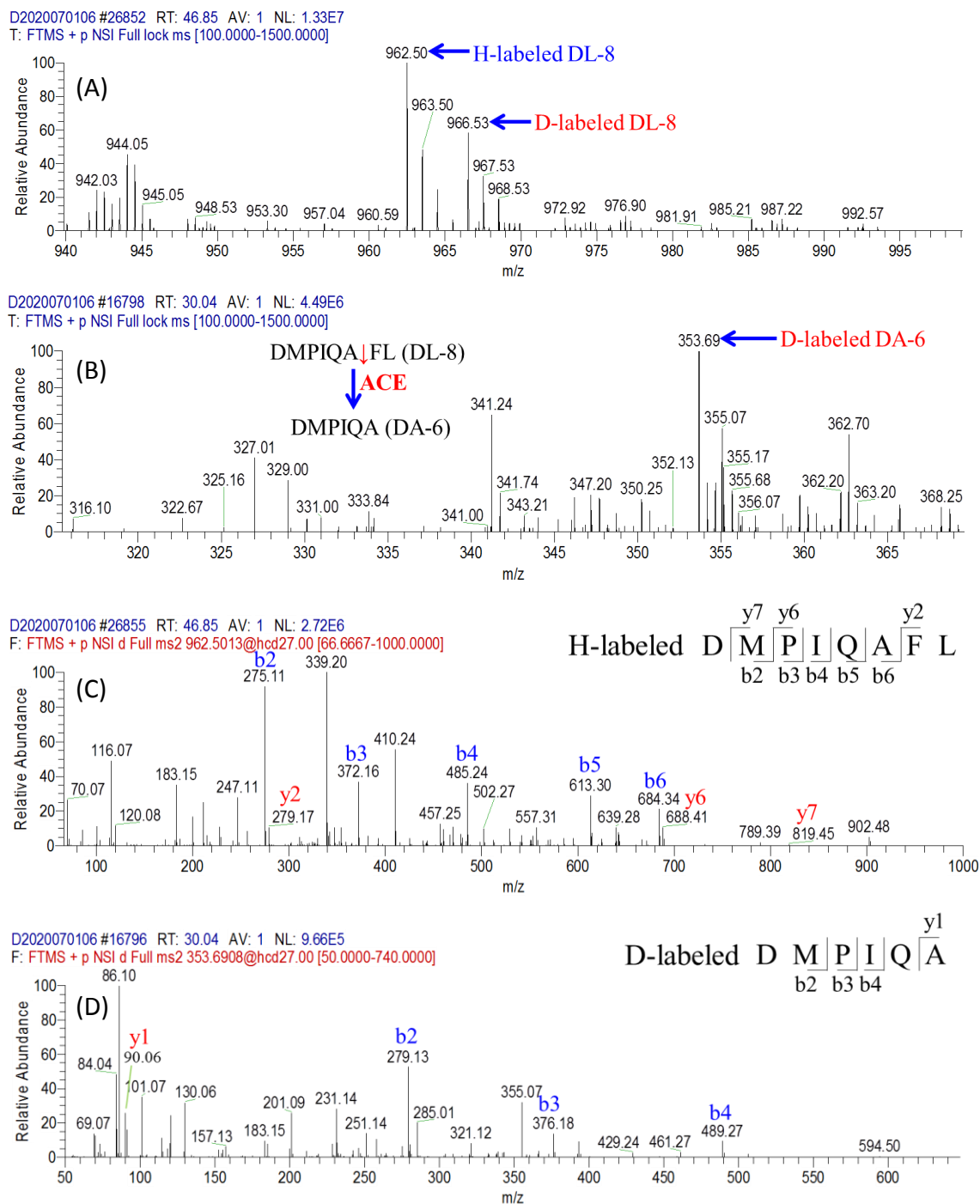


Figure S21. LC-MS/MS identification of ACE exogenous substrate DMPIQAF (DF-7) and its product. (A) MS spectrum of D- and H-labeled DF-7; (B) MS spectrum of the product DMPIQ (DQ-5); (C) MS/MS spectrum of DF-7 (m/z 849.3); (D) MS/MS of DQ-5 (m/z 635.3).

**Figure S22**



**Figure S22.** LC-MS/MS identification of ACE exogenous substrate DMPIQAFL (DL-8) and its product. (A) MS spectrum of D- and H-labeled DL-8; (B) MS spectrum of the product DMPIQA (DA-6); (C) MS/MS spectrum of DL-8 (m/z 962.5); (D) MS/MS of DA-6 (m/z 353.6).

**Figure S23-1**

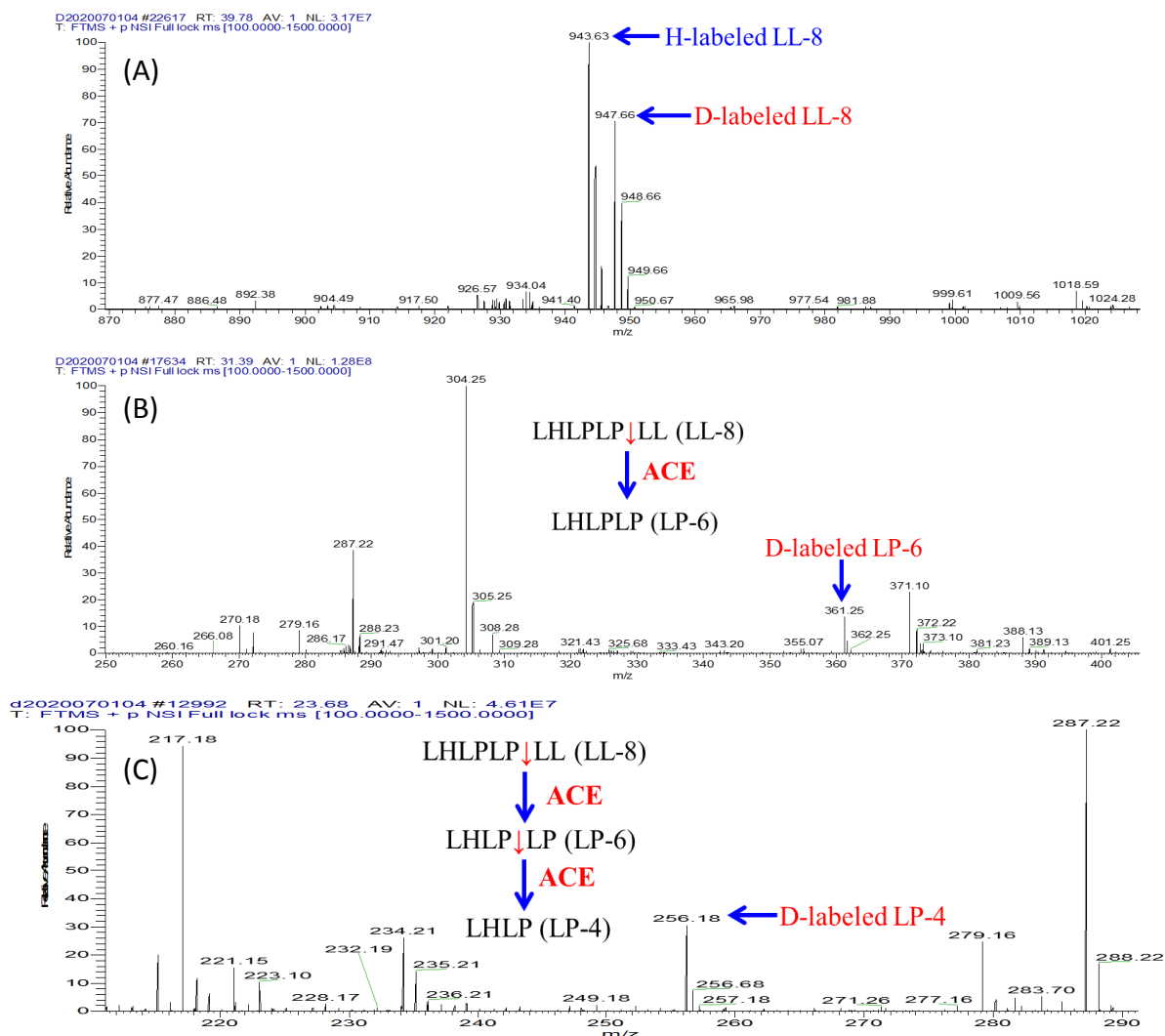


Figure S23-1. LC-MS/MS identification of ACE exogenous substrate LHLPLPLL (LL-8) and its products. (A) MS spectrum of D- and H-labeled LL-8; (B) MS spectrum of the product LHLPLP (LP-6); (C) MS spectrum of LHLPLP (LP-4).

**Figure S23-2**

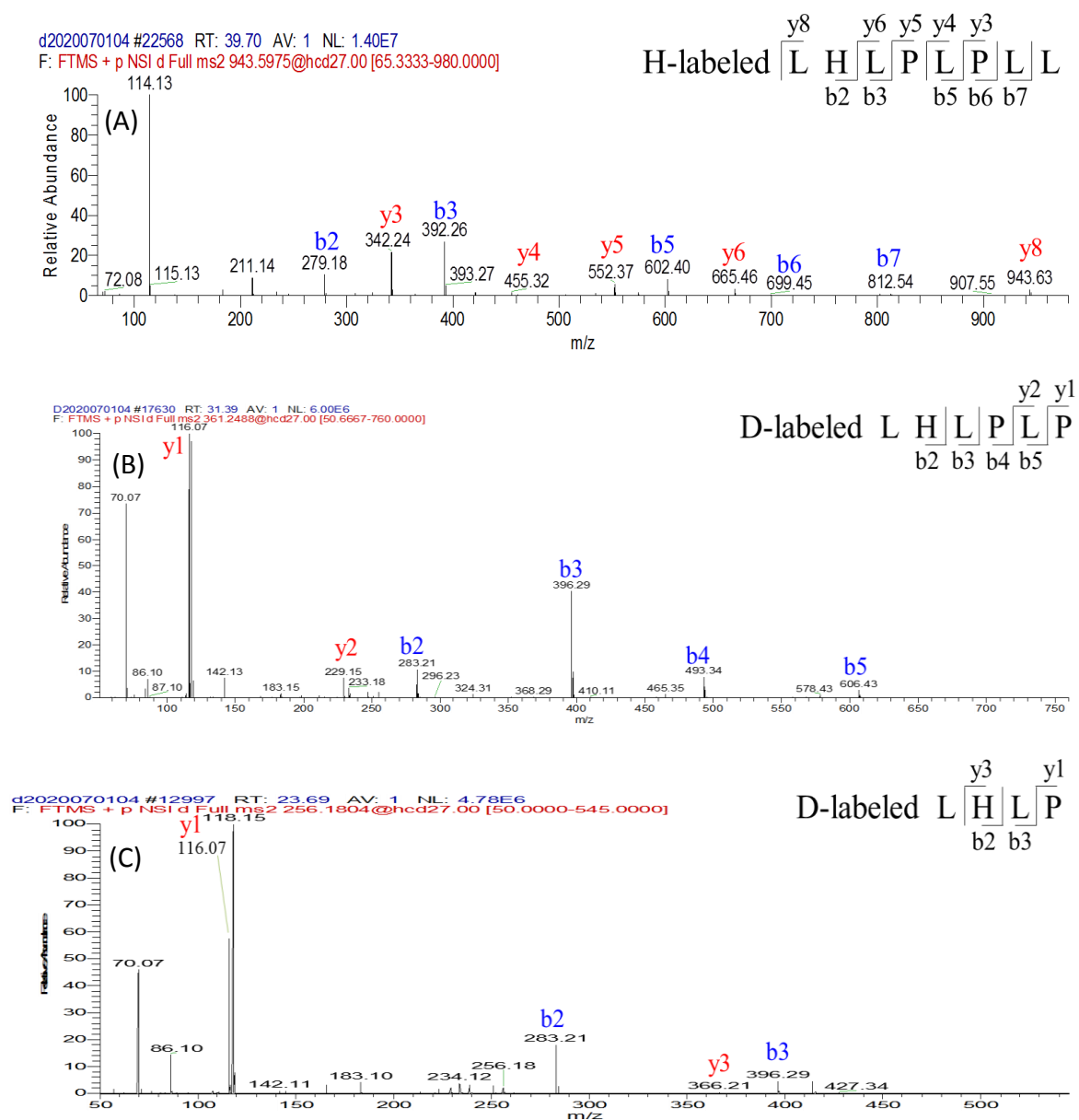
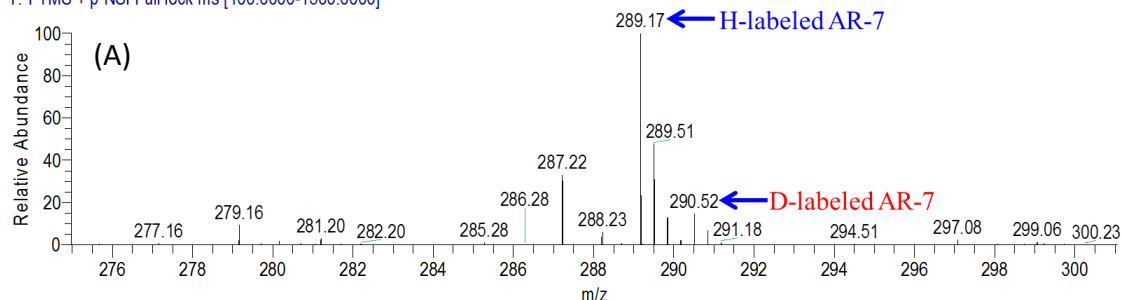


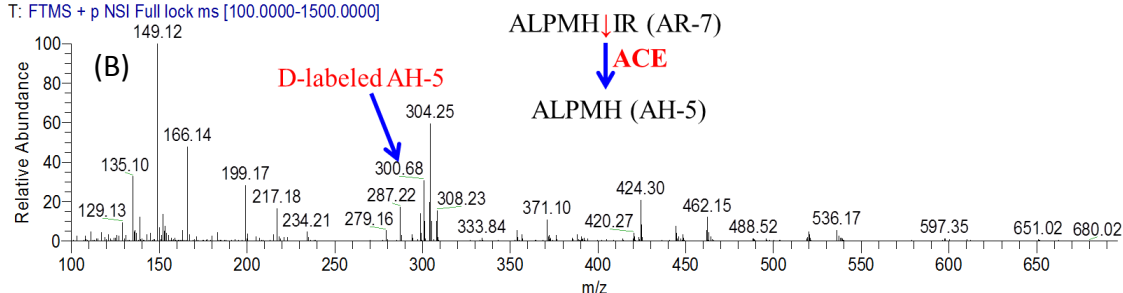
Figure S23-2. LC-MS/MS identification of ACE exogenous substrate LHLPLPLL (LL-8) and its products. (A) MS/MS spectrum of H-labeled LL-8 (m/z 943.5); (B) MS/MS spectrum of the product LP-6 (m/z 361.2); (C) MS spectrum of LP-4 (m/z 256.1).

**Figure S24**

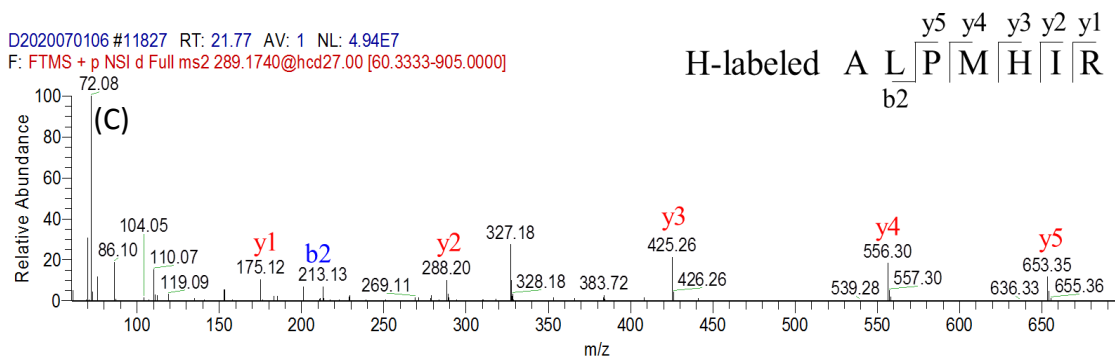
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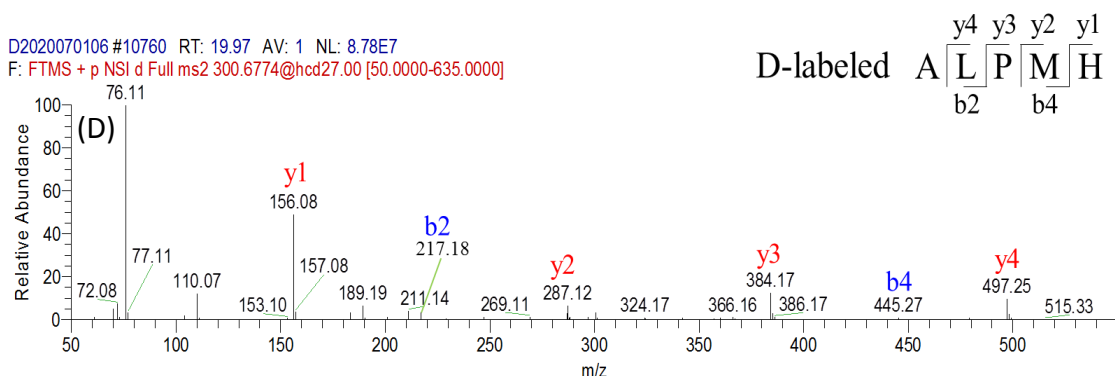
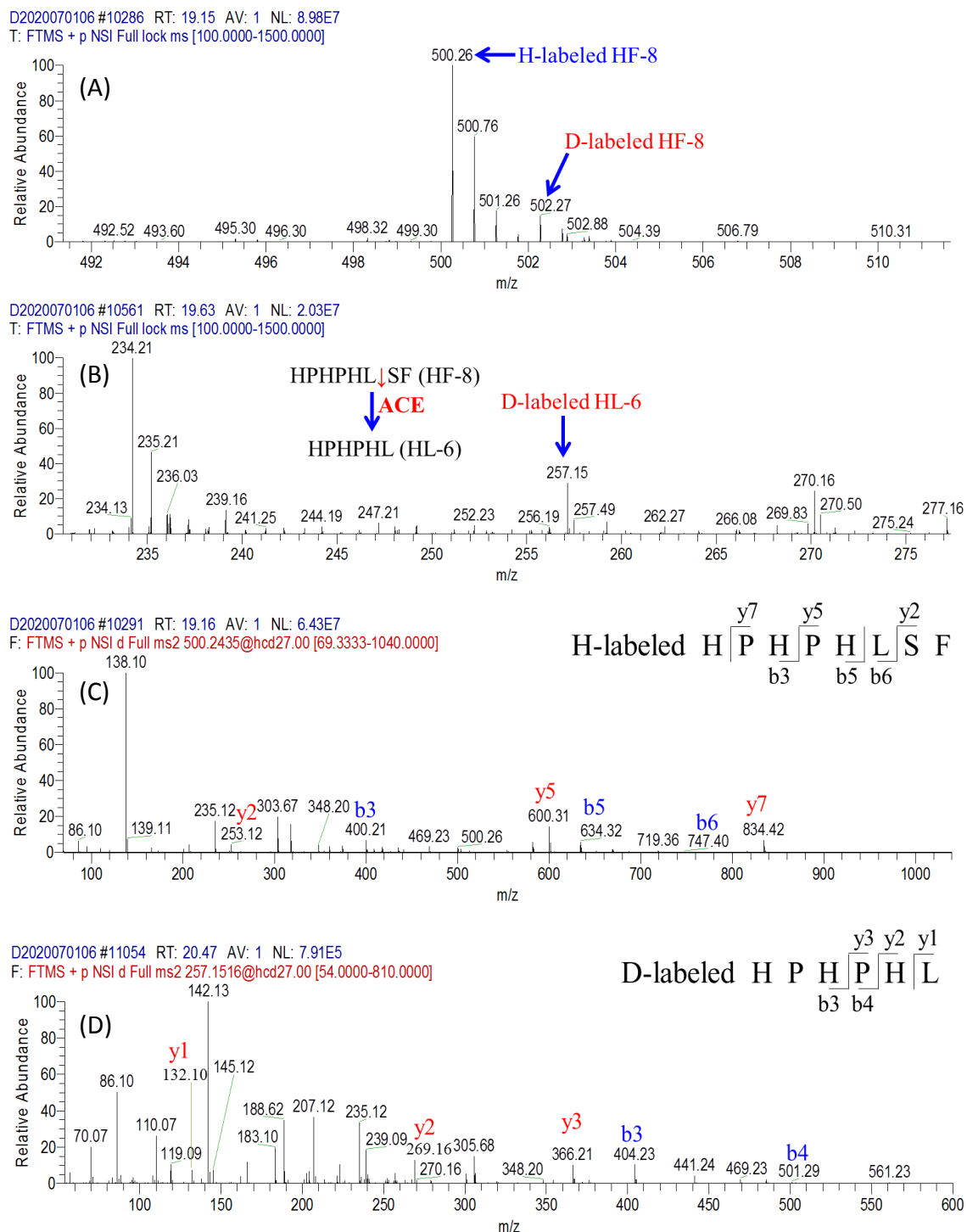


Figure S24. LC-MS/MS identification of ACE exogenous substrate ALPMHIR (AR-7) and its product. (A) MS spectrum of D- and H-labeled AR-7; (B) MS spectrum of the product ALPMH (AH-5); (C) MS/MS spectrum of AR-7 (m/z 289.1); (D) MS/MS of AH-5 (m/z 300.6).

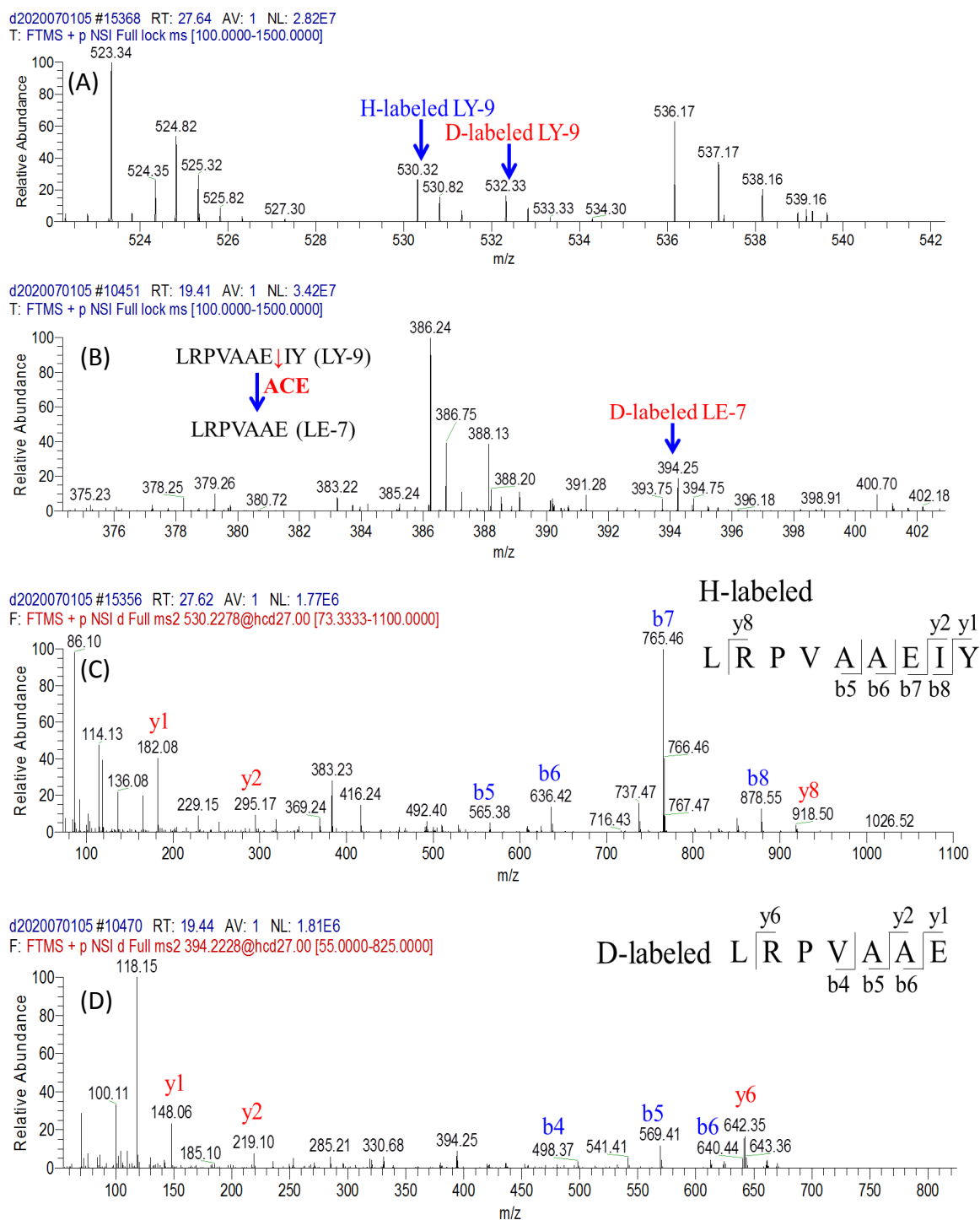


**Figure S25**



**Figure S25.** LC-MS/MS identification of ACE exogenous substrate HPHPHLSF (HF-8) and its product. (A) MS spectrum of D- and H-labeled HF-8; (B) MS spectrum of the product HPHPHL (HL-6); (C) MS/MS spectrum of HF-8 (m/z 500.2); (D) MS/MS of HL-6 (m/z 257.1).

**Figure S26**



**Figure S26.** LC-MS/MS identification of ACE exogenous substrate LRPVAAEIIY (LY-9) and its product. (A) MS spectrum of D- and H-labeled LY-9; (B) MS spectrum of the product LRPVAAE (LE-7); (C) MS/MS spectrum of LY-9 (m/z 530.2); (D) MS/MS of LE-7 (m/z 394.2).

**Figure S27**

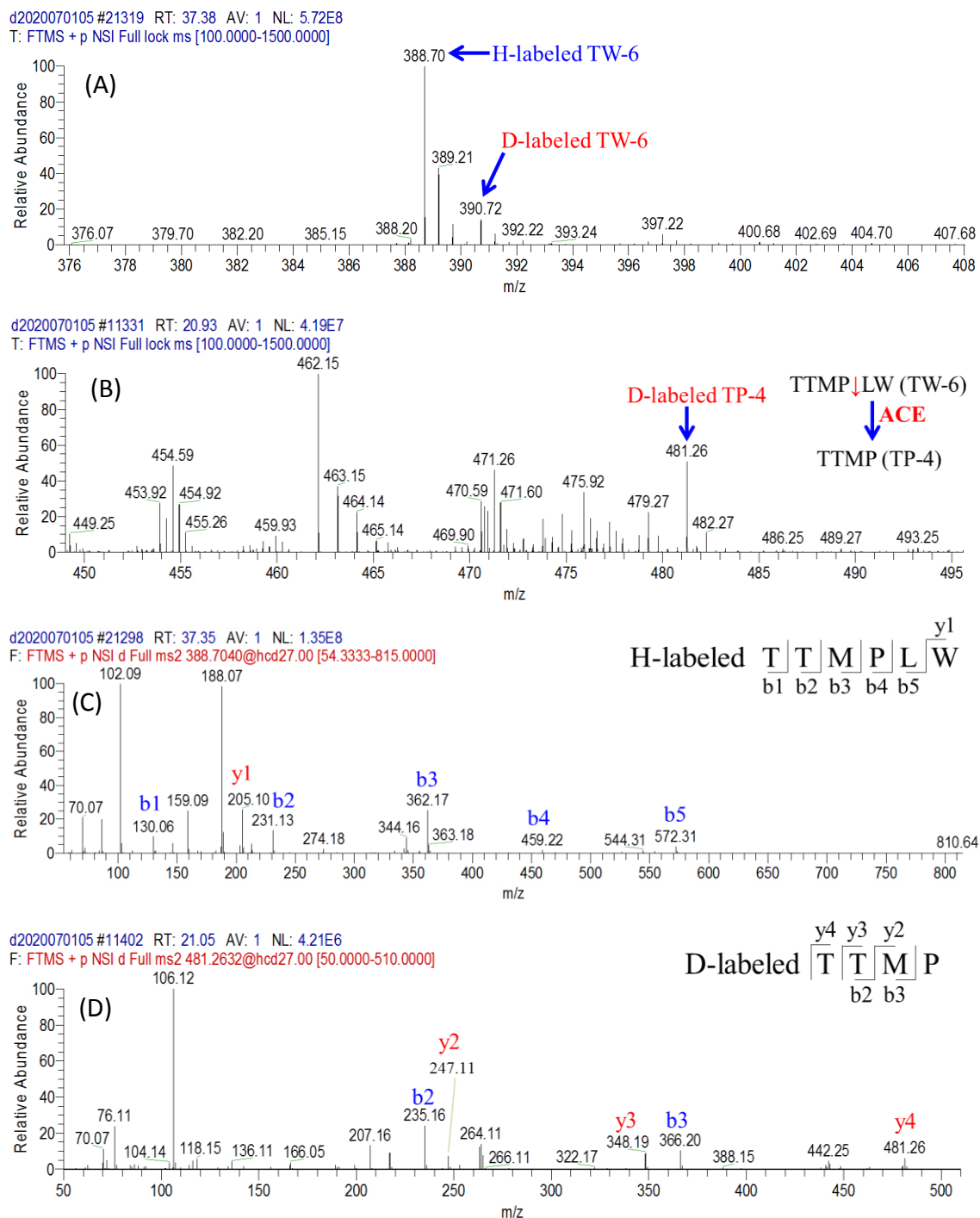
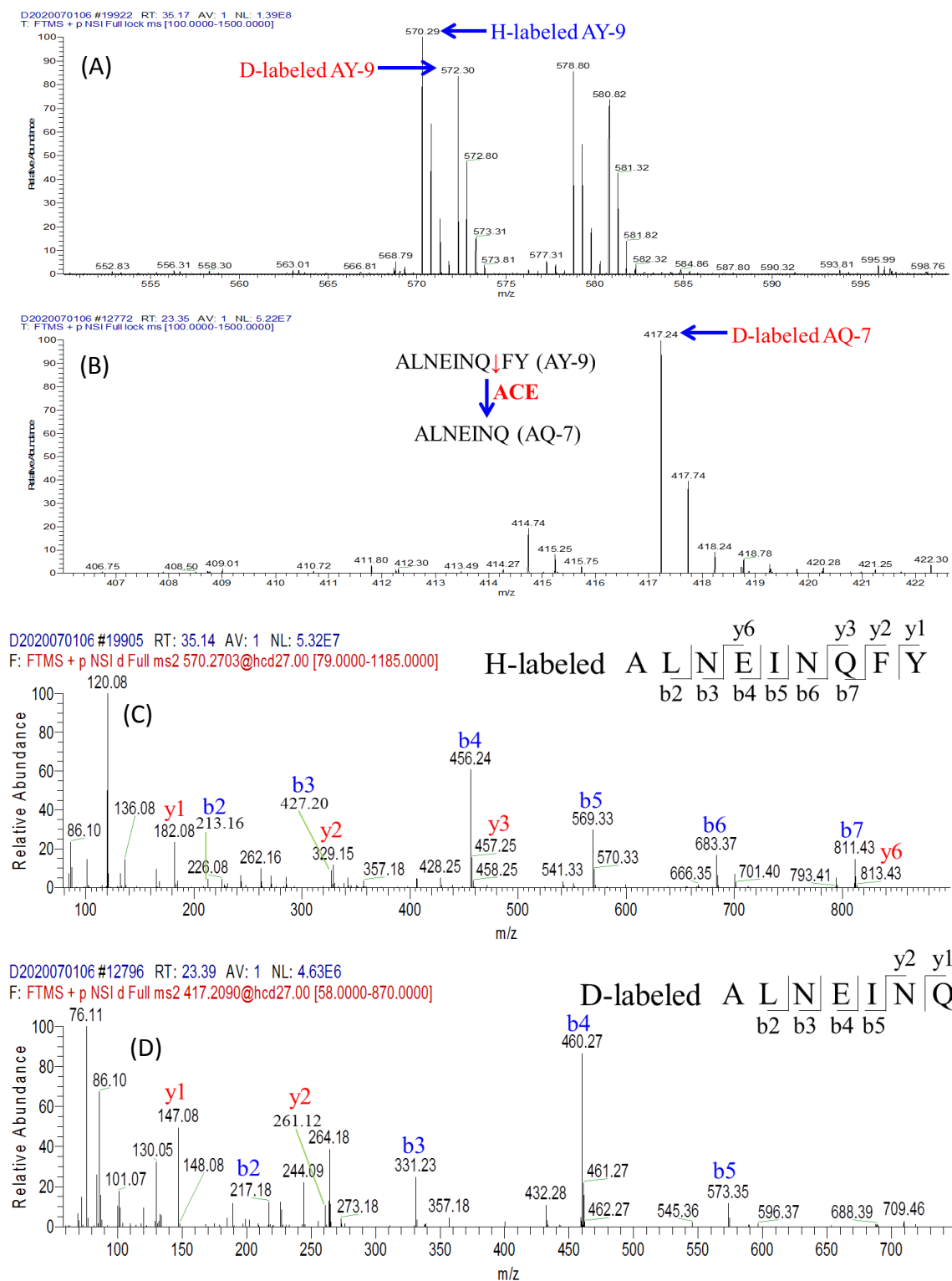


Figure S27. LC-MS/MS identification of ACE exogenous substrate TTMP<sub>L</sub>W (TW-6) and its product. (A) MS spectrum of D- and H-labeled TW-6; (B) MS spectrum of the product TTMP (TP-4); (C) MS/MS spectrum of TW-6 (m/z 388.7); (D) MS/MS of TP-4 (m/z 481.2).

**Figure S28**



**Figure S28.** LC-MS/MS identification of ACE exogenous substrate ALNEINQFY (AY-9) and its product. (A) MS spectrum of D- and H-labeled AY-9 (B) MS spectrum of the product ALNEINQ (AQ-7); (C) MS/MS spectrum of AY-9 (m/z 570.2); (D) MS/MS of AQ-7 (m/z 417.2).

**Figure S29**

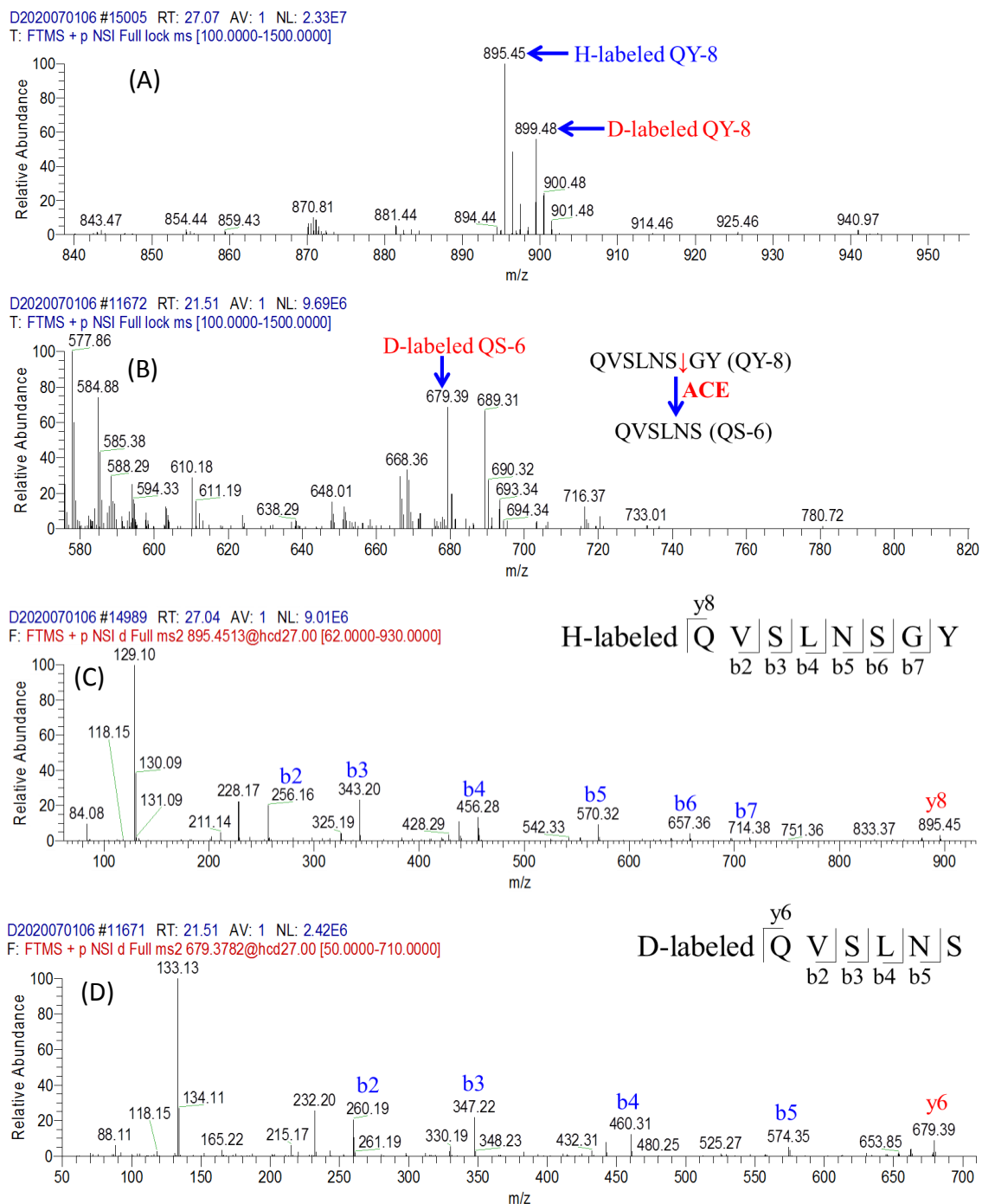


Figure S29. LC-MS/MS identification of ACE exogenous substrate QVSLNSGY (QY-8) and its product. (A) MS spectrum of D- and H-labeled QY-8 (B) MS spectrum of the product QVSLNS (QS-6); (C) MS/MS spectrum of QY-8 (m/z 895.4); (D) MS/MS of QS-6 (m/z 679.3).

**Figure S30**

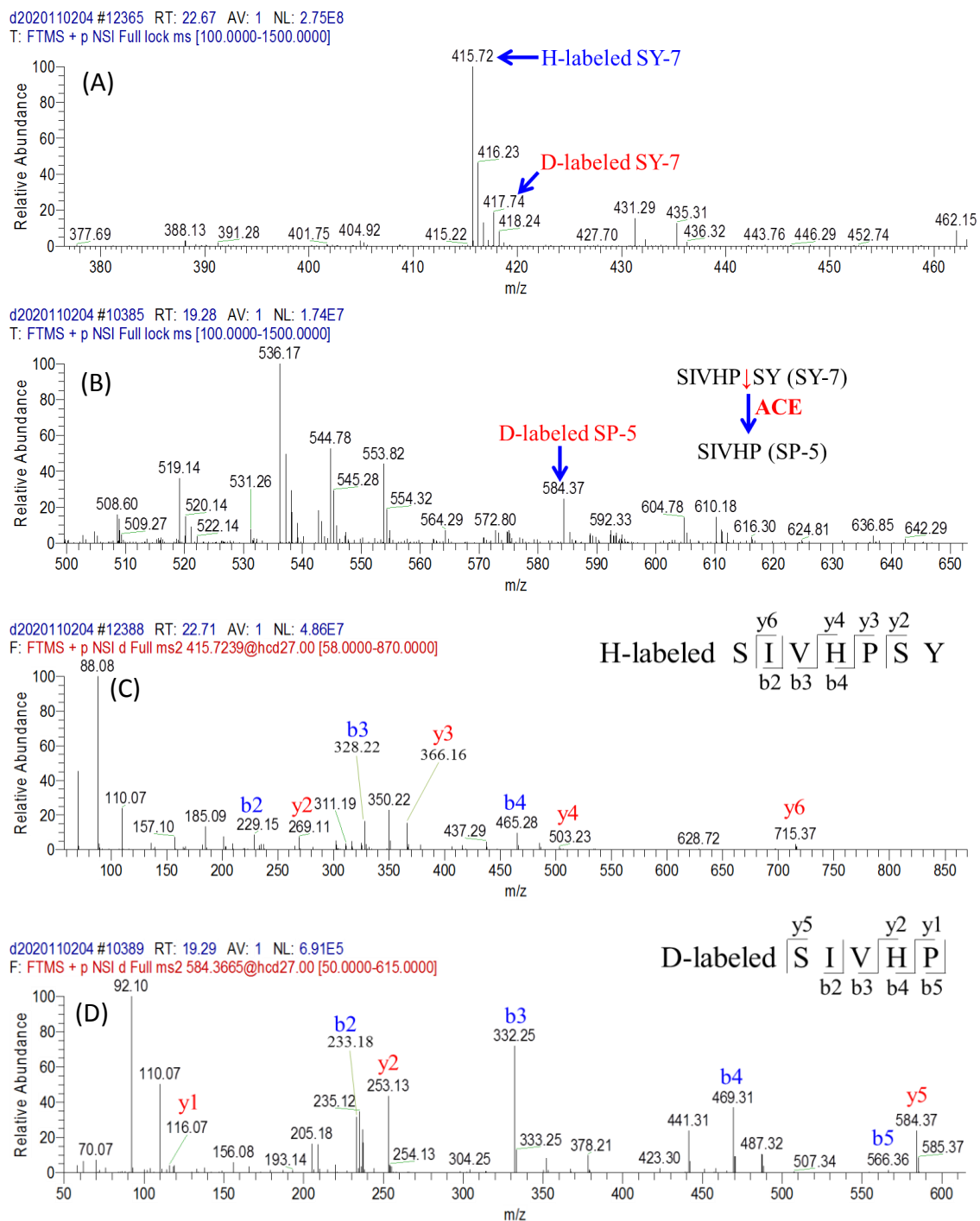
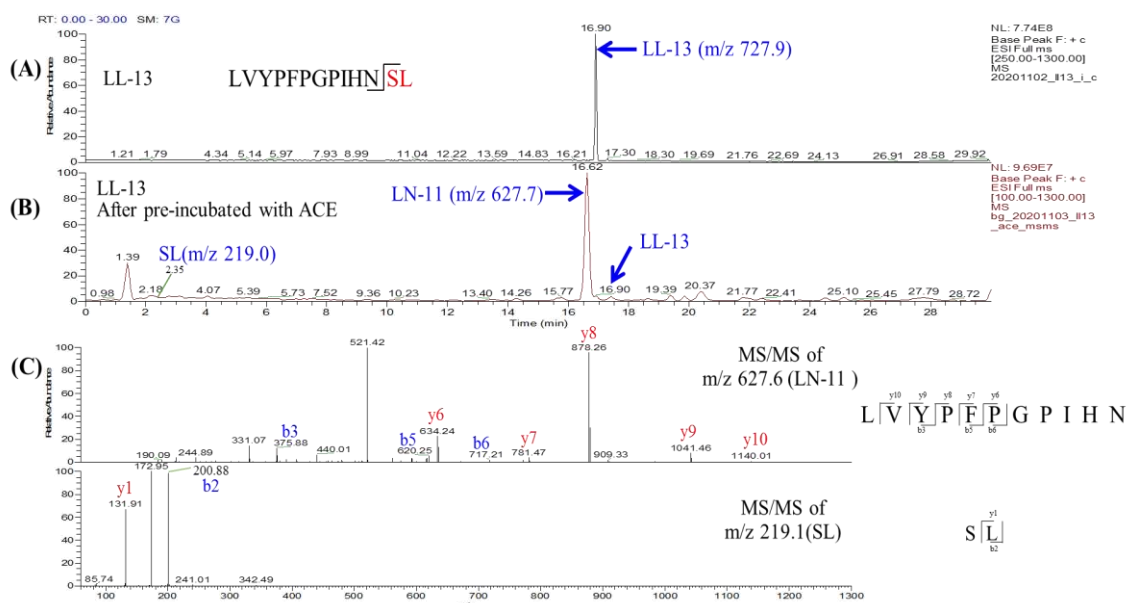


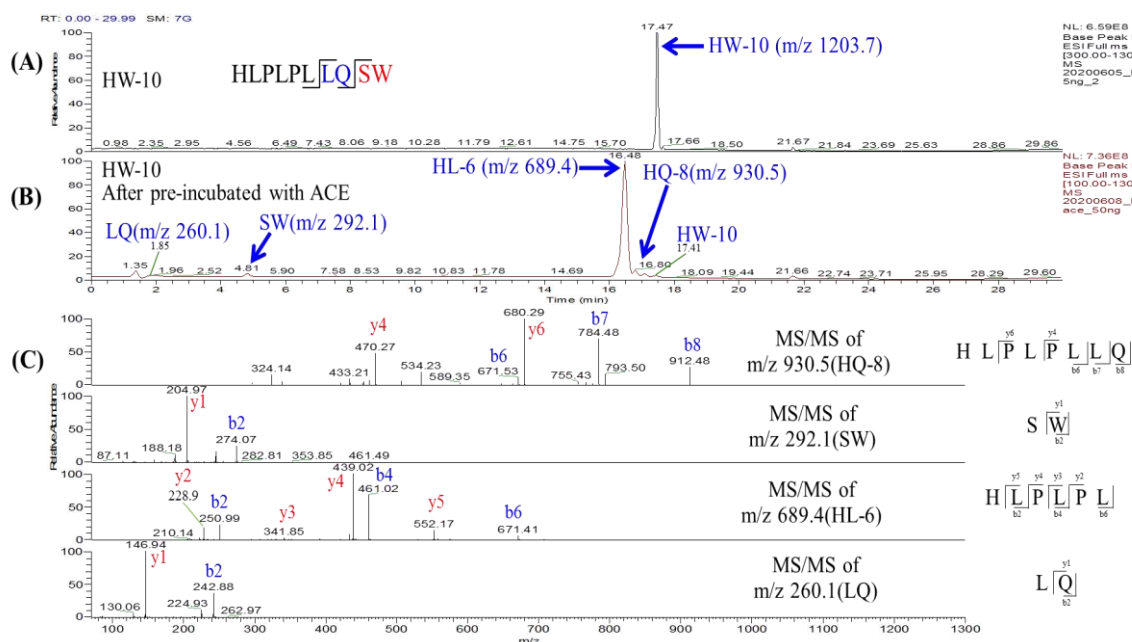
Figure S30. LC-MS/MS identification of ACE exogenous substrate SIVHPSY (SY-7) and its product. (A) MS spectrum of D- and H-labeled SY-7 (B) MS spectrum of the product SIVHP (SP-5); (C) MS/MS spectrum of SY-7 (m/z 415.7); (D) MS/MS of SP-5 (m/z 584.3).

**Figure S31**



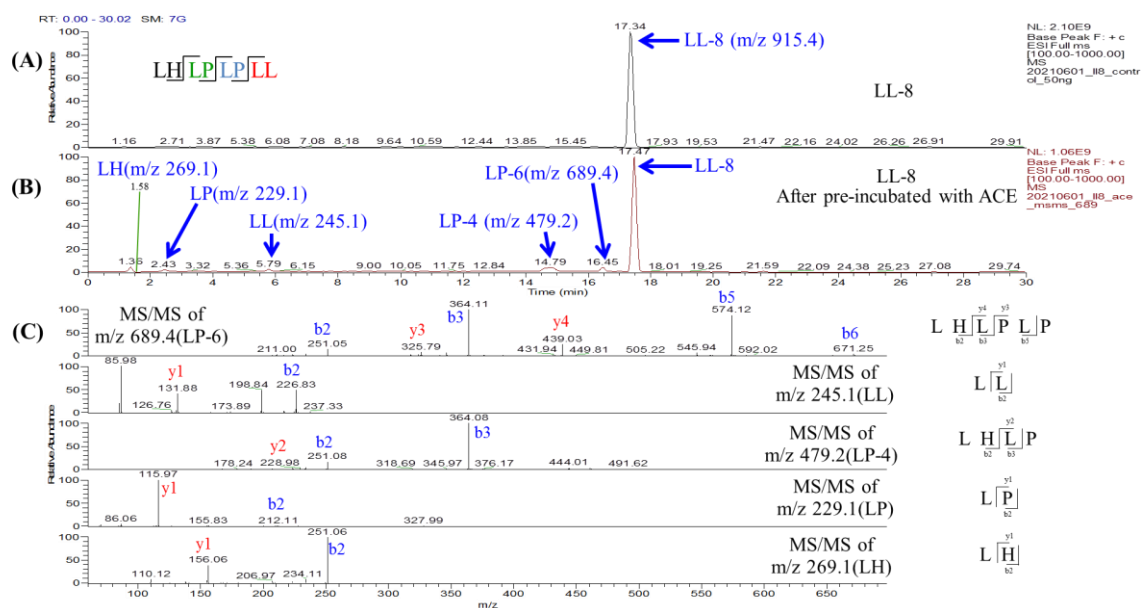
**Figure S31.** The reactivity confirmation of LVYPPFGPIHNSL (LL-13). (A) The full chromatogram of LL-13 (without ACE hydrolysis). (B) The full chromatogram of LL-13 after ACE incubation for 3h. (C) The MS/MS spectrum of LVYPPFGPIHNSL (LN-11) and SL.

**Figure S32**



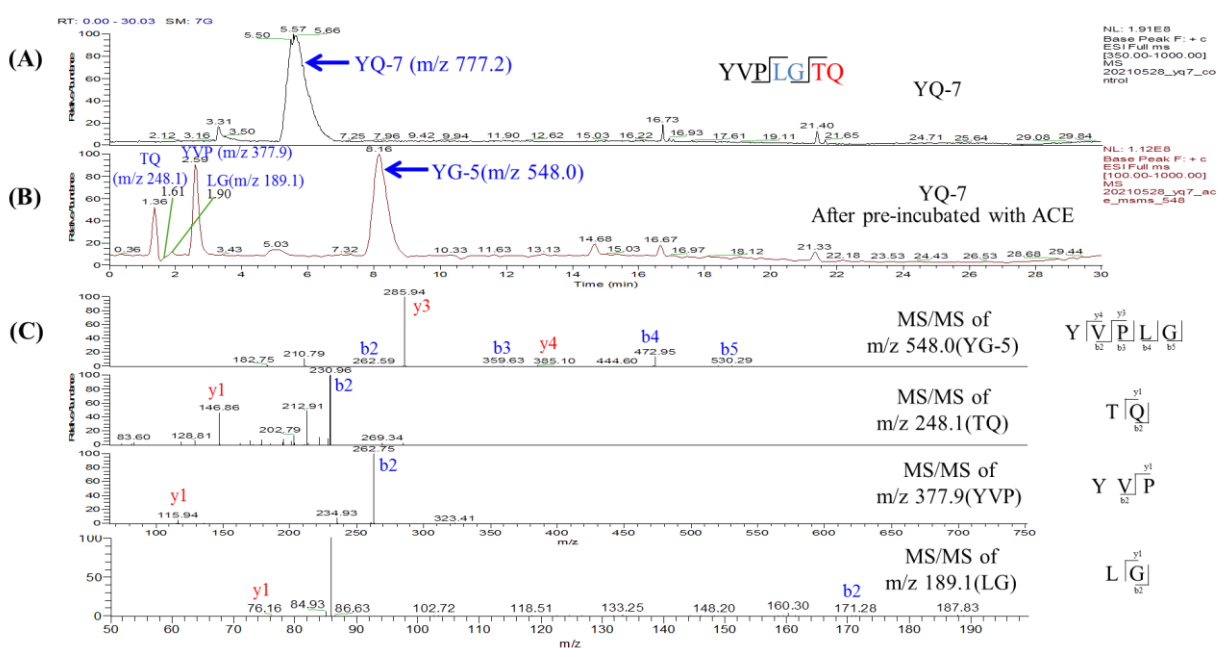
**Figure S32.** The reactivity confirmation of HLPLPLQLSW (HW-10). (A) The full chromatogram of HW-10 (without ACE hydrolysis). (B) The full chromatogram of HW-10 after ACE incubation for 3h. (C) The MS/MS spectra of HW-10's products.

**Figure S33**



**Figure S33.** The reactivity confirmation of LHLPLPLL (LL-8). (A) The full chromatogram of LL-8 (without ACE hydrolysis). (B) The full chromatogram of LL-8 after ACE incubation for 3h. (C) The MS/MS spectra of LL-8's products.

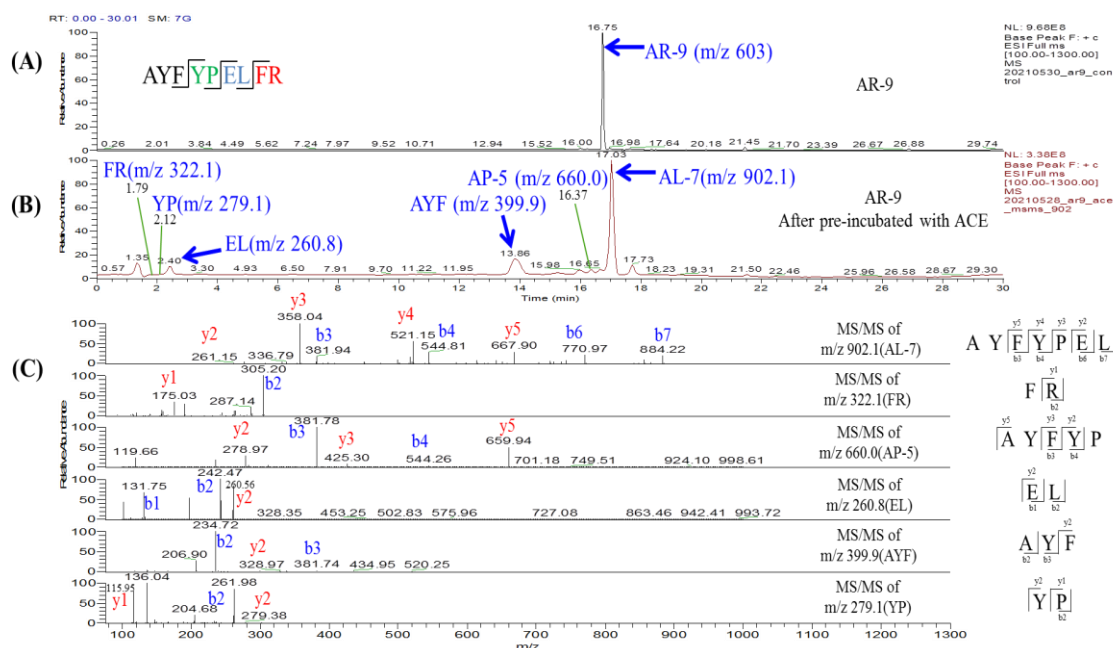
**Figure S34**



**Figure S34.** The reactivity confirmation of YVPLGTQ (YQ-7). (A) The full chromatogram of YQ-7 (without ACE hydrolysis). (B) The full chromatogram of YQ-7 after ACE incubation for 3h. (C) The MS/MS spectra of YQ-7's products.

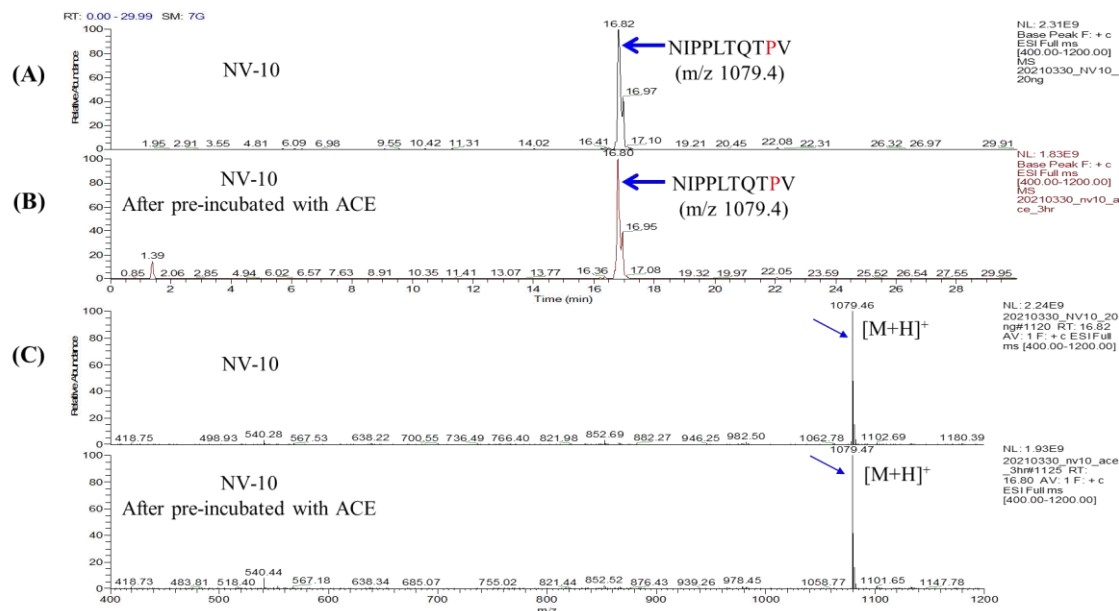


**Figure S35**



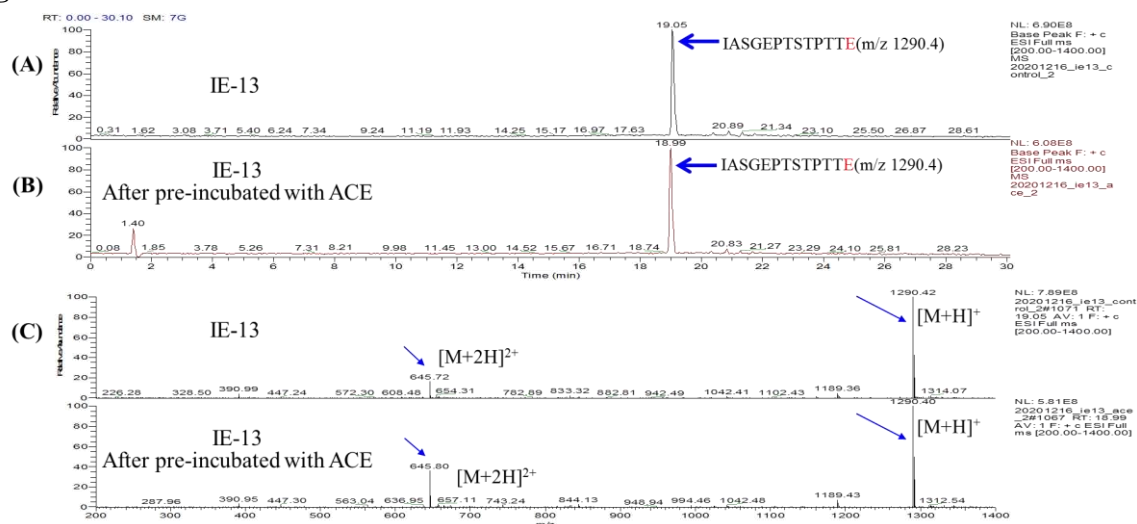
**Figure S35.** The reactivity confirmation of AYFYPELFR (AR-9). (A) The full chromatogram of AR-9 (without ACE hydrolysis). (B) The full chromatogram of AR-9 after ACE incubation for 3h. (C) The MS/MS spectra of AR-9's products.

**Figure S36**



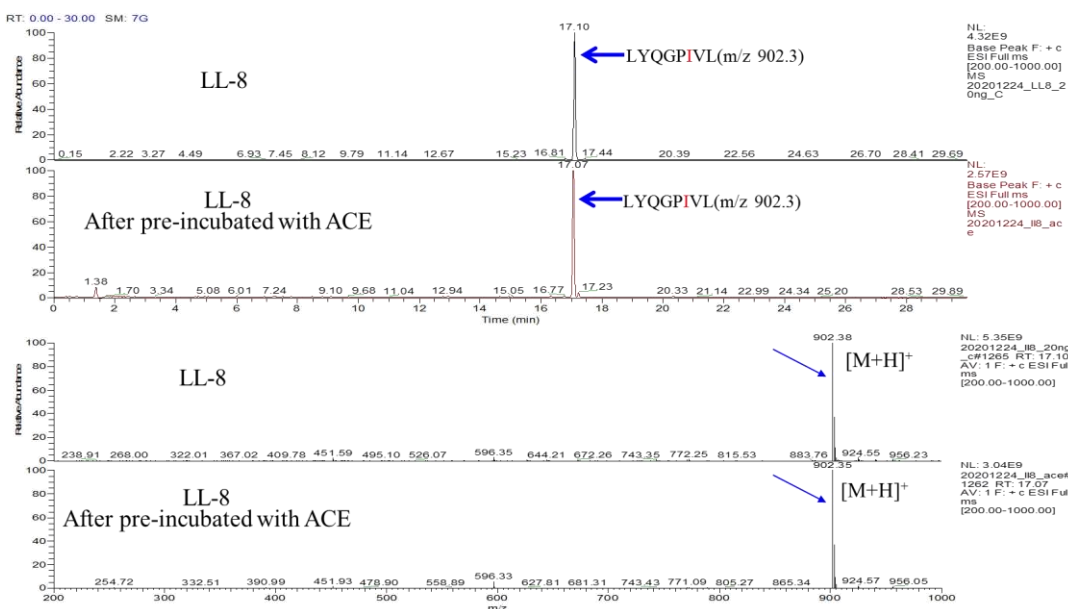
**Figure S36.** The reactivity confirmation of NIPPLTQTPV (NV-10) (Pro at P1' position). (A) The full chromatogram of NV-10 (without ACE hydrolysis). (B) The full chromatogram of NV-10 after ACE incubation for 3h (No any detectable product was observed). (C) The MS spectra of (A) and (B).

**Figure S37**



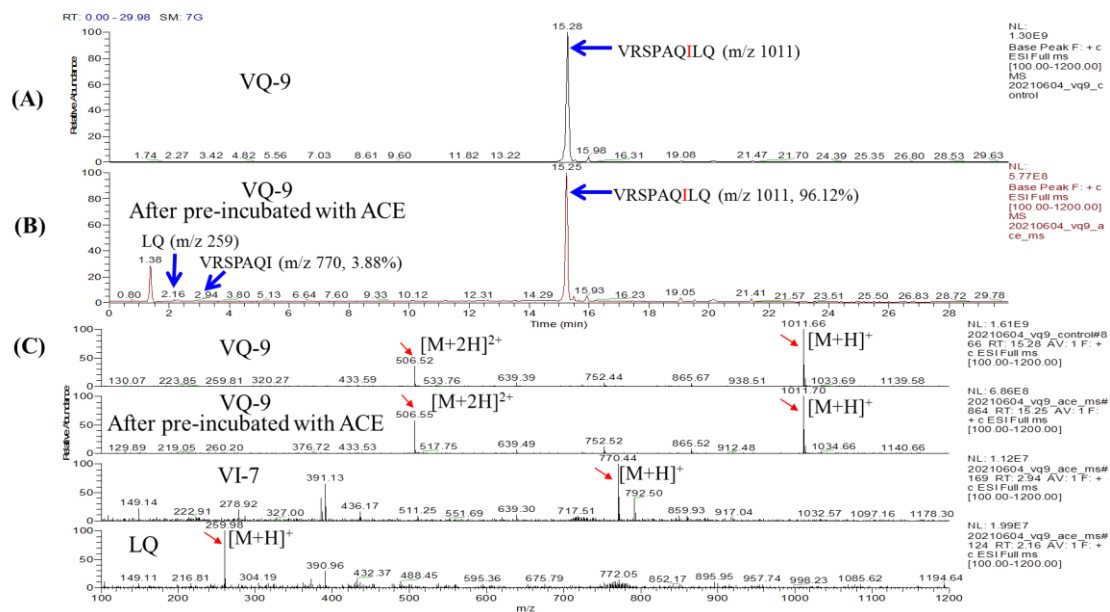
**Figure S37.** The reactivity confirmation of IASGEPTSTPTE (IE-13) (Glu at P2' position). (A) The full chromatogram of IE-13 (without ACE hydrolysis). (B) The full chromatogram of IE-13 after ACE incubation for 3h (No any detectable product was observed). (C) The MS spectra of (A) and (B).

**F0gure S38**



**Figure S38.** The reactivity confirmation of LYQGPIVL (LL-8) (Ile at P1 position). (A) The full chromatogram of LL-8 (without ACE hydrolysis). (B) The full chromatogram of LL-8 after ACE incubation for 3h (No any detectable product was observed). (C) The MS spectra of (A) and (B).

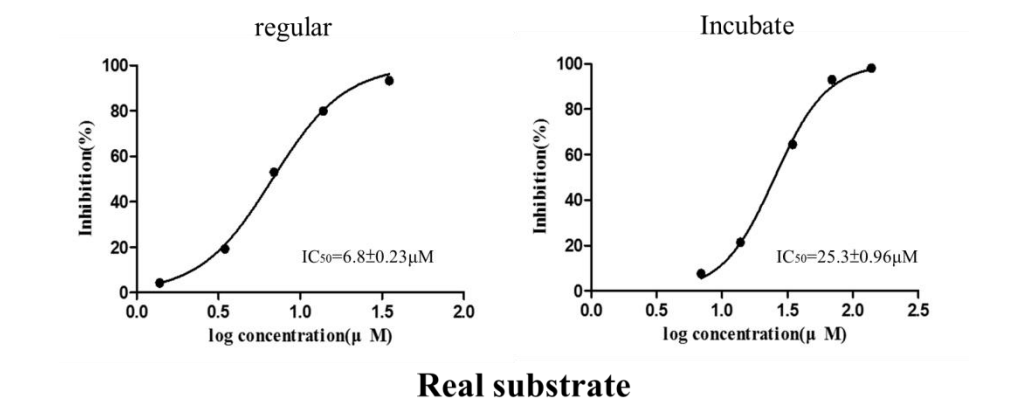
**Figure S39**



**Figure S39.** The reactivity confirmation of VRSPAQILQ (VQ-9) (Ile at P1 position). (A) The full chromatogram of VQ-9 (without ACE hydrolysis). (B) The full chromatogram of VQ-9 after ACE incubation for 3h (Trace amount of product was observed). (C) The MS spectra of (A), (B) and trace amount of products.

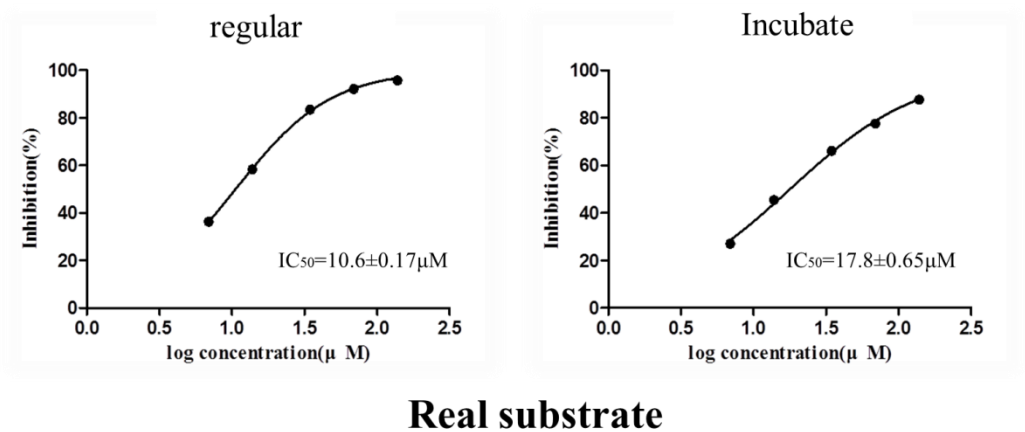
Figure S40  
(A)

ACE inhibitor type of AR-9



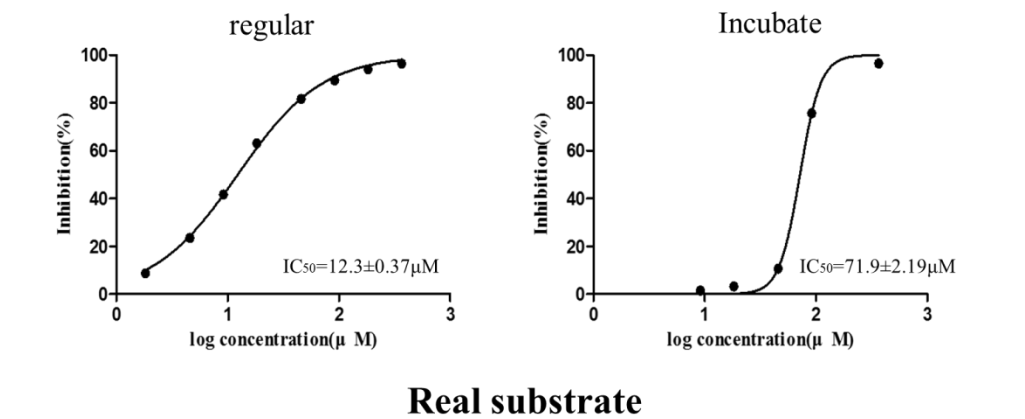
(B)

ACE inhibitor type of HW-10



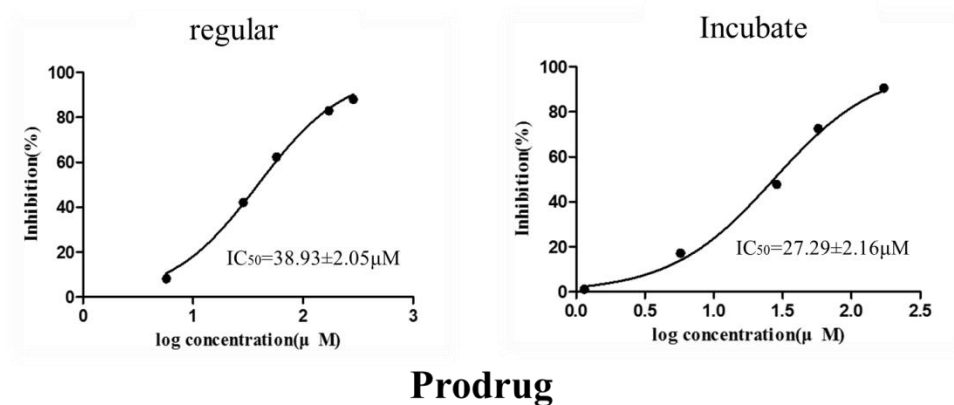
(C)

ACE inhibitor type of LL-8



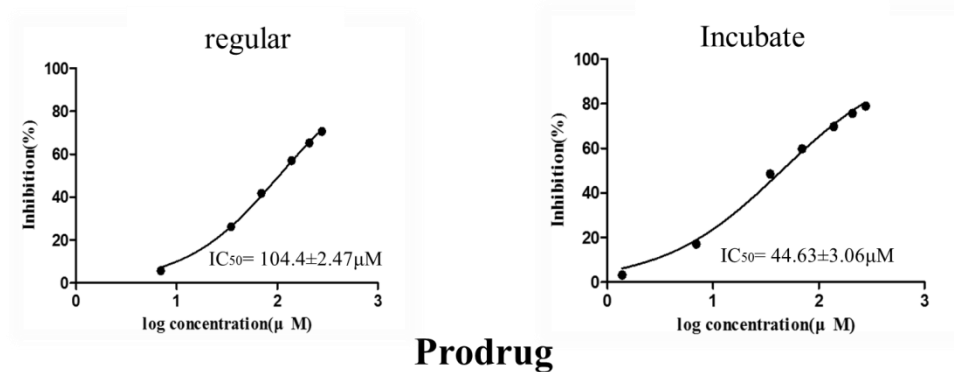
(D)

### ACE inhibitor type of LL-13



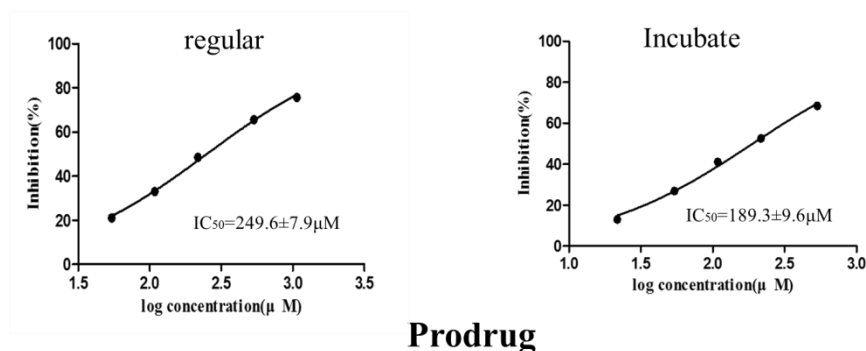
(E)

### ACE inhibitor type of FF-10



(F)

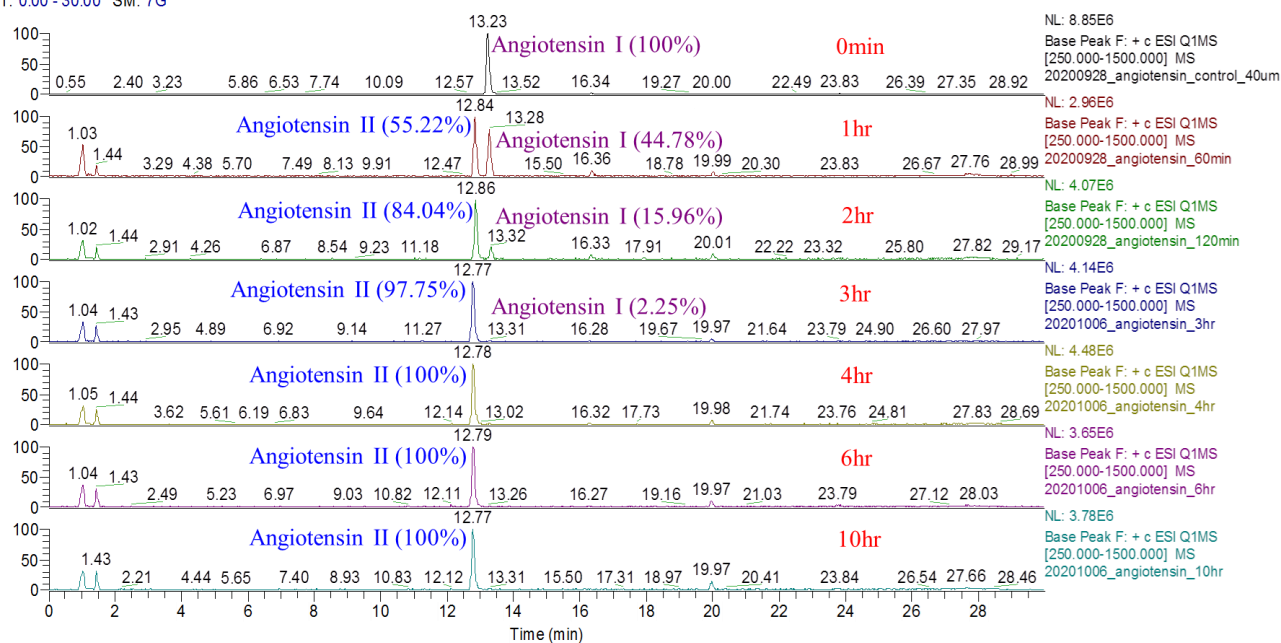
### ACE inhibitor type of YQ-7



**Figure S40.** IC<sub>50</sub> values of six peptides with and without ACE preincubation. (A) AR-9. (B) HW-10. (C) LL-8. (D) LL-13. (E) FF-10. (F) YQ-7.

**Figure S41**

RT: 0.00 - 30.00 SM: 7G



**Figure S41.** The remained abundance of synthetic Ang-I during the ACE hydrolysis monitored using LC-MS at various pre-incubation times.