

# **Cross-linking and modification of fibronectin by peroxynitrous acid: mapping and quantification of damage provides a new model for domain interactions**

Michele Mariotti<sup>1</sup>, Adelina Rogowska-Wrzesinska<sup>2</sup>, Per Hägglund<sup>1\*</sup>, Michael J. Davies<sup>1\*</sup>

<sup>1</sup>Department of Biomedical Sciences, Panum Institute, University of Copenhagen, Copenhagen, Denmark

<sup>2</sup>Department of Biochemistry and Molecular Biology and VILLUM Center for Bioanalytical Sciences, University of Southern Denmark, Odense, Denmark

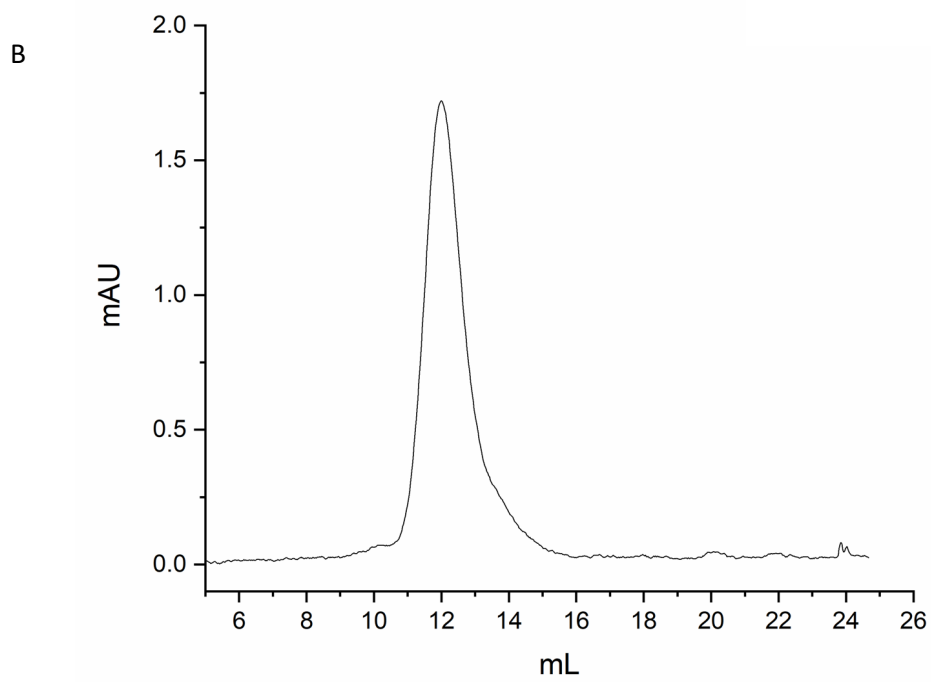
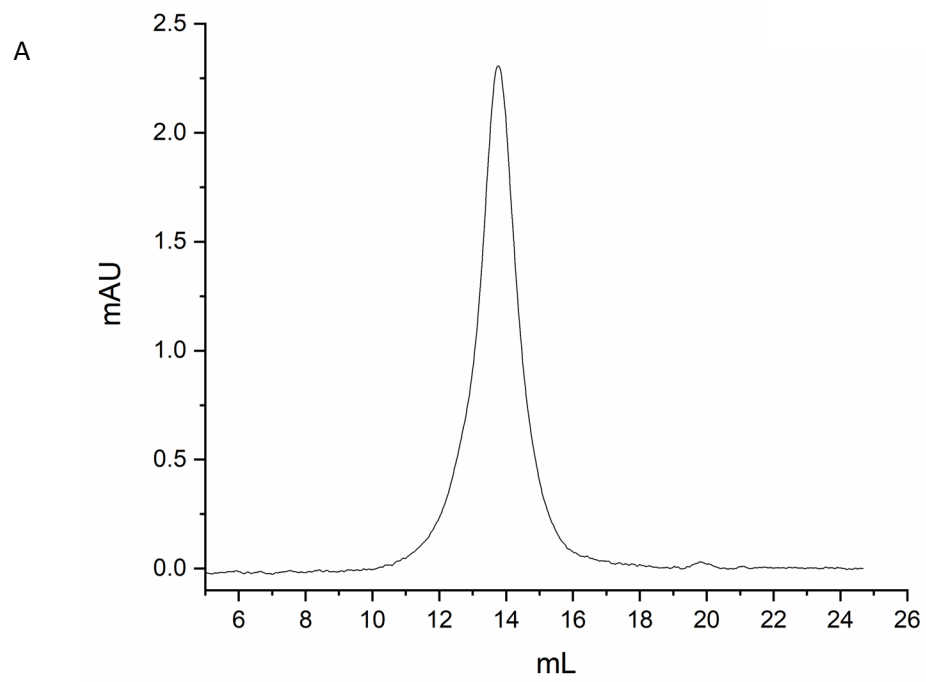
\* Joint senior and corresponding authors: M.J. Davies ([davies@sund.ku.dk](mailto:davies@sund.ku.dk)), P. Hägglund ([pjh@sund.ku.dk](mailto:pjh@sund.ku.dk))

**Running title:** Modification and cross-linking of fibronectin

## **SUPPLEMENTARY DATA**

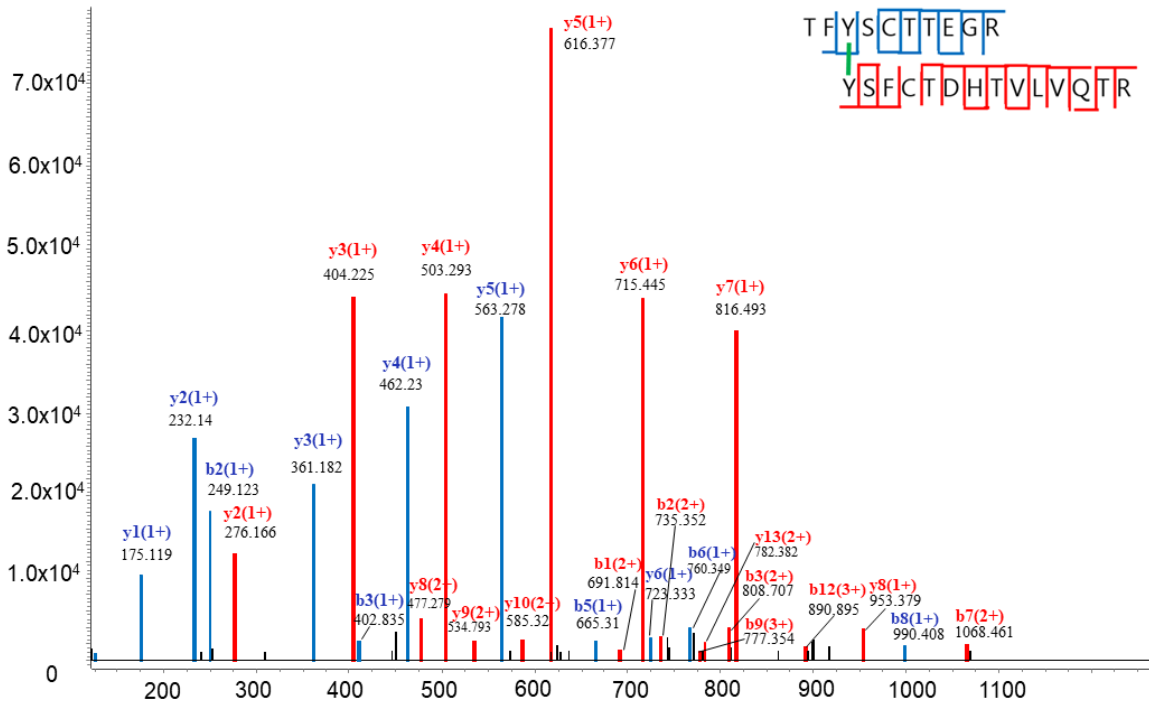
**Keywords:** Fibronectin, extracellular matrix, cross-links, nitration, oxidation, peroxynitrous acid, di-tyrosine, 3-nitrotyrosine, 6-nitrotryptophan



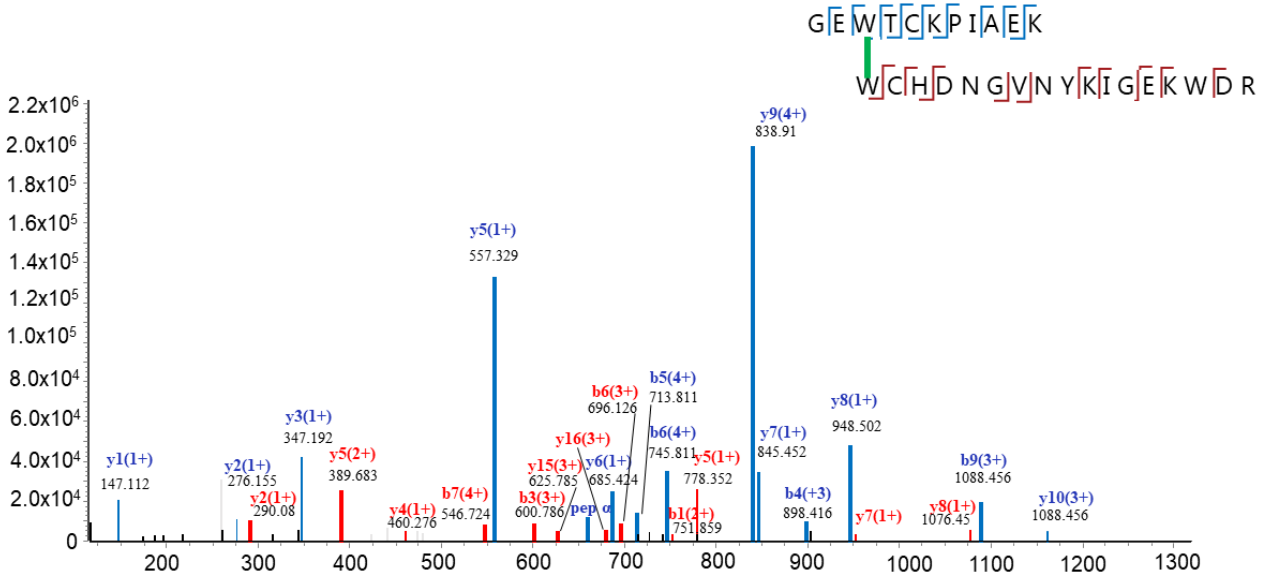


**Supplementary Figure 1.** SEC of 50  $\mu$ g native FN in 150 mM (A) and 750 mM (B) NaCl-containing buffer.

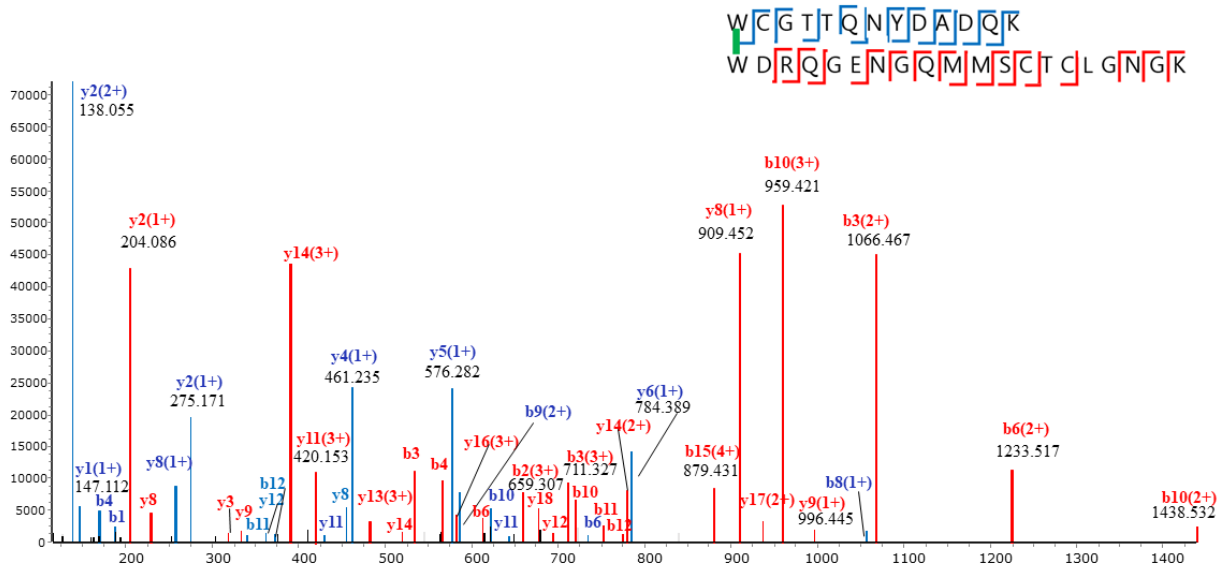
A)



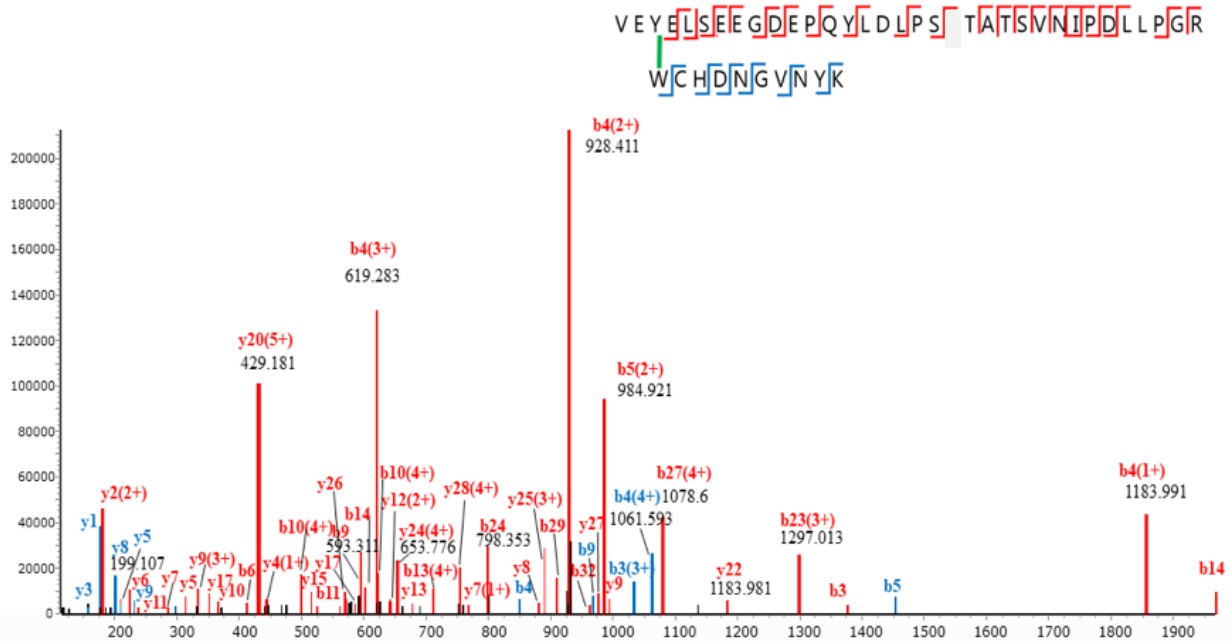
B)



C)



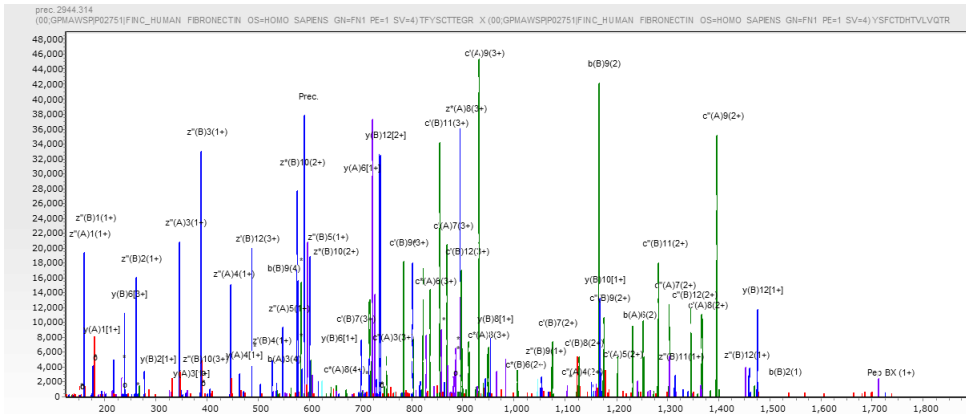
D)



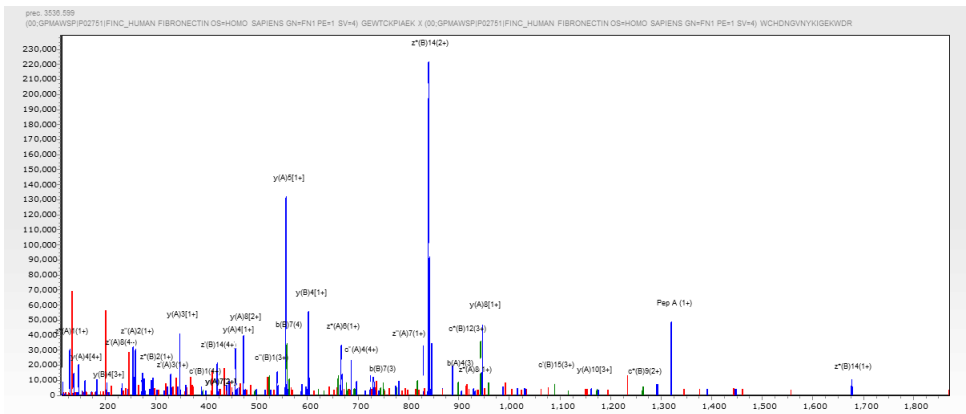
**Supplementary Figure 2.** HCD tandem mass spectra of cross-linked peptides. A) Spectrum of the quintuply-charged di-Tyr cross-linked peptide (TFY<sup>372</sup>SC TTEGR) (Y<sup>398</sup>SFCTDHTVLVQTR), with precursor ion of  $m/z$  589.87. B) Spectrum of the quintuply-charged di-Tyr cross-linked peptide (GEW<sup>177</sup>TCKPIAEK) (W<sup>2250</sup>CHDNGVNYKIGEKWDR), with nitration of W<sup>2264</sup> matching the precursor ion of  $m/z$  708.527. C) Spectrum of the di-Trp cross-linked peptide (W<sup>445</sup>CGTTQNYDADQK) (W<sup>2264</sup>DRQGENGMMSCTCLGN GK), with precursor ion having  $m/z$  801.326. D) Spectrum of the Tyr-Trp cross-linked peptide (VEY<sup>754</sup>ELSEEGDEPQYLDLPSTATSVNIPDLLPGR) (W<sup>2250</sup>CHDNGVNYK), with the precursor ion of  $m/z$  997.005.

Blue y and b fragments correspond to the  $\alpha$  (shorter) peptide, while red y and b fragments correspond to the  $\beta$  (longer) peptide.

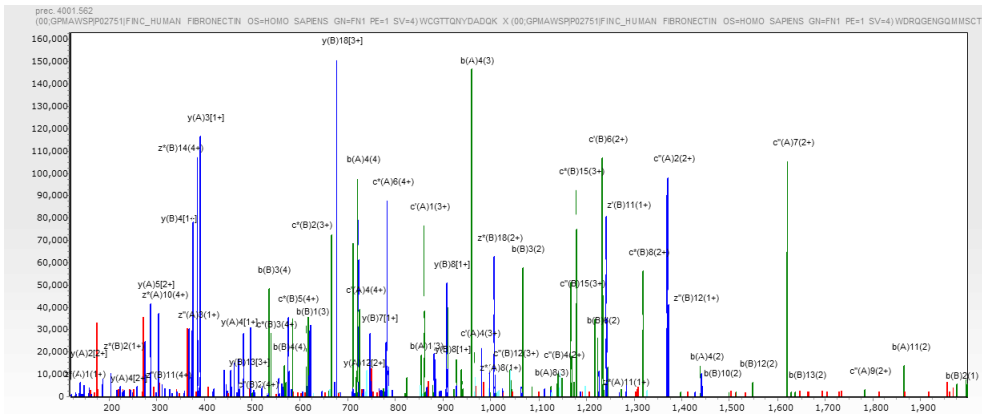
A)



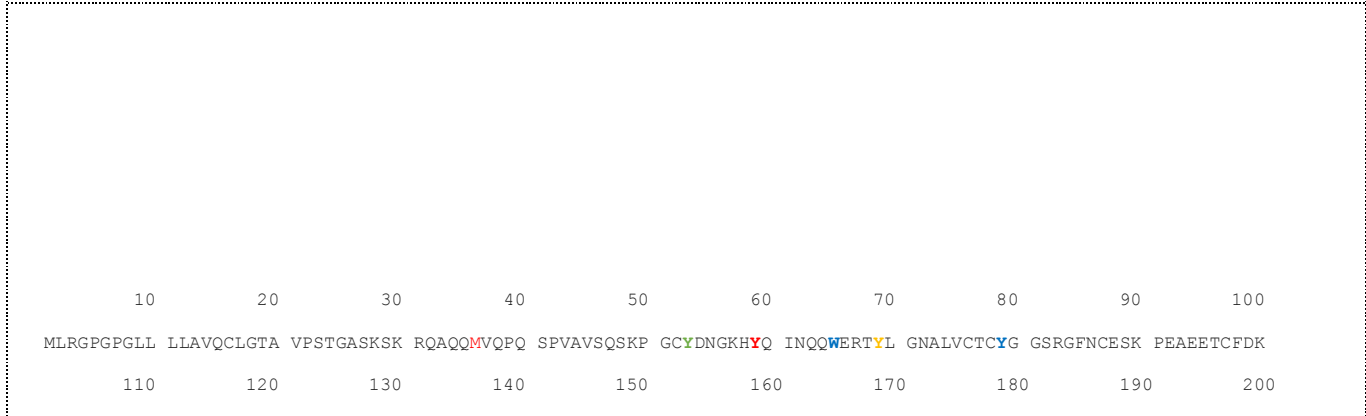
B)



C)



**Supplementary Figure 3.** EtHCD spectra of cross-linked peptides. A) Spectrum of the di-Tyr cross-linked peptide (TFY<sup>372</sup>SCTEGR) (Y<sup>398</sup>SFCTDHTVLVQTR). B) Spectrum of the di-Tyr cross-linked peptide (GEW<sup>177</sup>TCKPIAEK) (W<sup>2250</sup>CHDNGVNYKIGEKWDR). C) Spectrum of the di-Trp cross-linked peptide (W<sup>445</sup>CGTTQNYDADQK) (W<sup>2264</sup>DRQGENGQMMSCCLGNGK). Spectra were obtained from the software MassAI. Peaks matching y and z fragment ions are indicated in blue, whereas those matching blue b and c ions are shown in green. Peaks in red are unassigned ions.

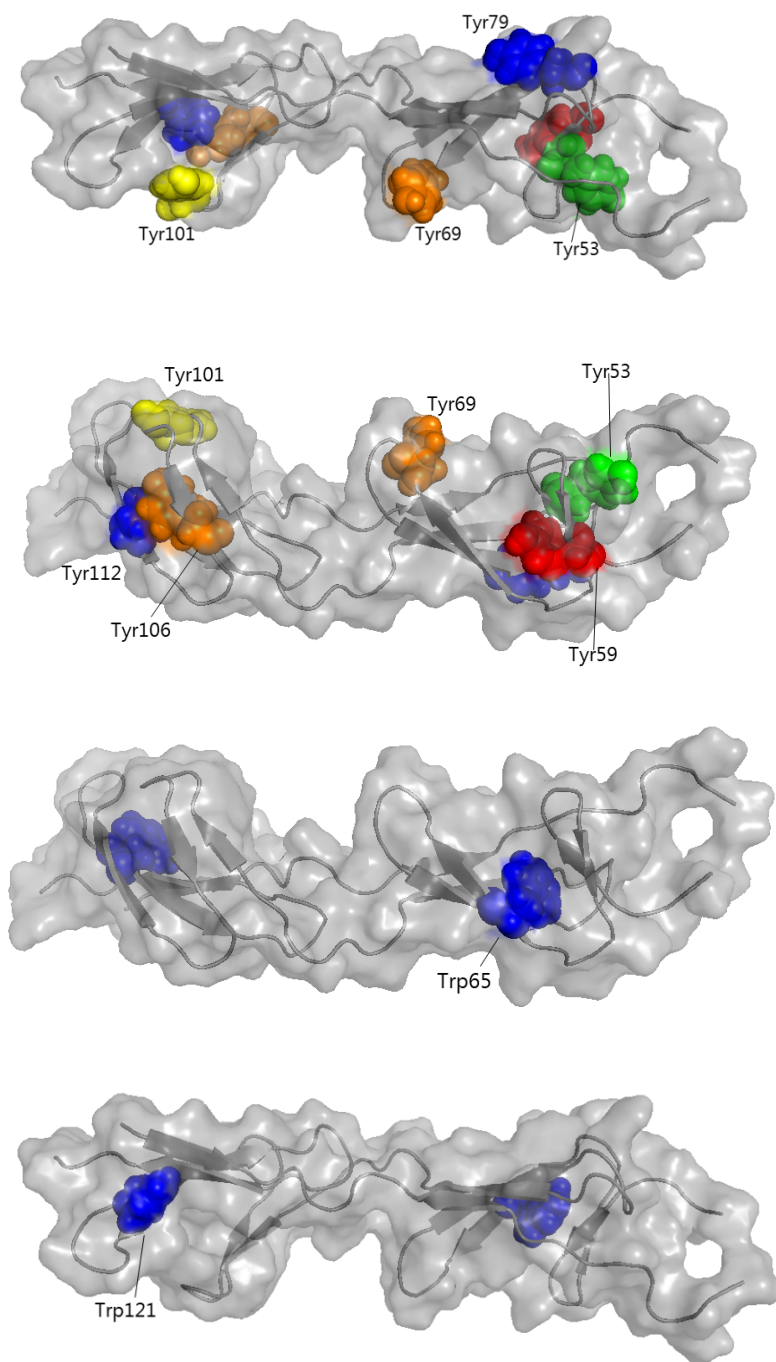


YTGNTYRVGD TYERPKDSMI WDCTCIGAGR GRISCTIANR CHEGGQSYKI GDTWRRPHET GGYMLECVCL GNGKGEWTCK PIAEKCFDHA AGTSYVVGET  
 210 220 230 240 250 260 270 280 290 300  
 WEKPYQGWM VDCTCLGEGS GRITCTSRNR CNDQDTRTSY RIGDTWSKKD NRGNLLQCIC TGNGRGEWK ERHTSVQTTS SGSGPFTDVR AAVYQPQPHP  
 310 320 330 340 350 360 370 380 390 400  
 QPPPYGHCVT DSGVVYSVGM QWLKTQGNKQ MLCTCLNGV SCQETAVTQT YGGNSNGEPC VLPFTYNGRT FYSCCTTEGRQ DGHLCSTTS NYEQDQKYSF  
 410 420 430 440 450 460 470 480 490 500  
 CTDHTVLVQT RGGNSGALC HFFFLYNNHN YTDCTSEGR DNMKWCGTTQ NYDADQKFGF CPMAAHEEIC TTNEGVMYRI GDQWQKQDM GHMMRCTCVG  
 510 520 530 540 550 560 570 580 590 600  
 NGRGEWTCIA YSQLRDQCIV DDITYNVNDT FHKRHEEGHM LNCTCFGQGR GRWKCDPVDQ CQDSETGTFY QIGDSWEKYV HGVRYQCXYC GRGIGEWHCQ  
 610 620 630 640 650 660 670 680 690 700  
 PLQTYPSSSG PVEVFITETP SQPNSHPIQW NAPQPSHISK YILRWRPKNS VGRWKEATIP GHLNSYTIK LKPGVVYEGQ LISIQQYGHQ EVTRFDFTTT  
 710 720 730 740 750 760 770 780 790 800  
 STSTPVTSNT VTGETTFFSP LVATSESVTE ITASSFVVSU VSASDTVSGF RVEYELSEEG DEPOYLDLPS TATSVNIPDL LPGRKYIVNV YQISEDEGQS  
 810 820 830 840 850 860 870 880 890 900  
 LILSTSQTTA PDAPPDITVD QVDDTSIVVR WSRPQAPITG YRIVYSPSVE GSSTELNLE TANSVTLSDL QPGVQYNITI YAVEENQEST PVVIQOETT  
 910 920 930 940 950 960 970 980 990 1000  
 TPRS DTVFSP RDLQFVEVTD VKVTIMWTPP ESAVTGYRVD VIPVNLPGEH GQRLPISRNT FAEVTGLSPG VTYYFKVFAV SHGRESKPLT AQTTKLDAP  
 1010 1020 1030 1040 1050 1060 1070 1080 1090 1100  
 TNLQFVNETD STVLVWTPP RAQITGYRLT VGLTRRGQPR QYNVGSPVSK YPLRNLPAS EYTVSLVAIK GNQESPKATG VFTTLQPGSS IPPYNTTEVTE  
 1110 1120 1130 1140 1150 1160 1170 1180 1190 1200  
 TTIVITWTPA PRIGFKLQVR PSQGGEAPRE VTSDSGSIVV SGLTPGVEYV YTIQVLRDQ ERDAPIVNKV VTPLSPPTNL HLEANPDTGV LTVSWERSTT  
 1210 1220 1230 1240 1250 1260 1270 1280 1290 1300  
 PDITGYRITT TPTNGQQGNS LEEVHADQS SCTFDNLSPG LEYVNSVYTV KDDKESVPIS DTIIPAVPPP TDLRFTNIGP DTMRVTWAPP PSIDLNTFLV  
 1310 1320 1330 1340 1350 1360 1370 1380 1390 1400  
 RYSPVKNEED VAELSSPSD NAVVLTNLLP GTEYVVSVS VYEQHESTPL RGRQKTGLDS PTGIDFSDIT ANSFTVHWIA PRATITGYRI RHHPEHFSGR  
 1410 1420 1430 1440 1450 1460 1470 1480 1490 1500  
 PREDRVPHSR NSITLTNLTP GTEYVVSIVA LNGREESPLL IGQQSTVSDV PRDLEVVAAT PTSLLISWDA PAVTVRYRYRI TYGETGGNSP VQEFFVPGSK  
 1510 1520 1530 1540 1550 1560 1570 1580 1590 1600  
 STATISGLKP GVDYTTITVYA VTGRGDSPAS SKPISINYRT EIDKPSQMOV TDVQDNSISV KWLPSSSPVT GYRVTTTPKN GPPTTKTKTA GPDQTEMTIE  
 1610 1620 1630 1640 1650 1660 1670 1680 1690 1700  
 GLQPTVEYVV SVYAQNPSGE SQPLVQTAVT NIDRPKGLAF TDVDVDSIKI AWBSPQGQVS RYRVTYSSPE DGIHELFPAP DGEEDTAELO GLRPGSEYTV  
 1710 1720 1730 1740 1750 1760 1770 1780 1790 1800  
 SVVALHDDME SQPLIGTQST AIPAPTDLKF TQVTPTSLSA QWTPPNVQLT GYRVRVTPKE KTGPMKEINL APDSSSVVVS GLMVATKYEV SVYALKDTLT  
 1810 1820 1830 1840 1850 1860 1870 1880 1890 1900  
 SRPAQGVVTT LENVSPFRR RVTDATETTTI TISWRKTET ITGFQVDAVP ANGQTPPIQRT IKPDVRSYTI TGLQPGTDYK IYLYTLNDNA RSPVVIDAS  
 1910 1920 1930 1940 1950 1960 1970 1980 1990 2000  
 TAIDAPSNLR FLATTPNSLL VSWQPPRARI TGYIIKYEKP GSPPREVVPR PRPGVTEATI TGLEPGTEYT IYVIALKNNQ KSEPLIGRKK TDELPLQVTL  
 2010 2020 2030 2040 2050 2060 2070 2080 2090 2100  
 PHPNLHGPEI LDVPSTVQKT PFVTHPGYDT GNGIQLPGTS GOQPSVGGQM IFEEHGFRRT TPPTTATPIR HRPRPYPPNV GEEIQIGHIP REDVDYHLYP  
 2110 2120 2130 2140 2150 2160 2170 2180 2190 2200  
 HGPGLNPNAS TGQEALSQTT ISWAPFQDTS EYIISCHPVG TDEEPLQFRV PGTSTSATLT GLTRGATYNV IVEALKDQQR HKVREEVTV GNSVNEGLNQ  
 2210 2220 2230 2240 2250 2260 2270 2280 2290 2300

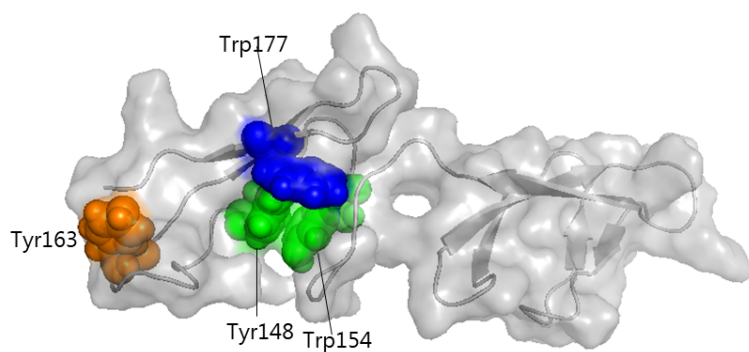


PTDDSCFD**Y** TVSH**Y**AVGDE **W**ERMSESGFK LLCQCLGFSG GHFRCDSSR**W** CHDNGV**N**Y**K**I GEK**W**DRQGEN G**Q****M****M**SCTCLG NGKGEFKCDP HEAT**C****Y**DDGK  
 2310 2320 2330 2340 2350 2360 2370 2380  
**T**YHVGE**Q****W**QK E**Y**LGAICSCT CFGGQR**G**WRC DNCRRPGGEP SPEGTTG**Q**S**Y** N**Q****Y**SQR**Y**HQR TNTNVNCP**I**E CF**M**PLDVQAD REDSRE

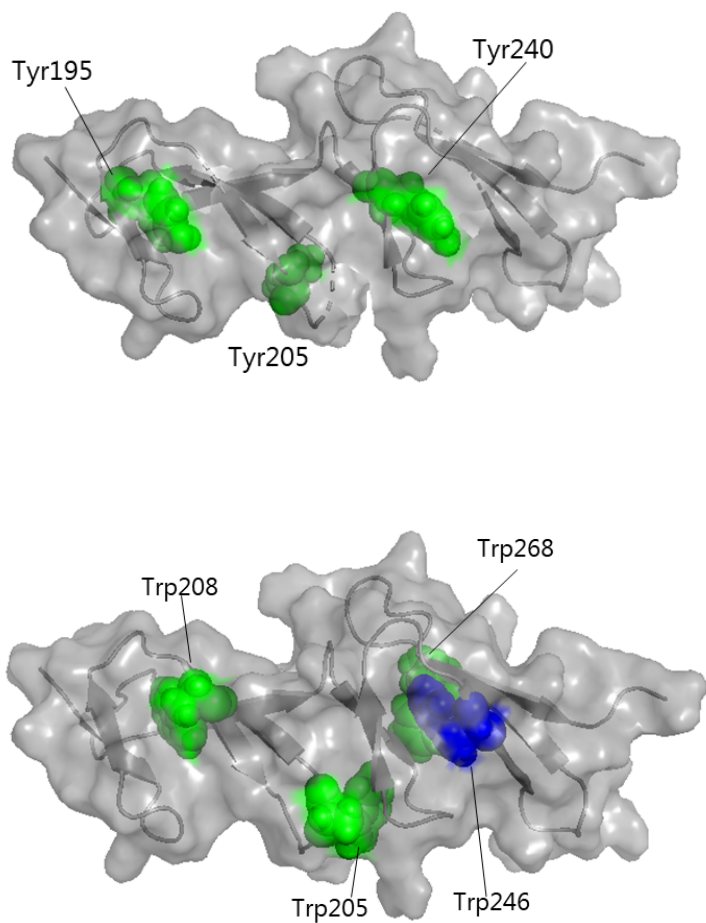
**Supplementary Figure 4.** Primary sequence of isoform 1 of human plasma fibronectin. Modified residues are highlighted in bold, and color coded corresponding to the extent of modification for the 1  $\mu$ M FN, 50  $\mu$ M ONOOH and 150 mM NaCl condition. Residues involved in cross-links are underlined. Y and W residues highlighted in red correspond to level of nitration higher than 30%; orange corresponds to a level of nitration of 20 - 30%; yellow corresponds to 10 - 20%; and blue corresponds to a level of 1 - 10% nitration. Green coloration corresponds to no detectable nitration; purple coloration corresponds to a residue that was not detected. M residues in red were detected as the oxidized (+16, sulfoxide) species.



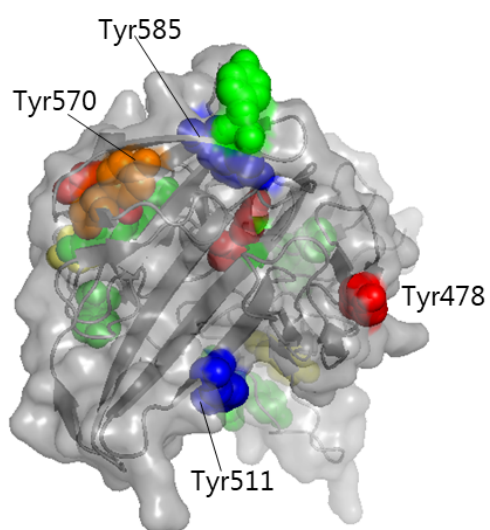
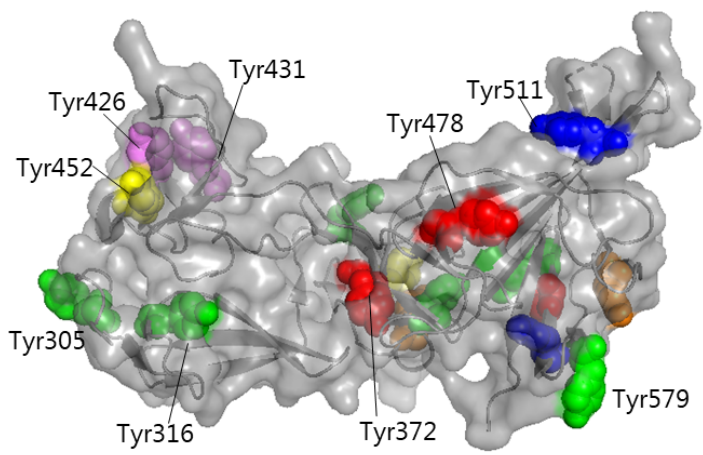
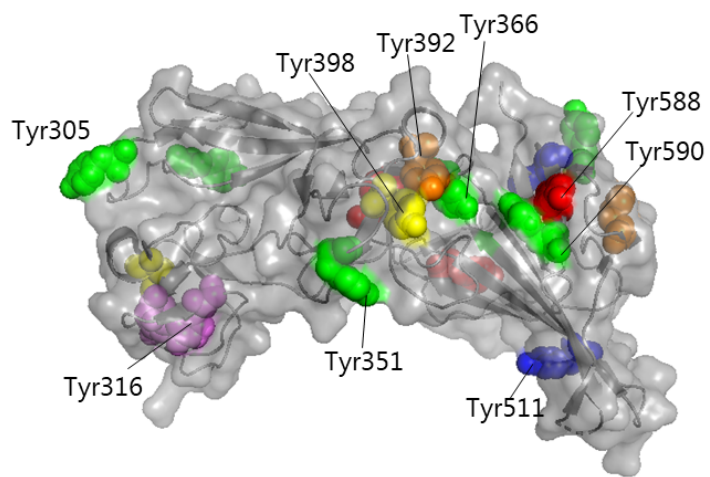
**Supplementary Figure 5.** Rendering of Tyr and Trp residues identified as nitrated in FN treated with 50  $\mu$ M ONOOH (150 mM NaCl). Red color corresponds to level of nitration higher than 30%; orange to nitration between 20 - 30%; yellow to nitration between 10 - 20%; and blue color corresponds to between 1 - 10% nitration. Green color corresponds to no detectable nitration. PDB structure: 1o9a (65), corresponding to domains FNI<sub>1-3</sub> and residues 48-140.

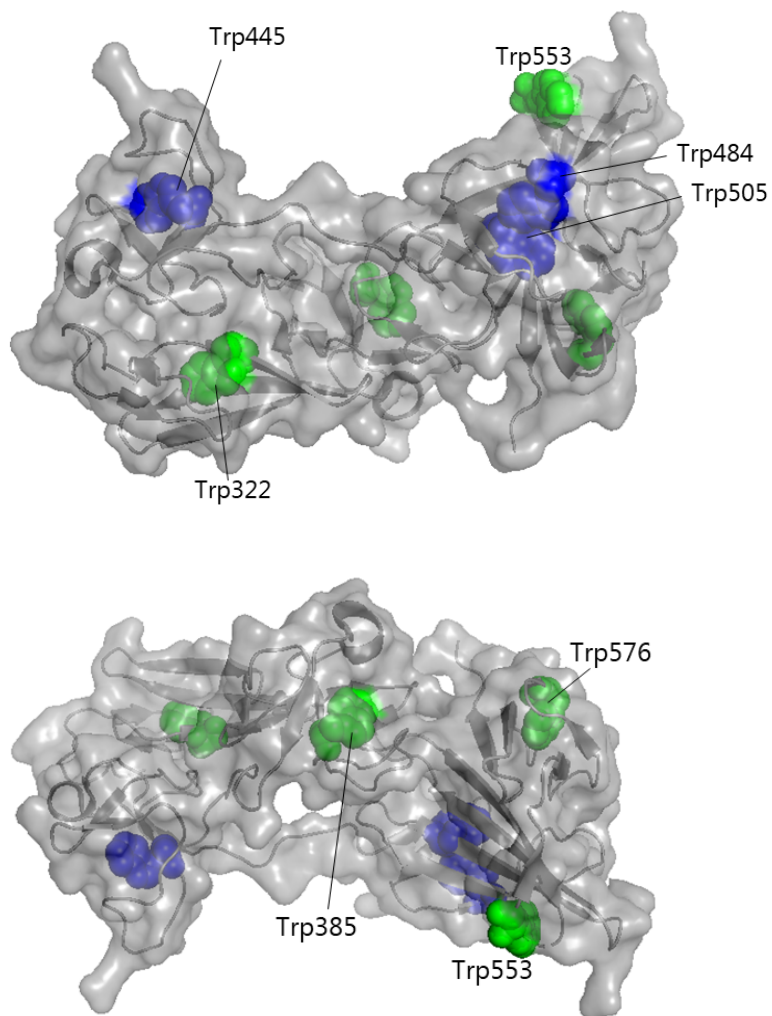


**Supplementary Figure 6.** Rendering of Tyr and Trp residues identified as nitrated in FN treated with 50  $\mu$ M ONOOH (150 mM NaCl). Red color corresponds to level of nitration higher than 30%; orange to nitration between 20 - 30%; yellow to nitration between 10 - 20%; and blue color corresponds to between 1 - 10% nitration. Green color corresponds to no detectable nitration. PDB structure: 2cku (63), corresponding to domains FN1<sub>2-3</sub> and residues 93-182.

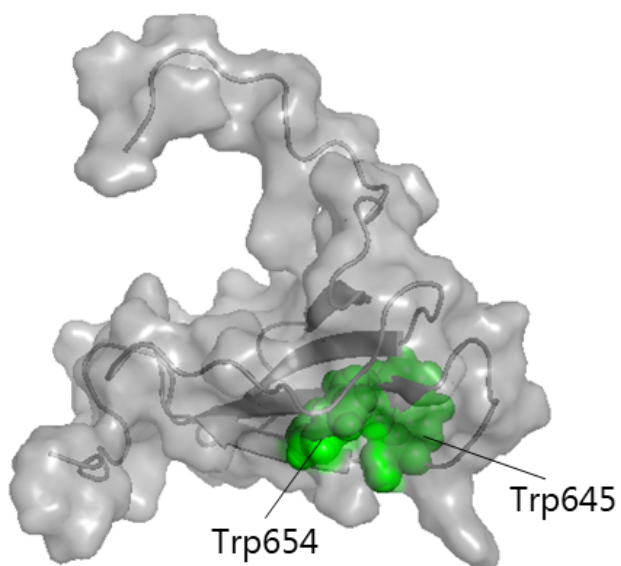
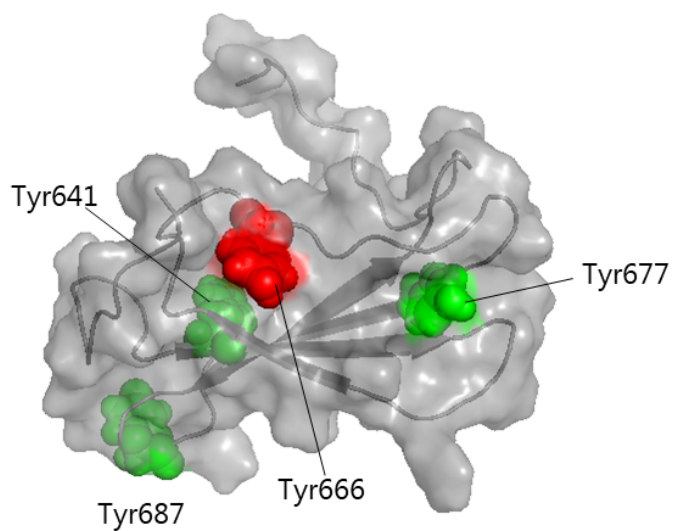


**Supplementary Figure 7.** Rendering of Tyr and Trp residues identified as nitrated in FN treated with 50 $\mu$ M ONOOH (150 mM NaCl). Red color corresponds to level of nitration higher than 30%; orange to nitration between 20 - 30%; yellow to nitration between 10 - 20%; and blue color corresponds to between 1 - 10% nitration. Green color corresponds to no detectable nitration. PDB structure: 1fbr (66) corresponding to domains FNI<sub>4-5</sub> and residues 183-275.

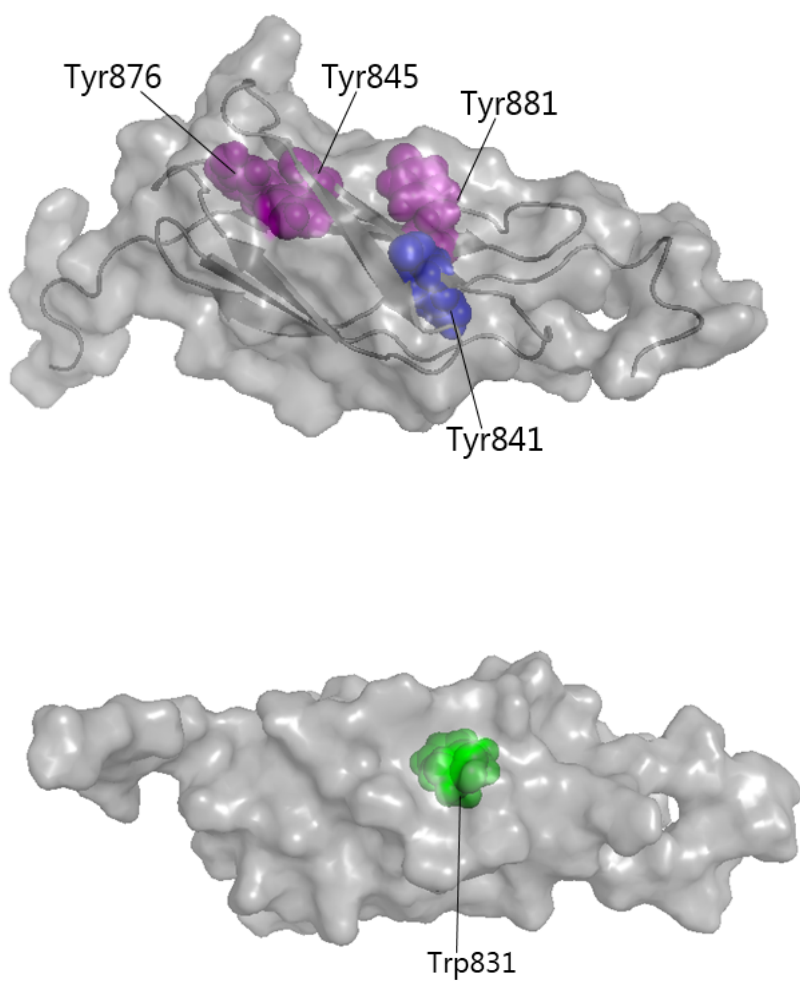




**Supplementary Figure 8.** Rendering of Tyr and Trp residues identified as nitrated in FN treated with 50 $\mu$ M ONOOH (150 mM NaCl). Red color corresponds to level of nitration higher than 30%; orange to nitration between 20 - 30%; yellow to nitration between 10 - 20%; and blue color corresponds to between 1 - 10% nitration. Green color corresponds to no detectable nitration. The purple color indicates that the residue was not detected in the tryptic digests. PDB structure: 3m7p (56) corresponding to domains FNI<sub>6</sub>, FNII<sub>1-2</sub>, FNI<sub>7-9</sub> and residues 297-604.

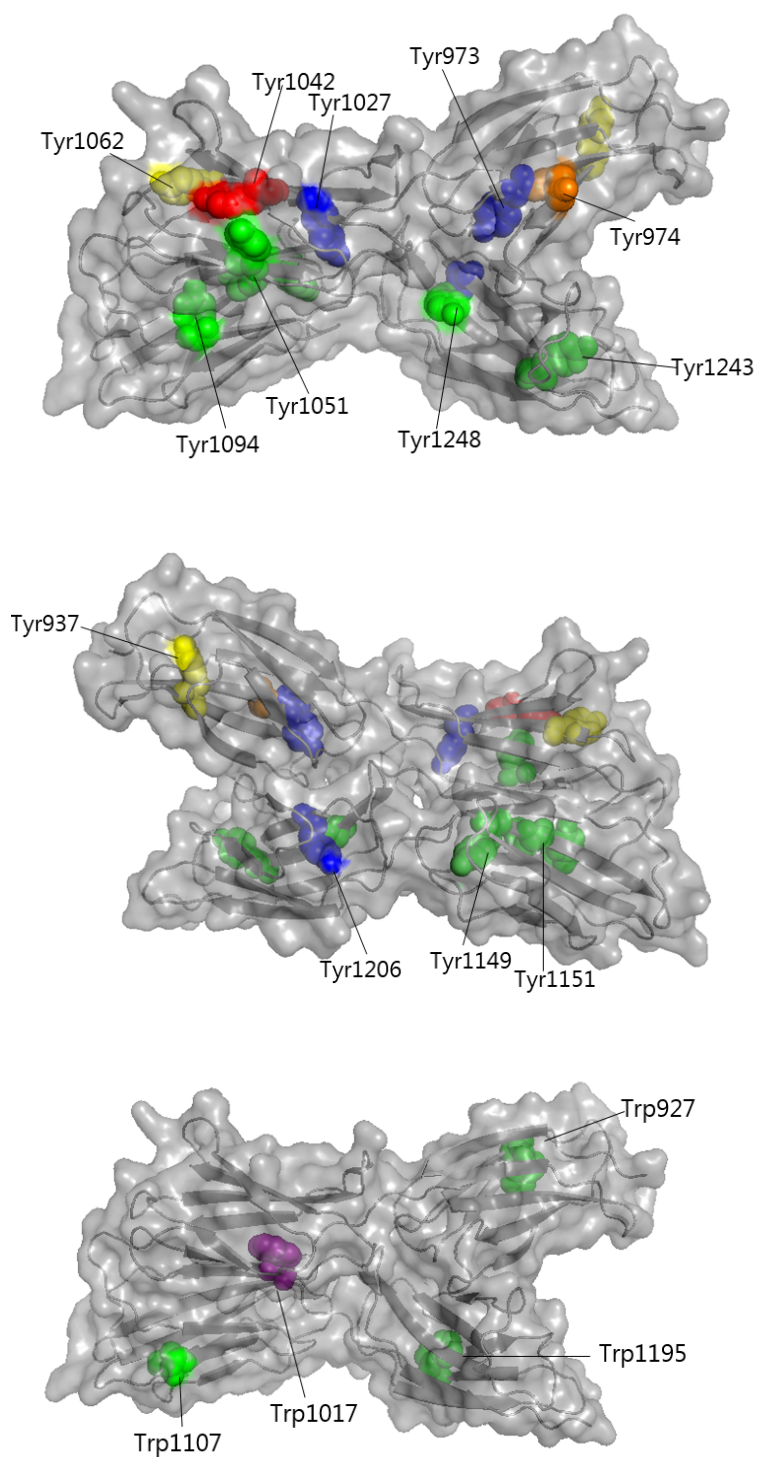


**Supplementary Figure 9.** Rendering of Tyr and Trp residues identified as nitrated in FN treated with 50 $\mu$ M ONOOH (150 mM NaCl). Red color corresponds to level of nitration higher than 30%; orange to nitration between 20 - 30%; yellow to nitration between 10 - 20%; and blue color corresponds to between 1 - 10% nitration. Green color corresponds to no detectable nitration. PDB structure: 1q38 (67), corresponding to FNIII<sub>1</sub> and residues 631-705.

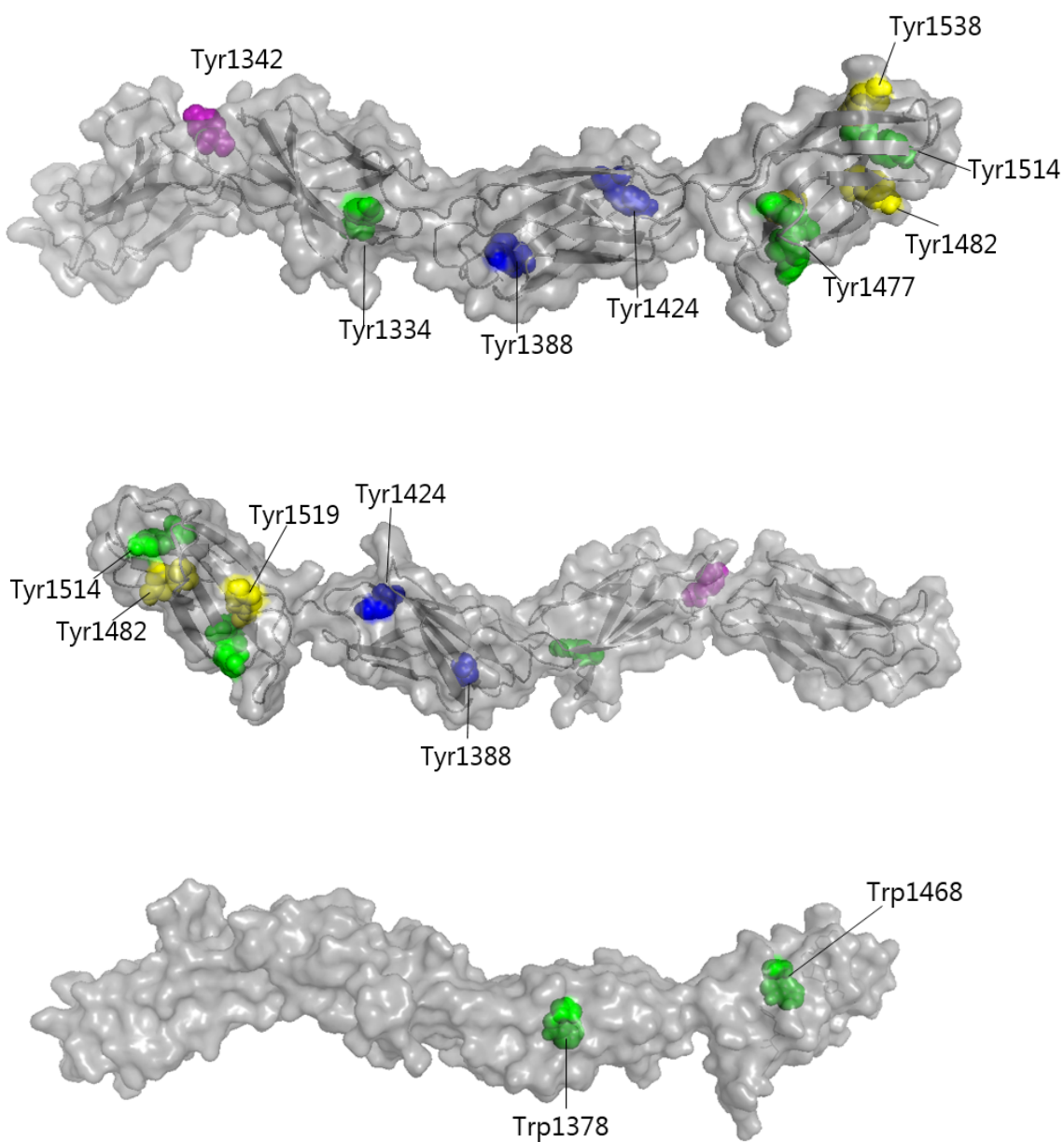


**Supplementary Figure 10.** Rendering of Tyr and Trp residues identified as nitrated in FN treated with 50 $\mu$ M ONOOH (150 mM NaCl). Red color corresponds to level of nitration higher than 30%; orange to nitration between 20 - 30%; yellow to nitration between 10 - 20%; and blue color corresponds to between 1 - 10% nitration. Green color corresponds to no detectable nitration. The purple color indicates that the residue was not detected in the tryptic digests. PDB structure: 2N1K (68) corresponding to domains FNIII<sub>2,3</sub> and residues 808-905.

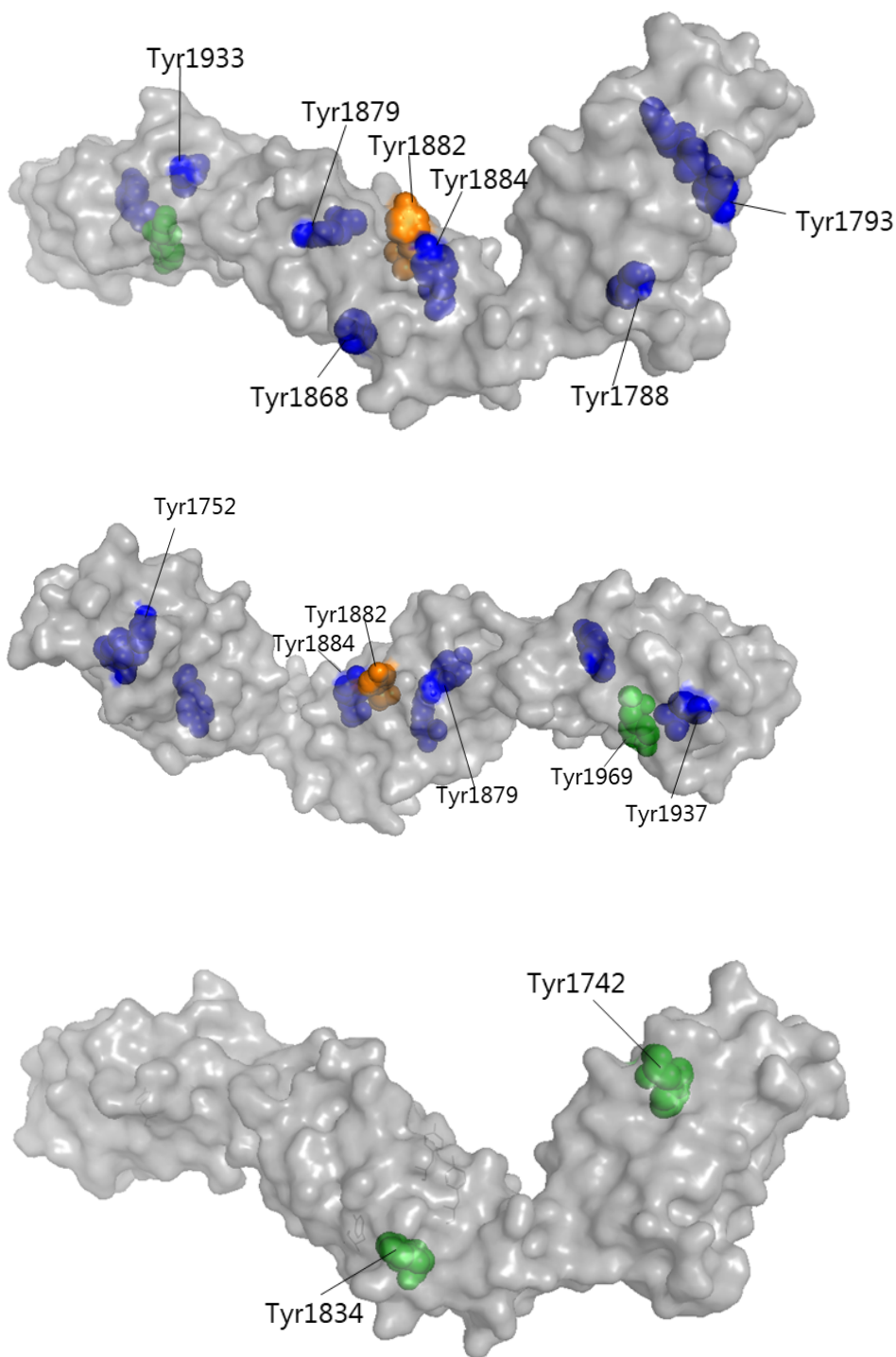




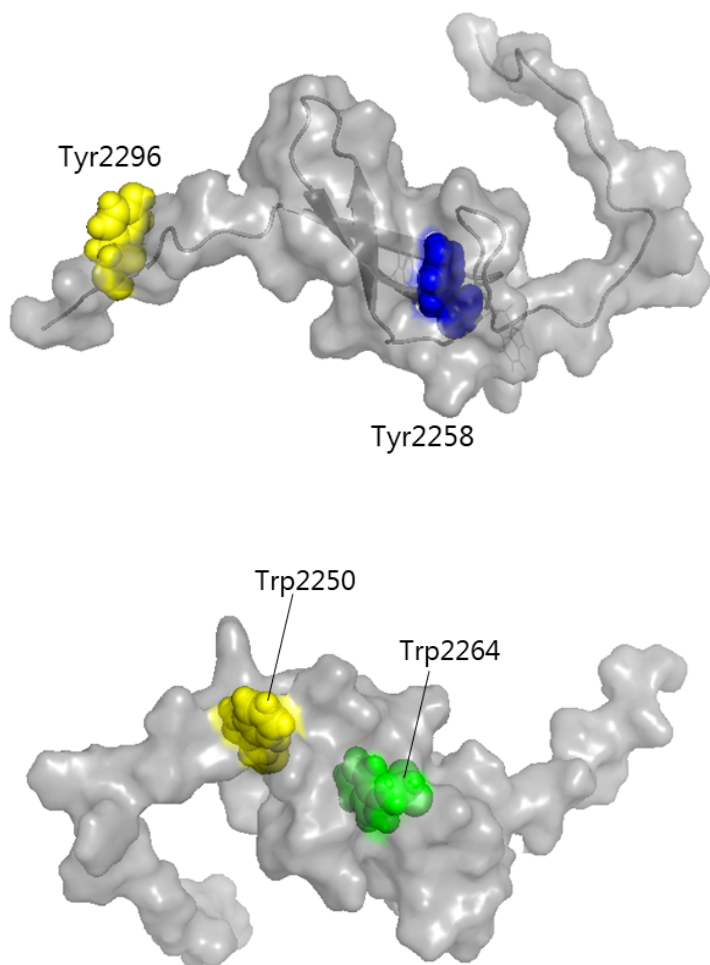
**Supplementary Figure 11.** Rendering of Tyr and Trp residues identified as nitrated in FN treated with 50 $\mu$ M ONOOH (150 mM NaCl). Red color corresponds to level of nitration higher than 30%; orange to nitration between 20 - 30%; yellow to nitration between 10 - 20%; and blue color corresponds to between 1 - 10% nitration. Green color corresponds to no detectable nitration. The purple color indicates that the residue was not detected in the tryptic digests. PDB structure: 6mfa (unpublished data deposited in PDB), corresponding to domains FNIII<sub>3-7</sub>, and residues 903-1268.



**Supplementary Figure 12.** Rendering of Tyr and Trp residues identified as nitrated in FN treated with 50 $\mu$ M ONOOH (150 mM NaCl). Red color corresponds to level of nitration higher than 30%; orange to nitration between 20 - 30%; yellow to nitration between 10 - 20%; and blue color corresponds to between 1 - 10% nitration. Green color corresponds to no detectable nitration. The purple color indicates that the residue was not detected in the tryptic digests. PDB structure: 1fnf (69) corresponding to domains FNIII<sub>6-10</sub> and residues 1173-1540.



**Supplementary Figure 13.** Rendering of Tyr and Trp residues identified as nitrated in FN treated with 50 $\mu$ M ONOOH (150 mM NaCl). Red color corresponds to level of nitration higher than 30%; orange to nitration between 20 - 30%; yellow to nitration between 10 - 20%; and blue color corresponds to between 1 - 10% nitration. Green color corresponds to no detectable nitration. PDB structure: 3r8q (unpublished data deposited in PDB) corresponding to domains FNIII<sub>12-15</sub>, and residues 1721-1991.



**Supplementary Figure 14.** Rendering of Tyr and Trp residues identified as nitrated in FN treated with 50 $\mu$ M ONOOH (150 mM NaCl). Red color corresponds to level of nitration higher than 30%; orange to nitration between 20 - 30%; yellow to nitration between 10 - 20%; and blue color corresponds to between 1 - 10% nitration. Green color corresponds to no detectable nitration, although Trp2264 was detected as nitrated in a cross-linked peptide, see Table 2). PDB structure: 2ec3 (unpublished data deposited in PDB) corresponding to domain FNI<sub>11</sub> (residues 2241-2299).