

# QUANTITATIVE MASS SPECTROMETRY REVEALS THE DYNAMICS OF FACTOR INHIBITING HIF (FIH)-CATALYZED HYDROXYLATION

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Running title: Characteristics of FIH-catalyzed asparaginyl hydroxylation

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## **Section B – Experimental Results**

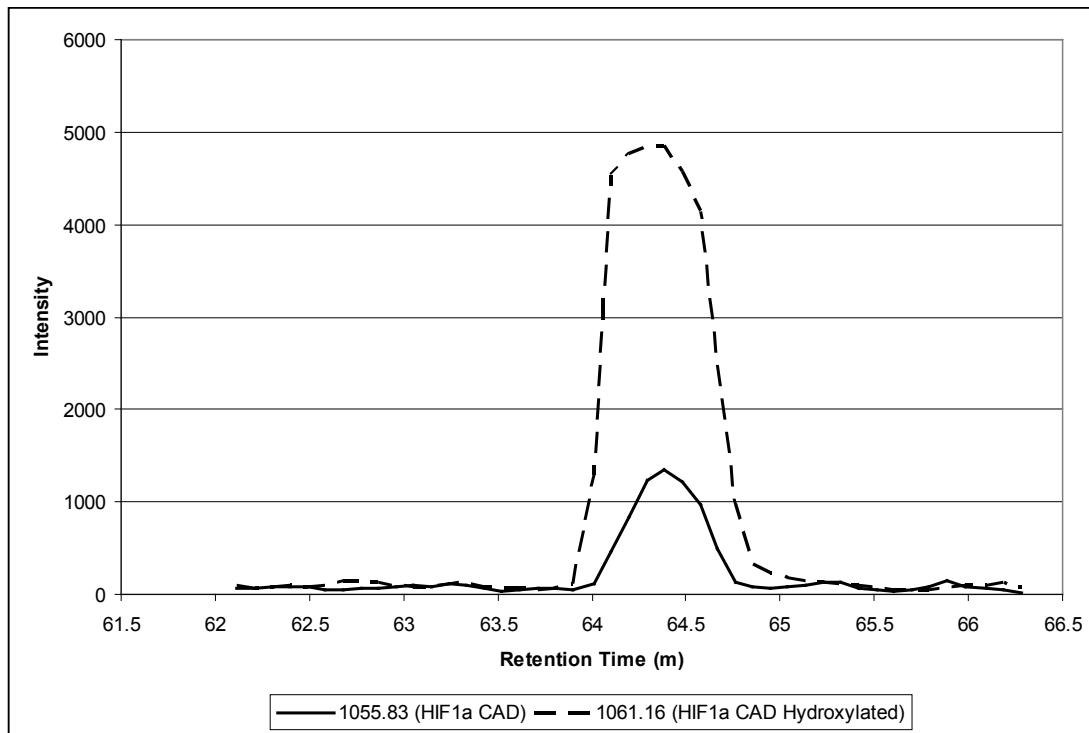
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## SUPPLEMENTARY FIGURES

### Section A – Establishing Peptide ID, Accurate Mass & Retention Time (AMT)



**Figure S1:** Extracted ion chromatograms showing retention times of HIF1-CAD peptides on Waters platform.

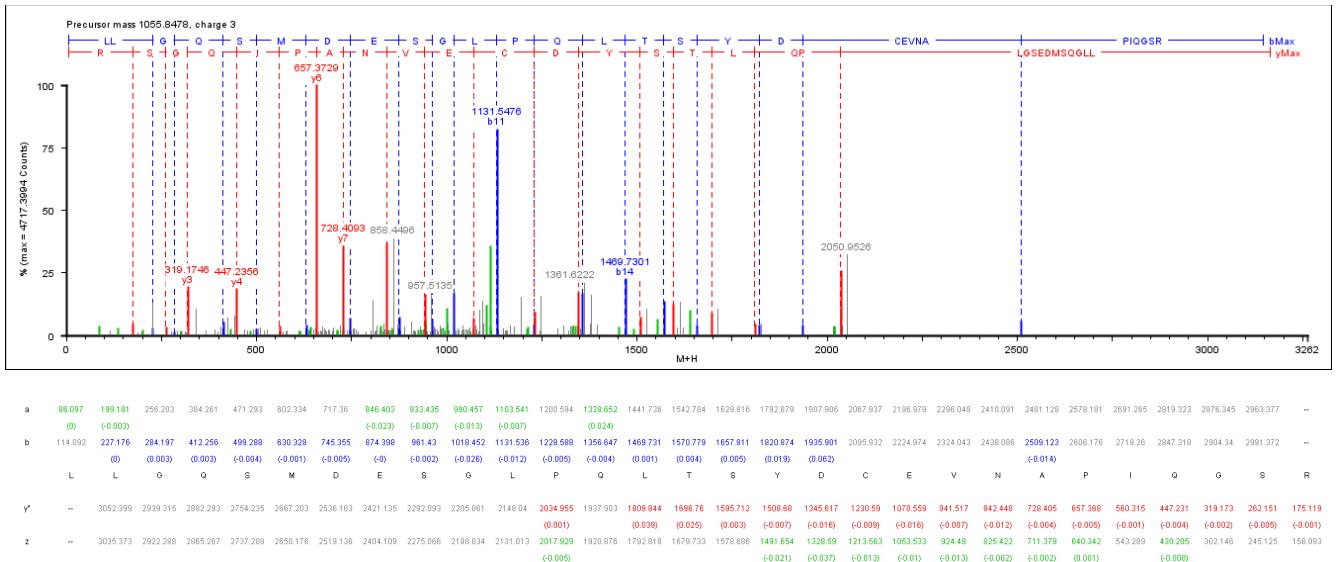


Figure S2: Annotated spectrum of HIF1-CAD peptide on Waters platform.

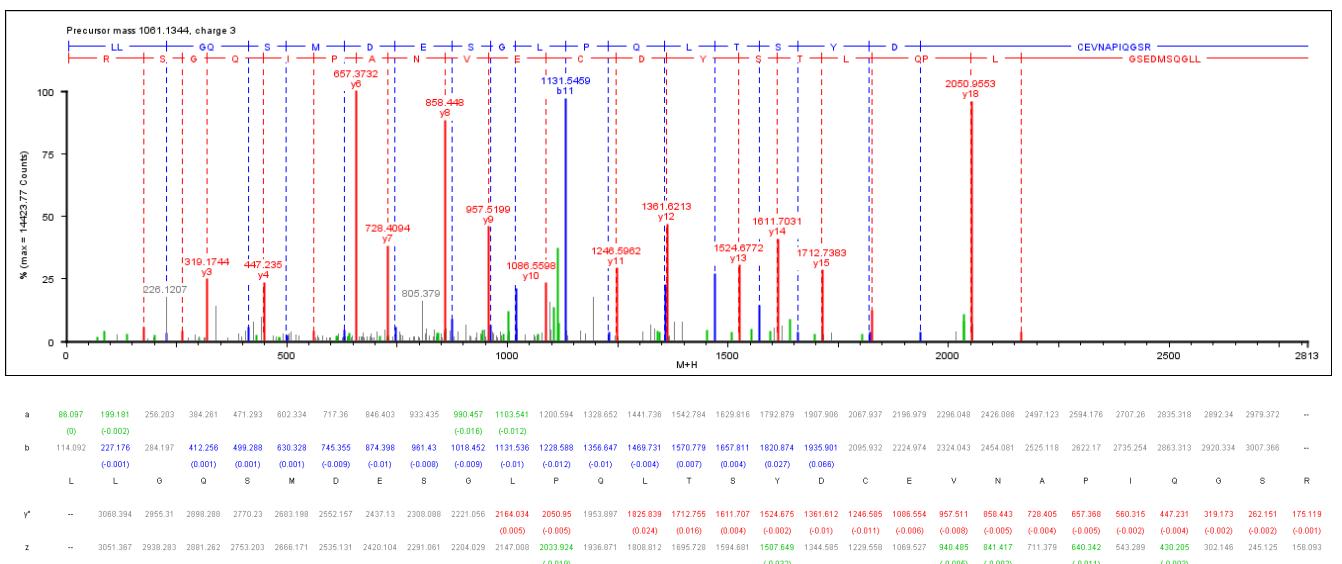
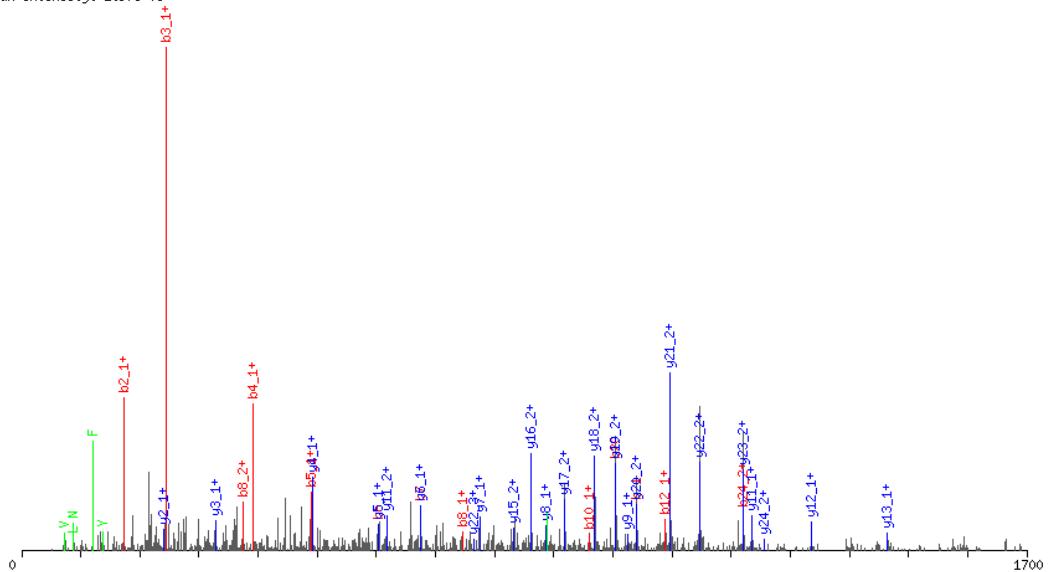


Figure S3: Annotated spectrum of hydroxylated HIF1-CAD peptide on Waters platform.

NGAFVNAATLGAQETPLHLVALYSSK(+6.02)

Charge: 3, m/z: 893.478

Max Intensity: 2.97e+03



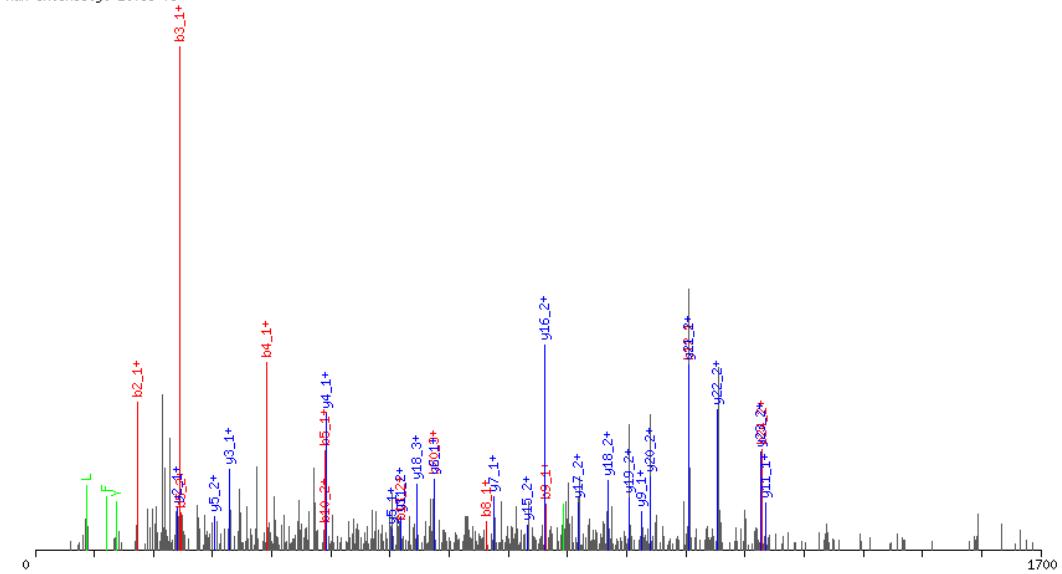
		b+	b++	b+++	y+	y++	y+++		
N	1	115.05	58.03	39.02				26	N
G	2	172.07	86.54	58.03	2564.38	1282.69	855.47	25	G
A	3	243.11	122.06	81.71	2507.36	1254.18	836.46	24	A
F	4	390.18	195.59	130.73	2436.32	1218.67	812.78	23	F
V	5	489.25	245.13	163.75	2289.25	1145.13	763.76	22	V
N	6	603.29	302.15	201.77	2190.19	1095.60	730.73	21	N
A	7	674.33	337.67	225.45	2076.14	1038.58	692.72	20	A
A	8	745.36	373.19	249.13	2005.11	1003.06	669.04	19	A
T	9	846.41	423.71	282.81	1934.07	967.54	645.36	18	T
L	10	959.49	480.25	320.50	1833.02	917.01	611.68	17	L
G	11	1016.52	508.76	339.51	1719.94	860.47	573.98	16	G
A	12	1087.55	544.28	363.19	1662.92	831.96	554.98	15	A
Q	13	1215.61	608.31	405.88	1591.88	796.44	531.30	14	Q
E	14	1344.65	672.83	448.89	1463.82	732.41	488.61	13	E
T	15	1445.70	723.35	482.57	1334.78	667.89	445.60	12	T
P	16	1542.75	771.88	514.92	1233.73	617.37	411.91	11	P
L	17	1655.84	828.42	552.62	1136.68	568.84	379.56	10	L
H	18	1792.90	896.95	598.30	1023.59	512.30	341.87	9	H
L	19	1905.98	953.49	636.00	886.53	443.77	296.18	8	L
V	20	2005.05	1003.03	669.02	773.45	387.23	258.49	7	V
A	21	2076.09	1038.55	692.70	674.38	337.69	225.47	6	A
L	22	2189.17	1095.09	730.40	603.34	302.18	201.79	5	L
Y	23	2352.23	1176.62	784.75	490.26	245.63	164.09	4	Y
S	24	2439.27	1220.14	813.76	327.20	164.10	109.74	3	S
S	25	2526.30	1263.65	842.77	240.16	120.59	80.73	2	S
K	26				153.13	77.07	51.72	1	K

**Figure S4:** Annotated spectrum of heavy-labelled Rabankyrin-5 Asn-316 peptide on Agilent platform.

NGAFVN(+15.99)AATLGAQETPLHLVALYSSK(+6.02)

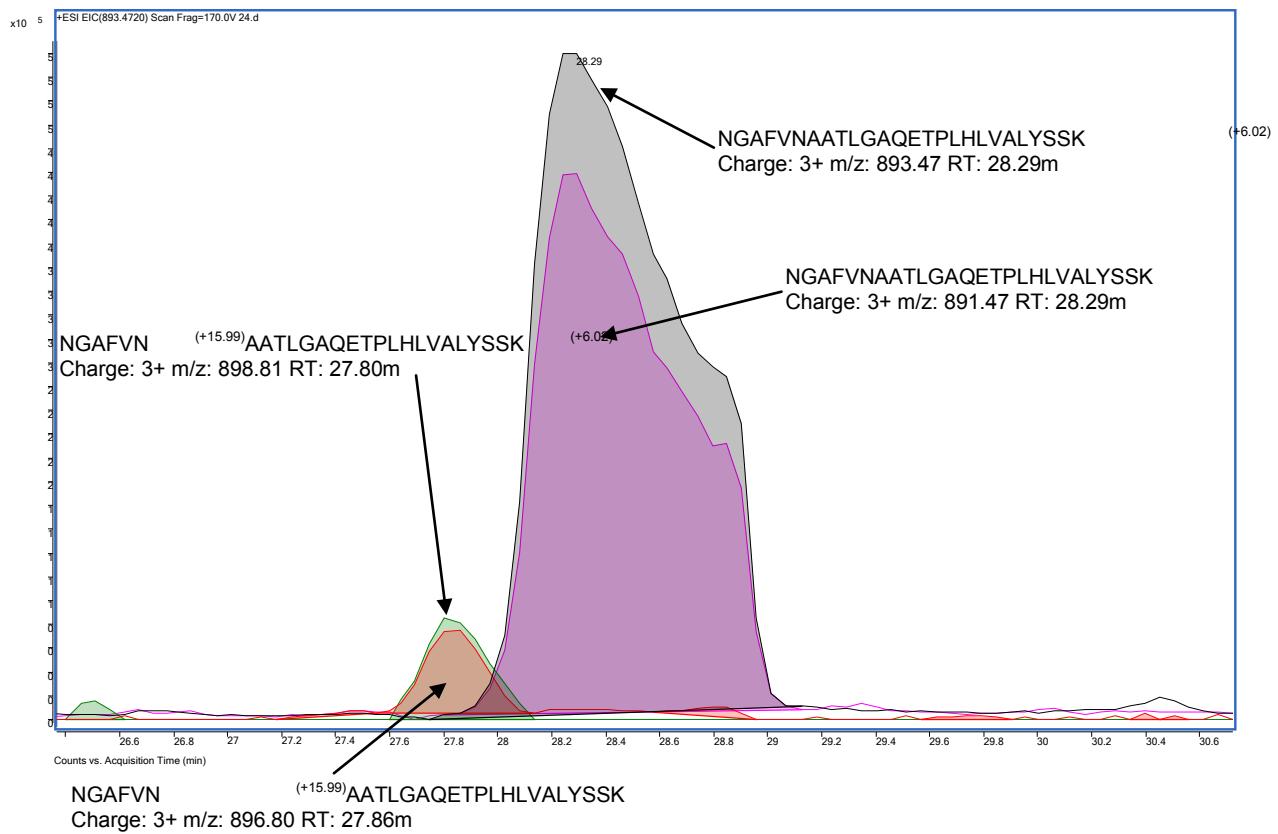
Charge: 3, m/z: 898.813

Max Intensity: 1.03e+03



		b+	b++	b+++	y+	y++	y+++		
N	1	115.05	58.03	39.02				26	N
G	2	172.07	86.54	58.03	2580.38	1290.69	860.80	25	G
A	3	243.11	122.06	81.71	2523.35	1262.18	841.79	24	A
F	4	390.18	195.59	130.73	2462.32	1226.66	818.11	23	F
V	5	489.25	245.13	163.75	2305.25	1153.13	769.09	22	V
N	6	619.28	310.15	207.10	2206.18	1103.59	736.07	21	N
A	7	690.32	345.66	230.78	2076.14	1038.58	692.72	20	A
A	8	761.36	381.18	254.46	2005.11	1003.06	669.04	19	A
T	9	862.41	431.71	288.14	1934.07	967.54	645.36	18	T
L	10	975.49	488.25	325.83	1833.02	917.01	611.68	17	L
G	11	1032.51	516.76	344.84	1719.94	860.47	573.98	16	G
A	12	1103.55	552.28	368.52	1662.92	831.96	554.98	15	A
Q	13	1231.61	616.31	411.21	1591.88	796.44	531.30	14	Q
E	14	1360.65	680.83	454.22	1463.82	732.41	488.61	13	E
T	15	1461.70	731.35	487.90	1334.78	667.89	445.60	12	T
P	16	1558.75	779.88	520.25	1233.73	617.37	411.91	11	P
L	17	1671.83	836.42	557.95	1136.68	568.84	379.56	10	L
H	18	1808.89	904.95	603.64	1023.59	512.30	341.87	9	H
L	19	1921.98	961.49	641.33	886.53	443.77	296.18	8	L
V	20	2021.05	1011.03	674.35	773.45	387.23	258.49	7	V
A	21	2092.08	1046.54	698.03	674.38	337.89	225.47	6	A
L	22	2205.17	1103.09	735.73	603.34	302.18	201.79	5	L
Y	23	2368.23	1184.62	790.08	490.26	245.63	164.09	4	Y
S	24	2465.26	1228.13	819.09	327.20	164.10	109.74	3	S
S	25	2542.29	1271.65	848.10	240.16	120.59	80.73	2	S
K	26				153.13	77.07	51.72	1	K

**Figure S5:** Annotated spectrum of heavy-labelled hydroxylated Rabankyrin-5 Asn-316 peptide on Agilent platform.

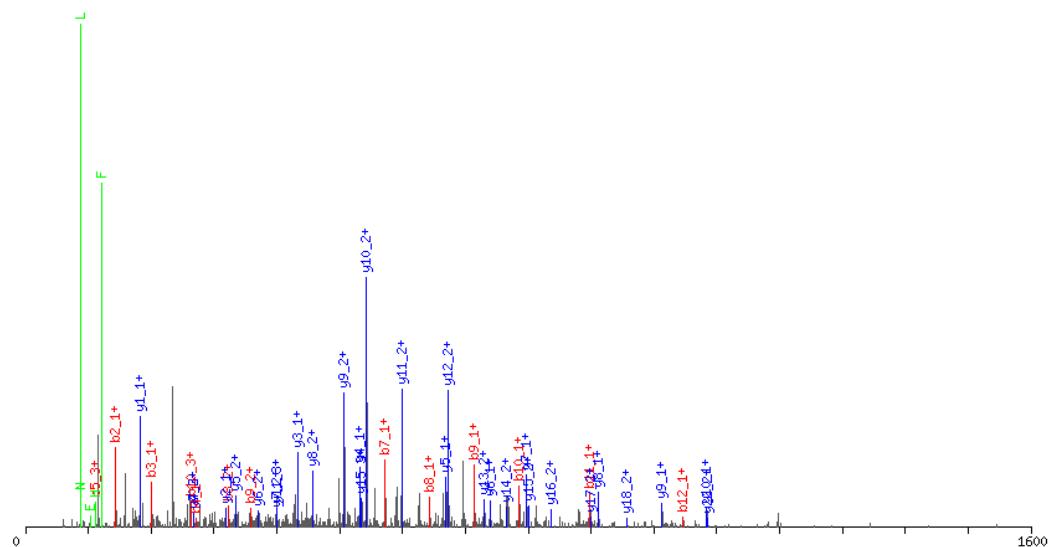


**Figure S6:** Extracted ion chromatograms showing retention times of Rabankyrin-5 Asn-316 peptides on Agilent platform.



Charge: 4, m/z: 560.543

Max Intensity: 4.12e+03



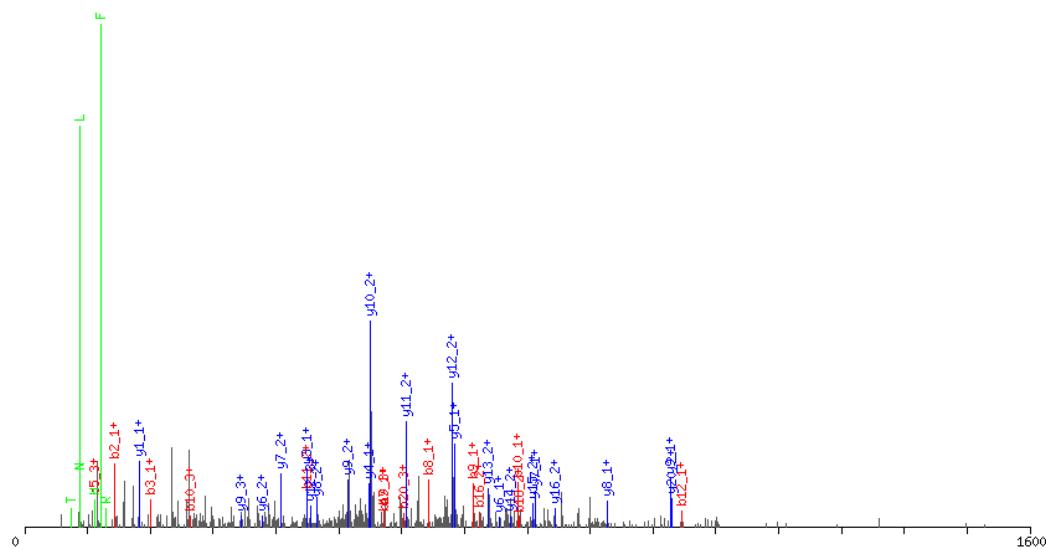
	b+	b++	b+++	y+	y++	y+++		
A	1	72.04	36.53	24.69				23 A
A	2	143.08	72.04	48.37	2168.11	1084.56	723.37	22 A
G	3	200.10	100.56	67.37	2097.07	1049.04	699.69	21 G
A	4	271.14	136.07	91.05	2040.05	1020.53	680.69	20 A
G	5	328.16	164.58	110.06	1969.01	985.01	657.01	19 G
N	6	442.20	221.61	148.07	1911.99	956.50	638.00	18 N
E	7	571.25	286.13	191.09	1797.95	899.48	599.99	17 E
A	8	642.28	321.65	214.77	1668.90	834.95	556.97	16 A
A	9	713.32	357.16	238.46	1597.87	799.44	533.29	15 A
A	10	784.36	392.68	262.12	1526.83	763.92	509.61	14 A
L	11	897.44	449.22	299.82	1455.79	728.40	485.94	13 L
F	12	1044.51	522.76	348.84	1342.71	671.86	448.24	12 F
L	13	1157.59	579.30	386.54	1195.64	598.32	399.22	11 L
A	14	1228.63	614.82	410.22	1082.55	541.78	361.52	10 A
T	15	1329.68	665.34	443.90	1011.52	506.26	337.84	9 T
N	16	1443.72	722.36	481.91	910.47	455.74	304.16	8 N
G	17	1500.74	750.88	500.92	796.43	398.72	266.15	7 G
A	18	1571.78	786.39	524.60	739.41	370.21	247.14	6 A
H	19	1708.84	854.92	570.28	668.37	334.69	223.46	5 H
V	20	1807.91	904.46	603.31	531.31	266.16	177.77	4 V
N	21	1921.95	961.48	641.32	432.24	216.62	144.75	3 N
H	22	2059.01	1030.01	687.01	318.20	159.60	106.74	2 H
R	23				181.14	91.07	61.05	1 R

**Figure S7:** Annotated spectrum of heavy-labelled Rabankyrin-5 Asn-485 peptide on Agilent platform.

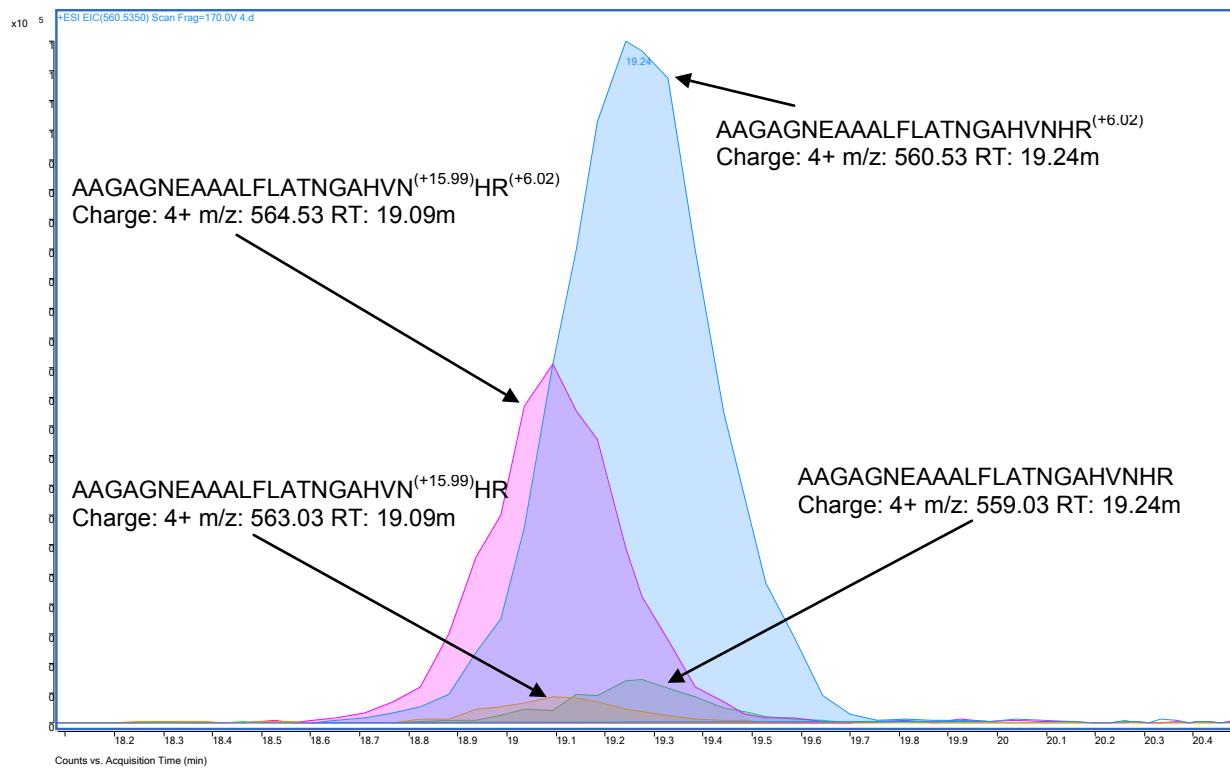
AAGAGNEAAALFLATNGAHVN(+15.99)HR(+6.02)

Charge: 4, m/z: 564.543

Max Intensity: 2.52e+03



**Figure S8:** Annotated spectrum of heavy-labelled hydroxylated Rabankyrin-5 Asn-485 peptide on Agilent platform.

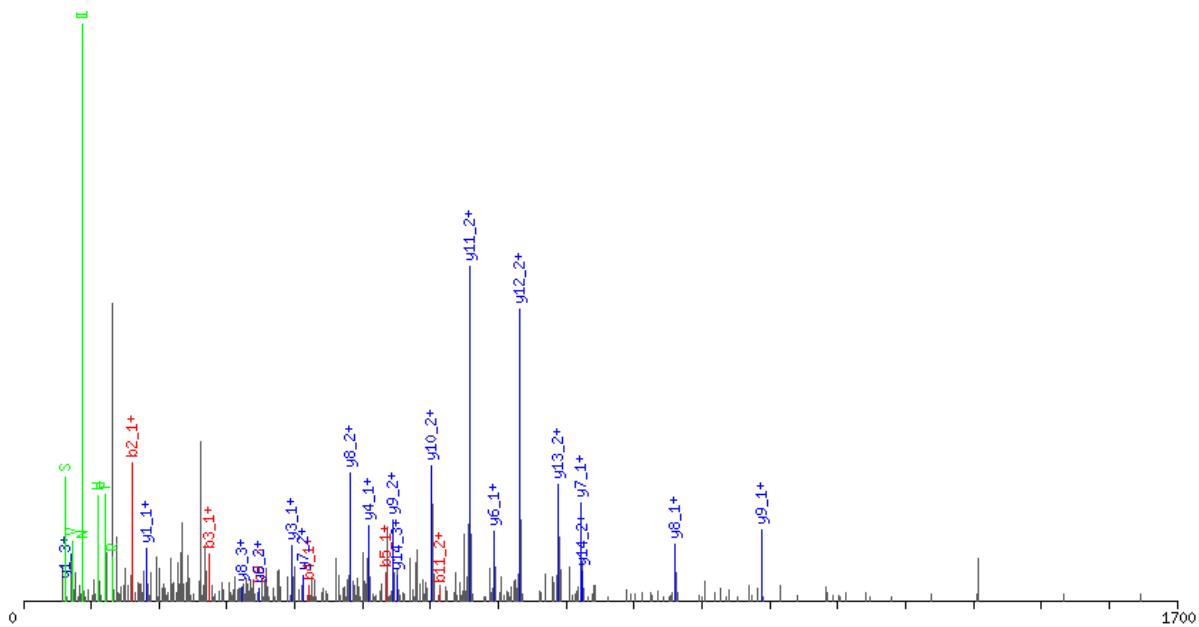


**Figure S9:** Extracted ion chromatograms showing retention times of Rabankyrin-5 Asn-485 peptides on Agilent platform.

SALFLLEHQADINVR(+6.02)

Charge: 3, m/z: 577.988

Max Intensity: 1.45e+03



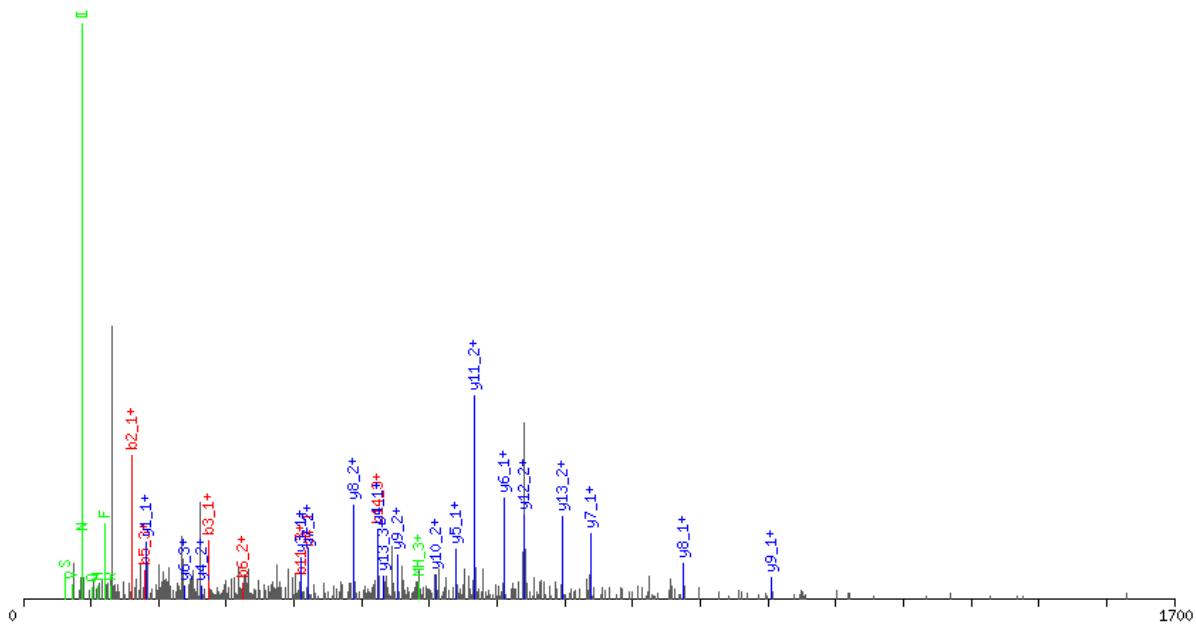
		b+	b++	b+++	y+	y++	y+++		
S	1	88.04	44.52	30.02				15	S
A	2	159.08	80.04	53.70	1644.92	822.96	548.98	14	A
L	3	272.16	136.58	91.39	1573.88	787.44	525.30	13	L
F	4	419.23	210.12	140.41	1460.80	730.90	487.60	12	F
L	5	532.31	266.66	178.11	1313.73	657.37	438.58	11	L
L	6	645.40	323.20	215.80	1200.64	600.82	400.89	10	L
E	7	774.44	387.72	258.82	1087.56	544.28	363.19	9	E
H	8	911.50	466.25	304.50	958.52	479.76	320.18	8	H
Q	9	1039.56	520.28	347.19	821.46	411.23	274.49	7	Q
A	10	1110.59	555.80	370.87	693.40	347.20	231.80	6	A
D	11	1225.62	613.31	409.21	622.36	311.68	208.13	5	D
I	12	1338.71	669.86	446.91	507.33	254.17	169.78	4	I
N	13	1452.75	726.88	484.92	394.25	197.63	132.09	3	N
V	14	1551.82	776.41	517.94	280.21	140.61	94.07	2	V
R	15				181.14	91.07	61.05	1	R

**Figure S10:** Annotated spectrum of heavy-labelled Rabankyrin-5 Asn-649 peptide on Agilent platform.

SALFLLEHQADIN(+15.99)VR(+6.02)

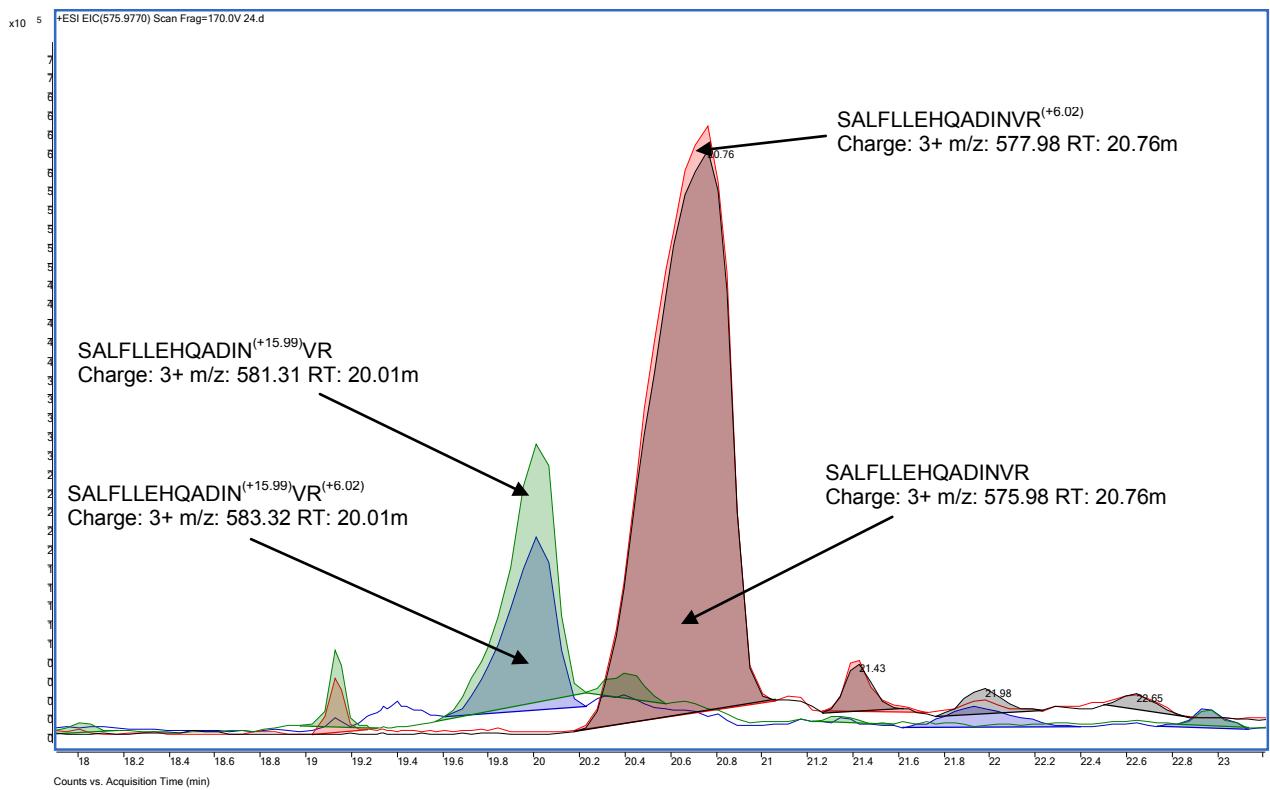
Charge: 3, m/z: 583.321

Max Intensity: 2.03e+03

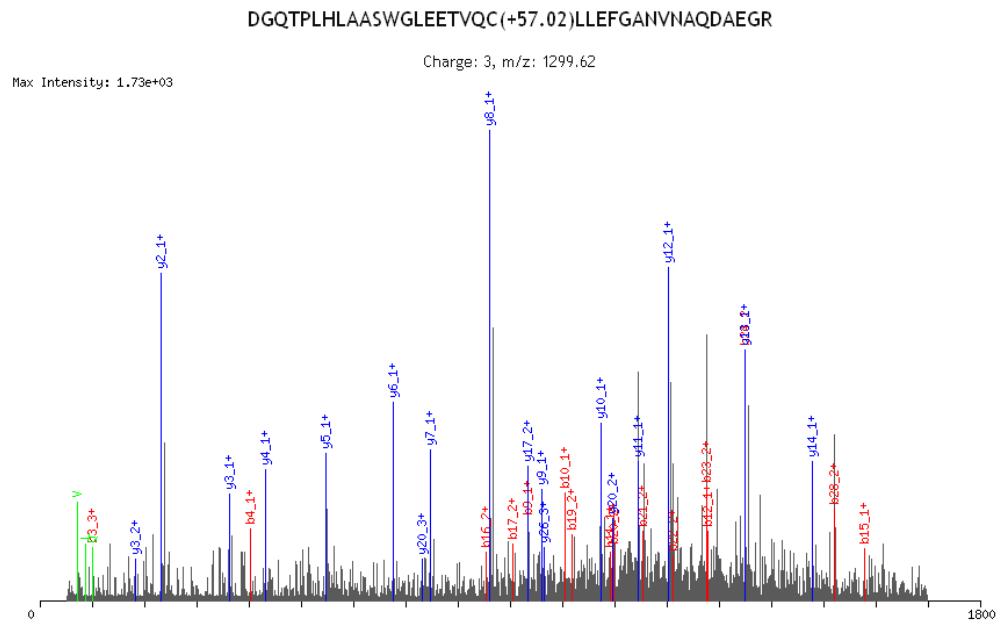


		b+	b++	b+++	y+	y++	y+++		
S	1	88.04	44.52	30.02				15	S
A	2	159.08	80.04	53.70	1860.91	830.96	554.31	14	A
L	3	272.16	136.58	91.39	1589.87	795.44	530.63	13	L
F	4	419.23	210.12	140.41	1476.79	738.90	492.93	12	F
L	5	532.31	266.66	178.11	1329.72	665.36	443.91	11	L
L	6	645.40	323.20	215.80	1216.64	608.82	406.22	10	L
E	7	774.44	387.72	258.82	1103.55	552.28	368.52	9	E
H	8	911.50	466.25	304.50	974.51	487.76	325.51	8	H
Q	9	1039.56	520.28	347.19	837.45	419.23	279.82	7	Q
A	10	1110.59	565.80	370.87	709.39	355.20	237.14	6	A
D	11	1225.62	613.31	409.21	638.36	319.68	213.46	5	D
I	12	1338.71	669.86	446.91	523.33	262.17	175.11	4	I
N	13	1468.74	734.88	490.25	410.25	205.63	137.42	3	N
V	14	1567.81	784.41	523.28	280.21	140.61	94.07	2	V
R	15				181.14	91.07	61.05	1	R

**Figure S11:** Annotated spectrum of heavy-labelled hydroxylated Rabankyrin-5 Asn-649 peptide on Agilent platform.

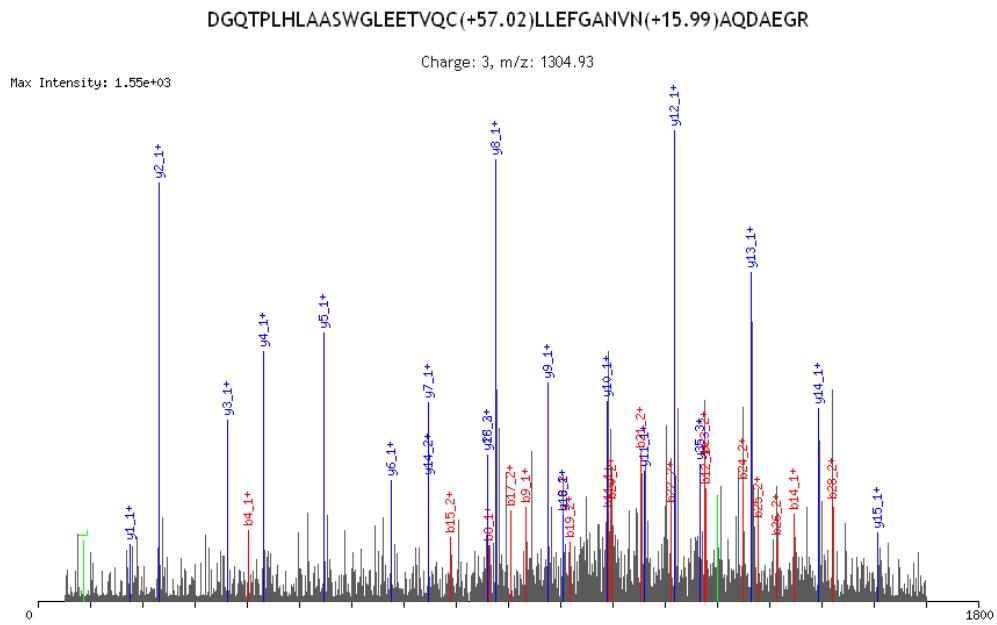


**Figure S12:** Extracted ion chromatograms showing retention times of Rabankyrin-5 Asn-649 peptides on Agilent platform.



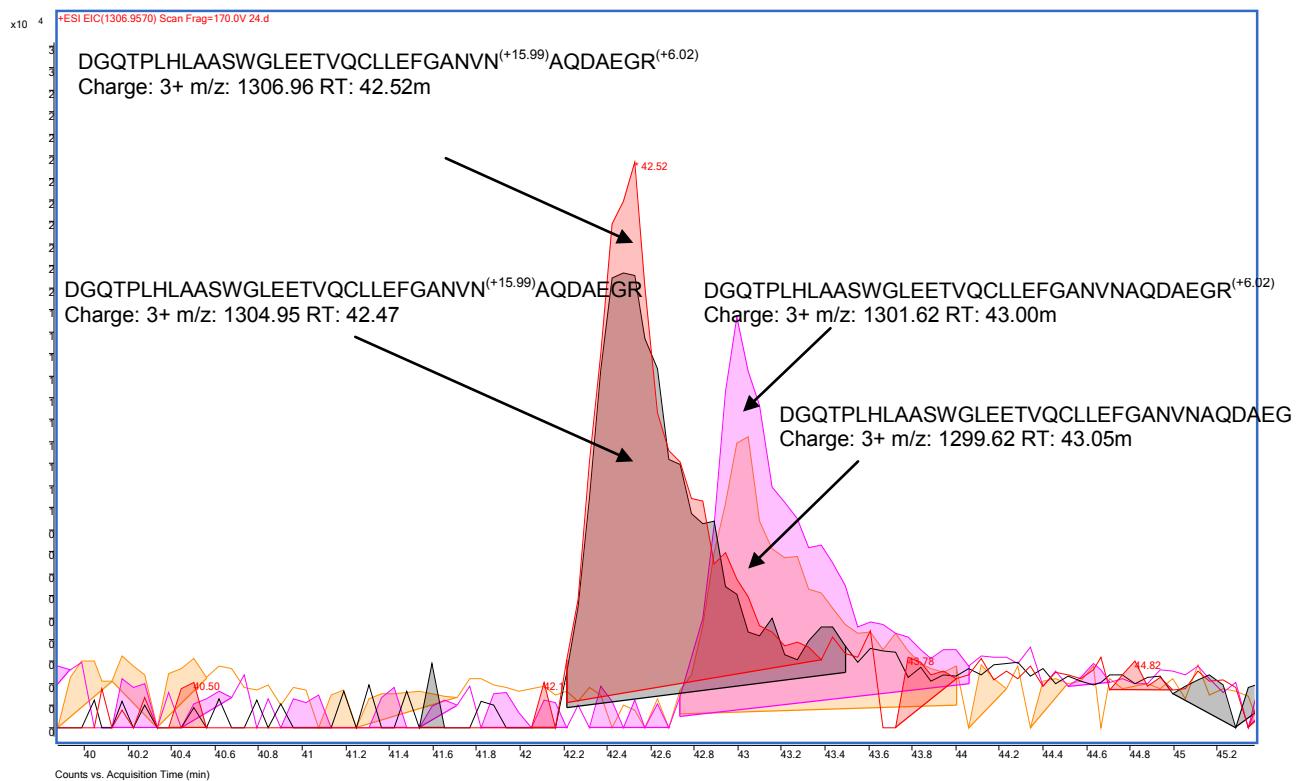
		b+	b++	b+++	y+	y++	y+++		
D	1	116.03	58.52	39.35				36	D
G	2	173.06	87.03	58.36	3781.82	1891.42	1261.28	35	G
Q	3	301.11	151.06	101.04	3724.80	1862.90	1242.27	34	Q
T	4	402.16	201.58	134.73	3596.74	1798.88	1199.59	33	T
P	5	499.21	250.11	167.08	3495.70	1749.35	1165.90	32	P
L	6	612.30	306.65	204.77	3398.64	1699.83	1133.55	31	L
H	7	749.36	375.18	250.46	3285.56	1643.28	1095.56	30	H
L	8	862.44	431.72	288.15	3148.50	1574.75	1050.17	29	L
A	9	933.48	467.24	311.83	3035.42	1518.21	1012.48	28	A
A	10	1004.52	502.76	335.51	2964.38	1482.69	998.80	27	A
S	11	1091.55	546.28	364.52	2893.34	1447.17	965.12	26	S
W	12	1277.63	639.32	426.55	2806.31	1403.66	936.11	25	W
G	13	1334.65	667.83	445.55	2620.23	1310.62	874.08	24	G
L	14	1447.73	724.37	483.25	2563.21	1282.11	855.07	23	L
E	15	1576.78	788.89	526.26	2450.13	1225.57	817.38	22	E
E	16	1705.82	853.41	569.28	2321.08	1161.04	774.37	21	E
T	17	1806.87	903.94	602.96	2192.04	1096.52	731.35	20	T
V	18	1905.93	953.47	635.98	2090.99	1046.00	697.67	19	V
Q	19	2033.99	1017.50	678.67	1991.92	996.47	664.65	18	Q
C	20	2194.02	1097.52	732.01	1863.87	932.44	621.98	17	C
L	21	2307.11	1154.06	769.71	1703.83	852.42	568.62	16	L
L	22	2420.19	1210.60	807.40	1590.75	795.88	530.92	15	L
E	23	2549.23	1275.12	850.42	1477.67	739.34	493.23	14	E
F	24	2666.30	1348.65	899.44	1348.62	674.82	450.21	13	F
G	25	2753.32	1377.17	918.45	1201.56	601.28	401.19	12	G
A	26	2824.36	1412.68	942.13	1144.53	572.77	382.18	11	A
N	27	2938.40	1469.71	980.14	1073.50	537.25	358.50	10	N
V	28	3037.47	1519.24	1013.16	959.45	480.23	320.49	9	V
N	29	3151.52	1576.26	1051.18	880.39	430.70	287.47	8	N
A	30	3222.55	1611.78	1074.86	746.34	373.68	249.45	7	A
Q	31	3350.61	1675.81	1117.54	675.31	338.16	225.77	6	Q
D	32	3465.64	1733.32	1155.88	547.25	274.13	183.09	5	D
A	33	3536.68	1768.84	1179.56	432.22	216.61	144.74	4	A
E	34	3665.72	1833.36	1222.58	361.18	181.10	121.07	3	E
G	35	3722.74	1861.87	1241.58	232.14	116.57	78.05	2	G
R	36				175.12	88.06	59.04	1	R

**Figure S13:** Annotated spectrum of Rabankyrin-5 Asn-797 peptide on Agilent platform.

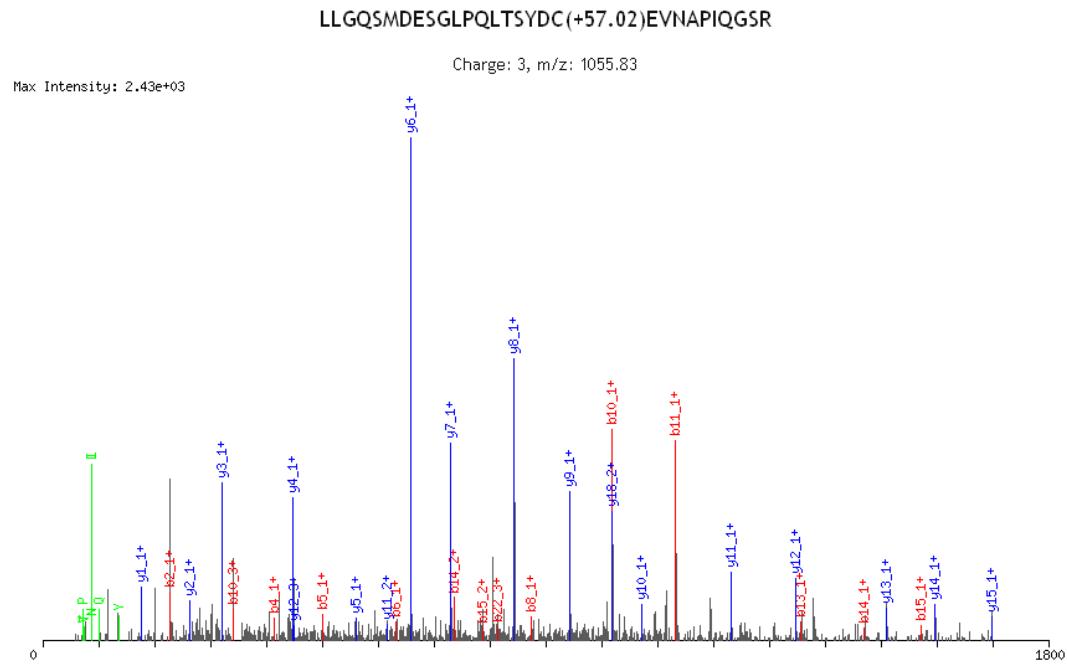


	b+	b++	b+++	y <sup>+</sup>	y <sup>++</sup>	y <sup>+++</sup>			
D	1	116.03	58.52	39.35			36	D	
G	2	173.06	87.03	58.36	3797.82	1899.41	1266.81	35	G
Q	3	301.11	151.06	101.04	3740.80	1870.90	1247.50	34	Q
T	4	402.16	201.58	134.73	3612.74	1808.87	1204.92	33	T
P	5	499.21	250.11	167.08	3511.69	1756.35	1171.24	32	P
L	6	612.30	306.65	204.77	3414.64	1707.82	1138.88	31	L
H	7	749.36	375.18	250.46	3301.55	1651.28	1101.19	30	H
L	8	882.44	431.72	288.15	3164.50	1582.75	1055.50	29	L
A	9	933.48	467.24	311.83	3051.41	1526.21	1017.81	28	A
A	10	1004.52	502.76	335.51	2980.37	1490.69	994.13	27	A
S	11	1091.55	546.28	364.52	2909.34	1455.17	970.45	26	S
W	12	1277.63	639.32	426.55	2822.31	1411.66	941.44	25	W
G	13	1334.65	667.83	445.55	2636.23	1318.62	879.41	24	G
L	14	1447.73	724.37	483.25	2579.20	1290.11	860.41	23	L
E	15	1576.78	788.89	526.26	2466.12	1233.56	822.71	22	E
E	16	1705.82	853.41	569.28	2337.08	1169.04	779.70	21	E
T	17	1808.87	903.94	602.96	2208.03	1104.52	736.68	20	T
V	18	1905.93	953.47	635.98	2106.99	1054.00	703.00	19	V
Q	19	2033.99	1017.50	678.67	2007.92	1004.46	669.98	18	Q
C	20	2194.02	1097.52	732.01	1879.86	940.43	627.29	17	C
L	21	2307.11	1154.06	769.71	1719.83	860.42	573.95	16	L
L	22	2420.19	1210.60	807.40	1606.75	803.88	536.25	15	L
E	23	2649.23	1275.12	850.42	1493.66	747.33	498.56	14	E
F	24	2696.30	1348.65	899.44	1364.62	682.81	455.54	13	F
G	25	2753.32	1377.17	918.45	1217.55	609.28	406.52	12	G
A	26	2824.36	1412.68	942.13	1160.53	580.77	387.51	11	A
N	27	2938.40	1469.71	980.14	1089.49	546.25	363.84	10	N
V	28	3037.47	1519.24	1013.16	975.45	488.23	325.82	9	V
N	29	3167.51	1584.26	1056.51	876.38	438.69	292.80	8	N
A	30	3238.55	1619.78	1080.19	746.34	373.68	249.45	7	A
Q	31	3366.61	1683.81	1122.87	679.31	338.16	225.77	6	Q
D	32	3481.63	1741.32	1161.22	547.25	274.13	183.09	5	D
A	33	3562.67	1776.84	1184.89	432.22	216.81	144.74	4	A
E	34	3681.71	1841.36	1227.91	361.18	181.10	121.07	3	E
G	35	3738.73	1869.87	1246.92	232.14	116.57	78.05	2	G
R	36				176.12	88.06	59.04	1	R

**Figure S14:** Annotated spectrum of hydroxylated Rabankyrin-5 Asn-797 peptide on Agilent platform.

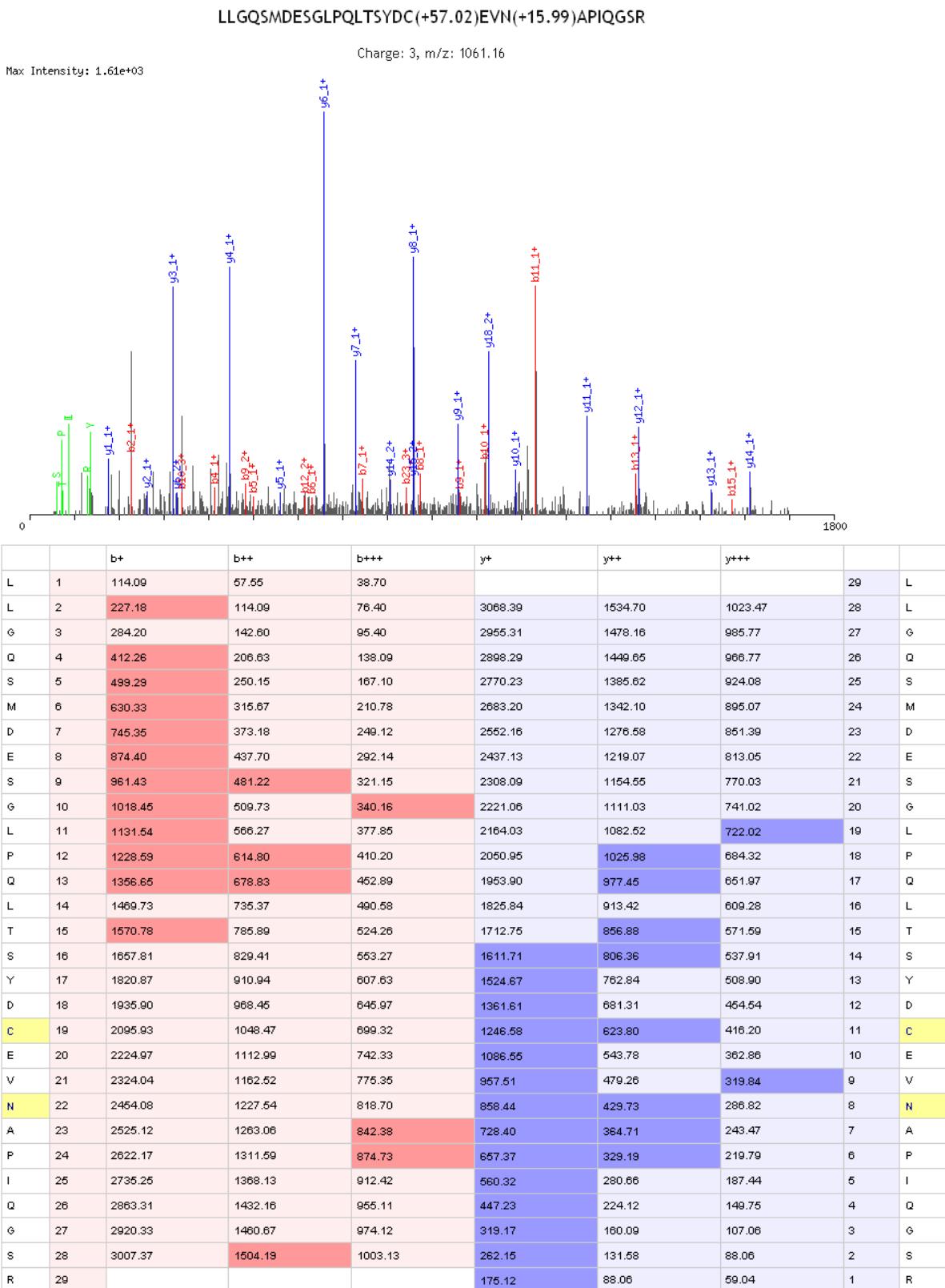


**Figure S15:** Extracted ion chromatograms showing retention times of Rabankyrin-5 Asn-797 peptides on Agilent platform.

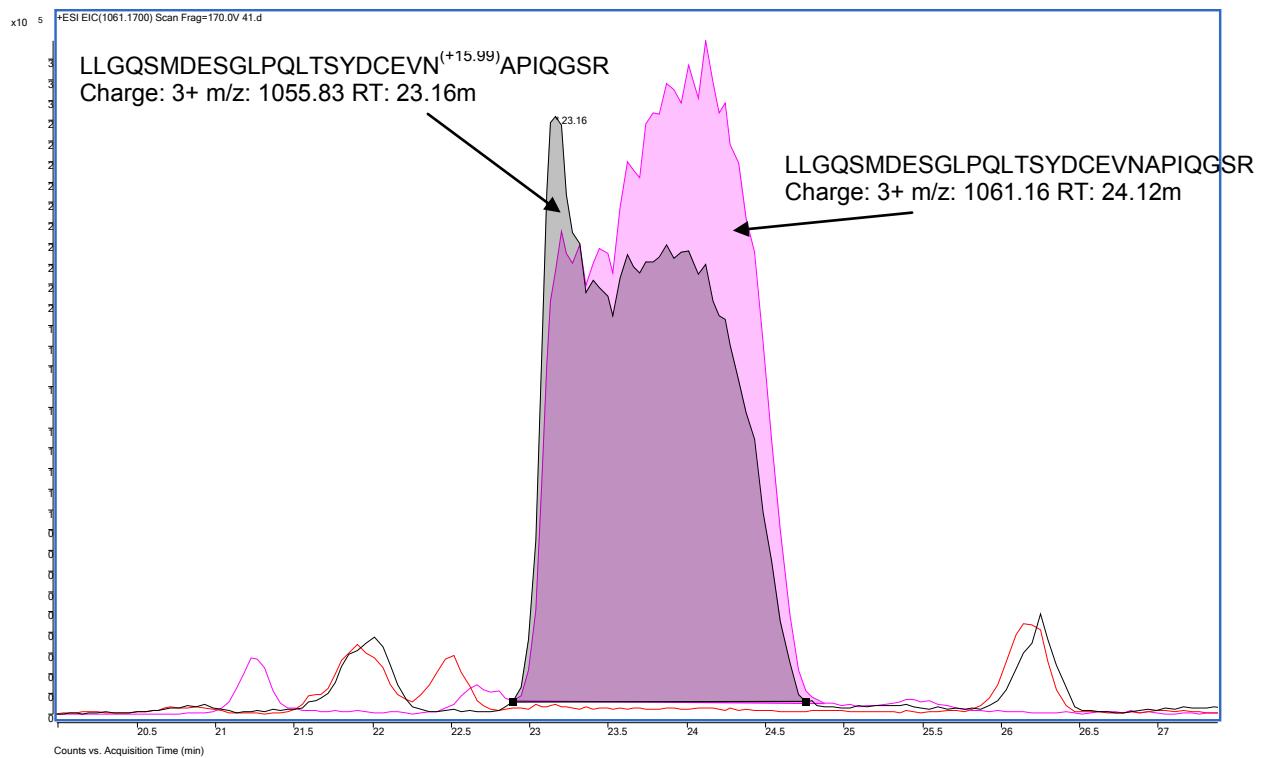


	b+	b++	b+++	y+	y++	y+++		
L	1	114.09	57.55	38.70				29 L
L	2	227.18	114.09	76.40	3052.40	1526.70	1018.14	28 L
G	3	284.20	142.60	95.40	2939.31	1470.16	980.44	27 G
Q	4	412.26	206.63	138.09	2882.29	1441.65	961.44	26 Q
S	5	499.29	250.15	167.10	2754.23	1377.82	918.75	25 S
M	6	630.33	315.67	210.78	2667.20	1334.10	889.74	24 M
D	7	745.35	373.18	249.12	2636.16	1268.58	846.06	23 D
E	8	874.40	437.70	292.14	2421.14	1211.07	807.72	22 E
S	9	961.43	481.22	321.15	2292.09	1146.55	764.70	21 S
G	10	1018.45	509.73	340.16	2205.06	1103.03	735.69	20 G
L	11	1131.54	566.27	377.85	2148.04	1074.52	716.68	19 L
P	12	1228.59	614.80	410.20	2034.95	1017.98	678.99	18 P
Q	13	1356.65	678.83	452.89	1937.90	969.45	646.64	17 Q
L	14	1469.73	735.37	490.58	1809.84	905.43	603.95	16 L
T	15	1570.78	785.89	524.26	1696.76	848.88	566.26	15 T
S	16	1657.81	829.41	553.27	1595.71	798.36	532.58	14 S
Y	17	1820.87	910.94	607.63	1508.88	754.84	503.56	13 Y
D	18	1935.90	968.45	645.97	1345.62	673.31	449.21	12 D
C	19	2095.93	1048.47	699.32	1230.59	615.80	410.87	11 C
E	20	2224.97	1112.99	742.33	1070.56	535.78	357.52	10 E
V	21	2324.04	1162.52	775.35	941.52	471.26	314.51	9 V
N	22	2438.09	1219.55	813.37	842.45	421.73	281.49	8 N
A	23	2509.12	1255.06	837.05	728.40	364.71	243.47	7 A
P	24	2606.17	1303.59	869.40	657.37	329.19	219.79	6 P
I	25	2719.26	1360.13	907.09	560.32	280.66	187.44	5 I
Q	26	2847.32	1424.16	949.78	447.23	224.12	149.75	4 Q
G	27	2904.34	1452.67	968.78	319.17	160.09	107.06	3 G
S	28	2991.37	1496.19	997.80	262.15	131.58	88.06	2 S
R	29				175.12	88.06	59.04	1 R

**Figure S16:** Annotated spectrum of HIF1-CAD peptide on Agilent platform.



**Figure S17:** Annotated spectrum of hydroxylated HIF1-CAD peptide on Agilent platform.

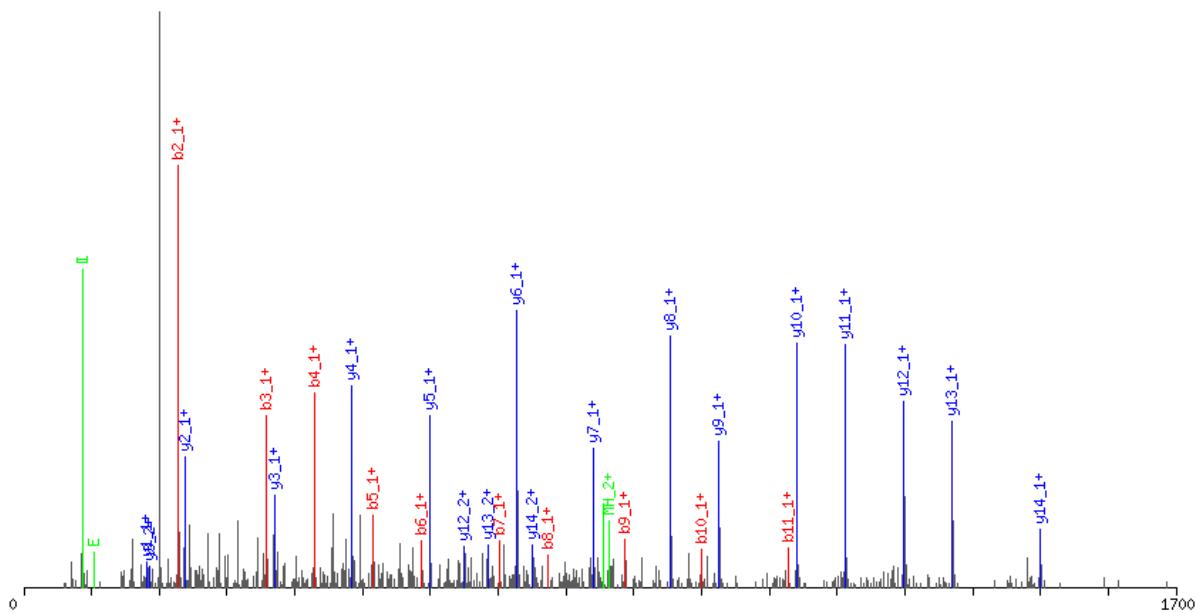


**Figure S18:** Extracted ion chromatograms showing retention times of HIF1-CAD peptides on Agilent platform.

LLEASADANIQDNMGR(+6.02)

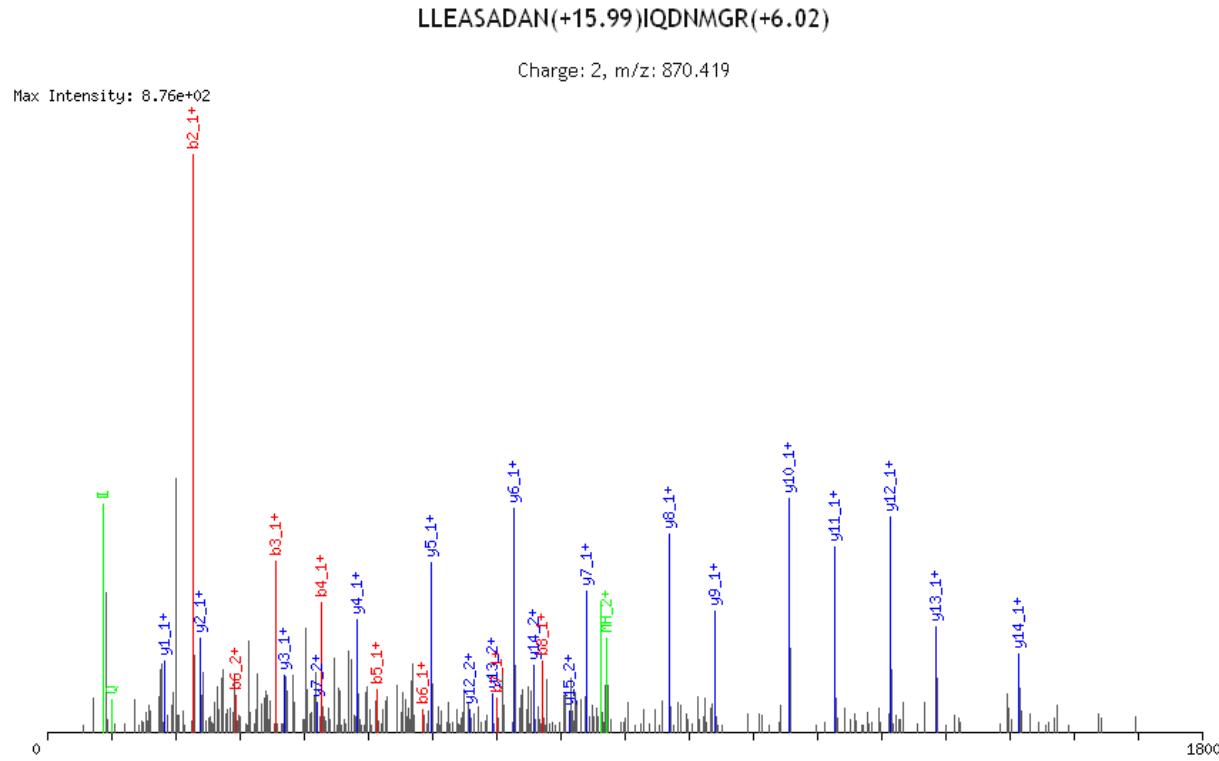
Charge: 2, m/z: 862.422

Max Intensity: 1.80e+03



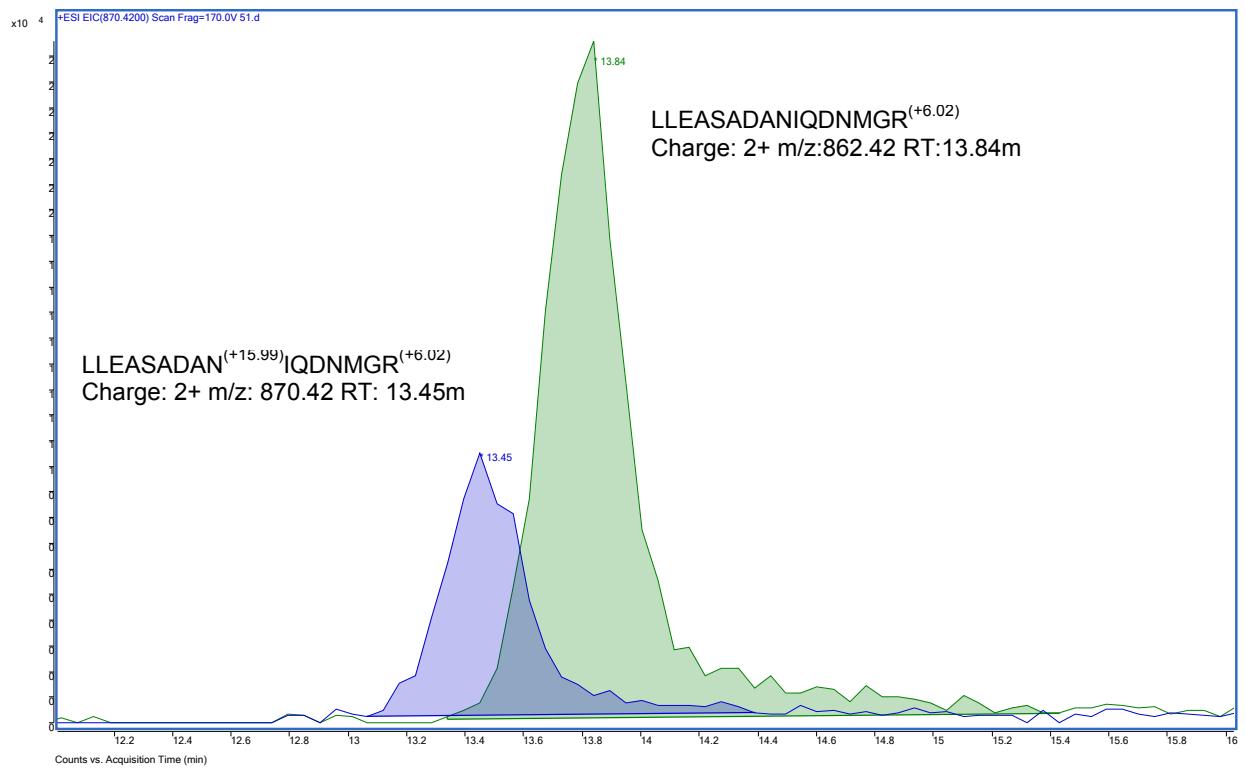
		b+	b++	y+	y++		
L	1	114.09	57.55			16	L
L	2	227.18	114.09	1610.75	805.88	15	L
E	3	356.22	178.61	1497.67	749.34	14	E
A	4	427.26	214.13	1368.63	684.82	13	A
S	5	514.29	257.65	1297.59	649.30	12	S
A	6	585.32	293.17	1210.56	605.78	11	A
D	7	700.35	350.68	1139.52	570.26	10	D
A	8	771.39	386.20	1024.49	512.75	9	A
N	9	885.43	443.22	953.46	477.23	8	N
I	10	998.52	499.76	839.41	420.21	7	I
Q	11	1126.57	563.79	726.33	363.67	6	Q
D	12	1241.60	621.30	598.27	299.64	5	D
N	13	1355.64	678.33	483.24	242.13	4	N
M	14	1486.68	743.85	369.20	185.10	3	M
G	15	1543.71	772.36	238.16	119.58	2	G
R	16			181.14	91.07	1	R

**Figure S19:** Annotated spectrum of heavy-labelled NOTCH DANI peptide on Agilent platform.



		b+	b++	y+	y++		
L	1	114.09	57.55			16	L
L	2	227.18	114.09	1626.75	813.88	15	L
E	3	356.22	178.61	1513.66	757.34	14	E
A	4	427.26	214.13	1384.62	692.81	13	A
S	5	514.29	257.65	1313.58	657.30	12	S
A	6	585.32	293.17	1226.55	613.78	11	A
D	7	700.35	350.68	1155.52	578.26	10	D
A	8	771.39	386.20	1040.49	520.75	9	A
N	9	901.43	451.22	969.45	485.23	8	N
I	10	1014.51	507.76	839.41	420.21	7	I
Q	11	1142.57	571.79	726.33	363.67	6	Q
D	12	1257.60	629.30	598.27	299.64	5	D
N	13	1371.64	686.32	483.24	242.13	4	N
M	14	1502.68	751.84	369.20	185.10	3	M
G	15	1559.70	780.35	238.16	119.58	2	G
R	16			181.14	91.07	1	R

**Figure S20:** Annotated spectrum of hydroxylated heavy-labelled NOTCH DANI peptide on Agilent platform.

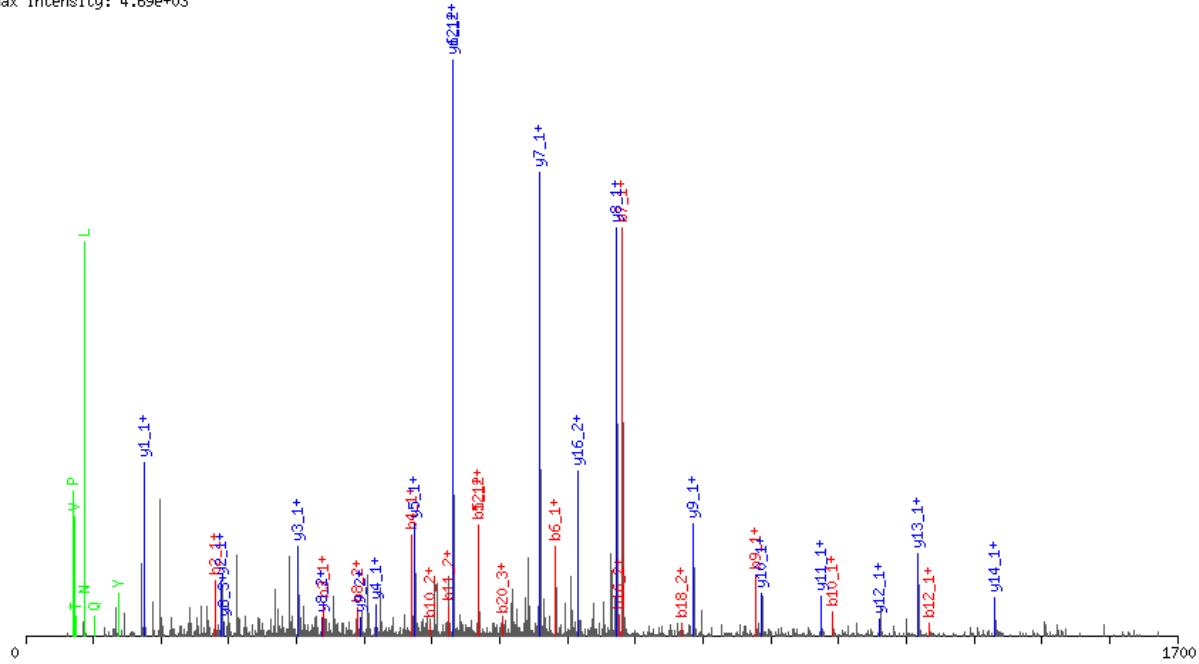


**Figure S21:** Extracted ion chromatograms showing retention times of NOTCH DANI peptides on Agilent platform.

YDC(+57.02)EVNVPVLGSSTLLQGGDLLR

Charge: 3, m/z: 835.759

Max Intensity: 4.69e+03



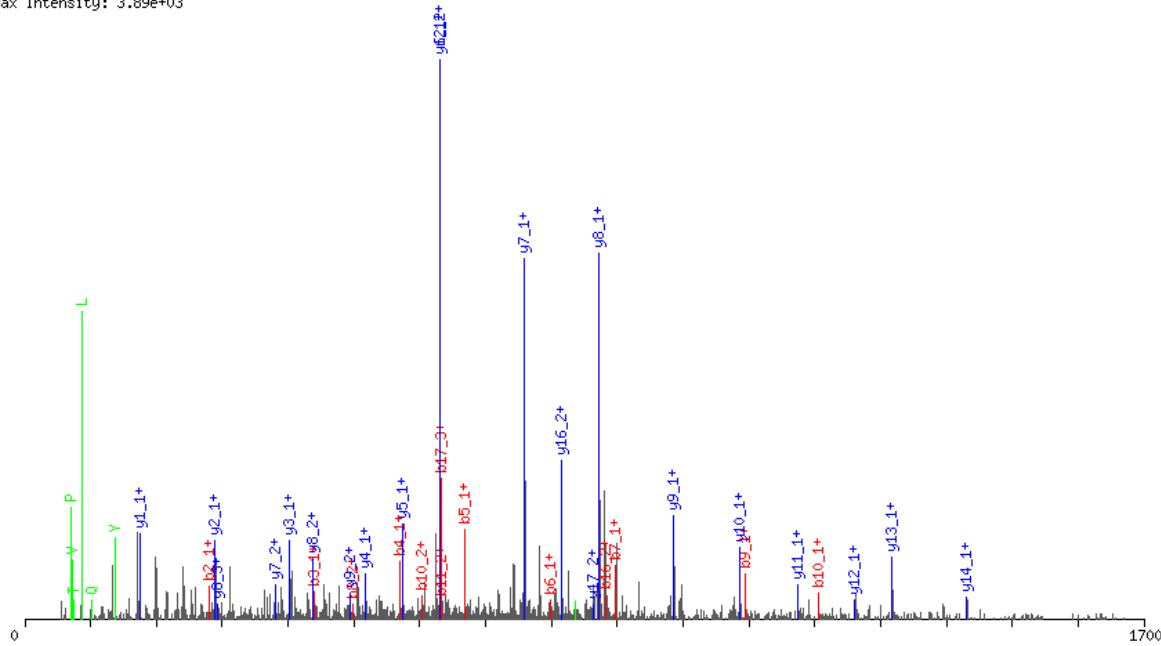
	b+	b++	b+++	y+	y++	y+++	
Y	1	164.07	82.54	55.36			23
D	2	279.10	140.05	93.70	2342.20	1171.60	781.41
C	3	439.13	220.07	147.05	2227.18	1114.09	743.06
E	4	568.17	284.59	190.06	2067.14	1034.08	689.72
V	5	667.24	334.12	223.08	1938.10	969.55	646.71
N	6	781.28	391.14	261.10	1839.03	920.02	613.68
V	7	880.35	440.68	294.12	1724.99	863.00	575.67
P	8	977.40	489.21	326.47	1625.92	813.46	542.65
V	9	1076.47	538.74	359.50	1528.87	764.94	510.29
L	10	1189.56	595.28	397.19	1429.80	715.40	477.27
G	11	1246.58	623.79	416.20	1316.72	668.86	439.58
S	12	1333.61	667.31	446.21	1259.70	630.35	420.57
S	13	1420.64	710.82	474.22	1172.66	586.84	391.56
T	14	1521.69	761.35	507.90	1085.63	543.32	362.55
L	15	1634.77	817.89	546.60	984.58	492.80	328.87
L	16	1747.86	874.43	583.29	871.50	436.25	291.17
Q	17	1875.92	938.46	625.98	758.42	379.71	253.48
G	18	1932.94	966.97	644.98	630.36	315.68	210.79
G	19	1989.96	995.48	663.99	573.34	287.17	191.78
D	20	2104.99	1053.00	702.33	516.31	268.66	172.78
L	21	2218.07	1109.54	740.03	401.29	201.15	134.43
L	22	2331.15	1166.08	777.72	288.20	144.61	96.74
R	23				175.12	88.06	59.04
							1
							R

**Figure S22:** Annotated spectrum of HIF2-CAD peptide on Agilent platform.

YDC(+57.02)EVN(+15.99)VPVLGSSTLLQGGDLLR

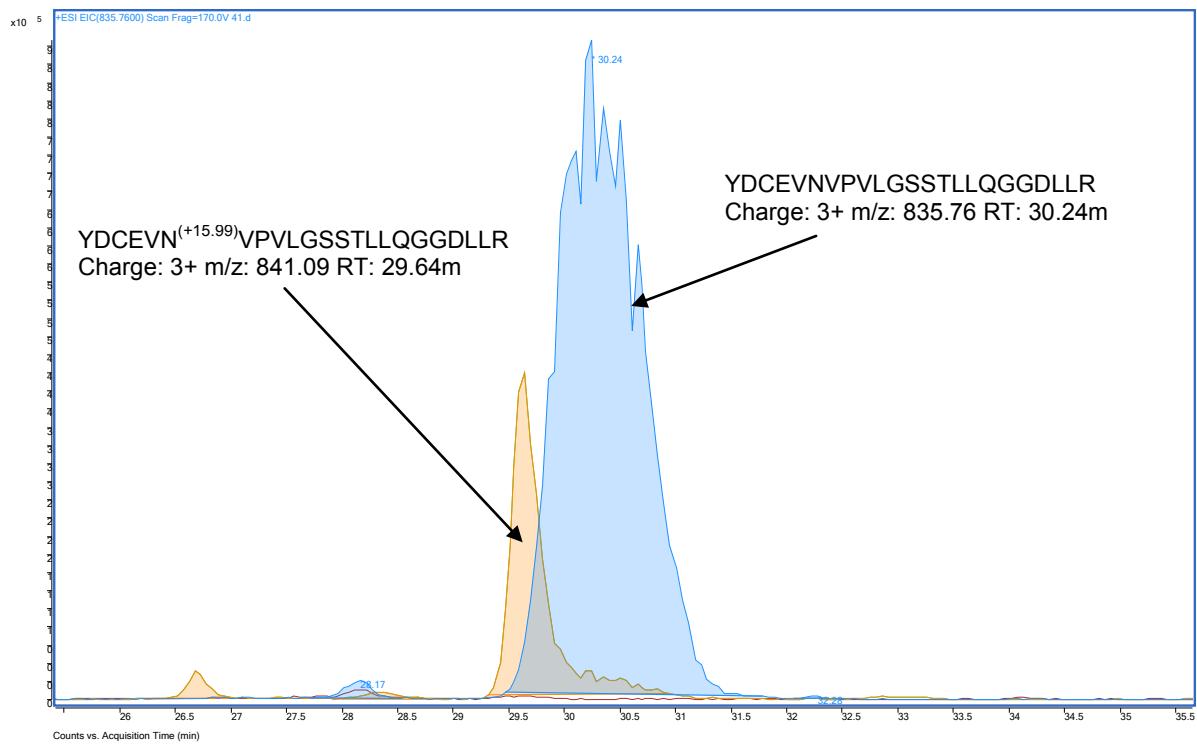
Charge: 3, m/z: 841.09

Max Intensity: 3.89e+03



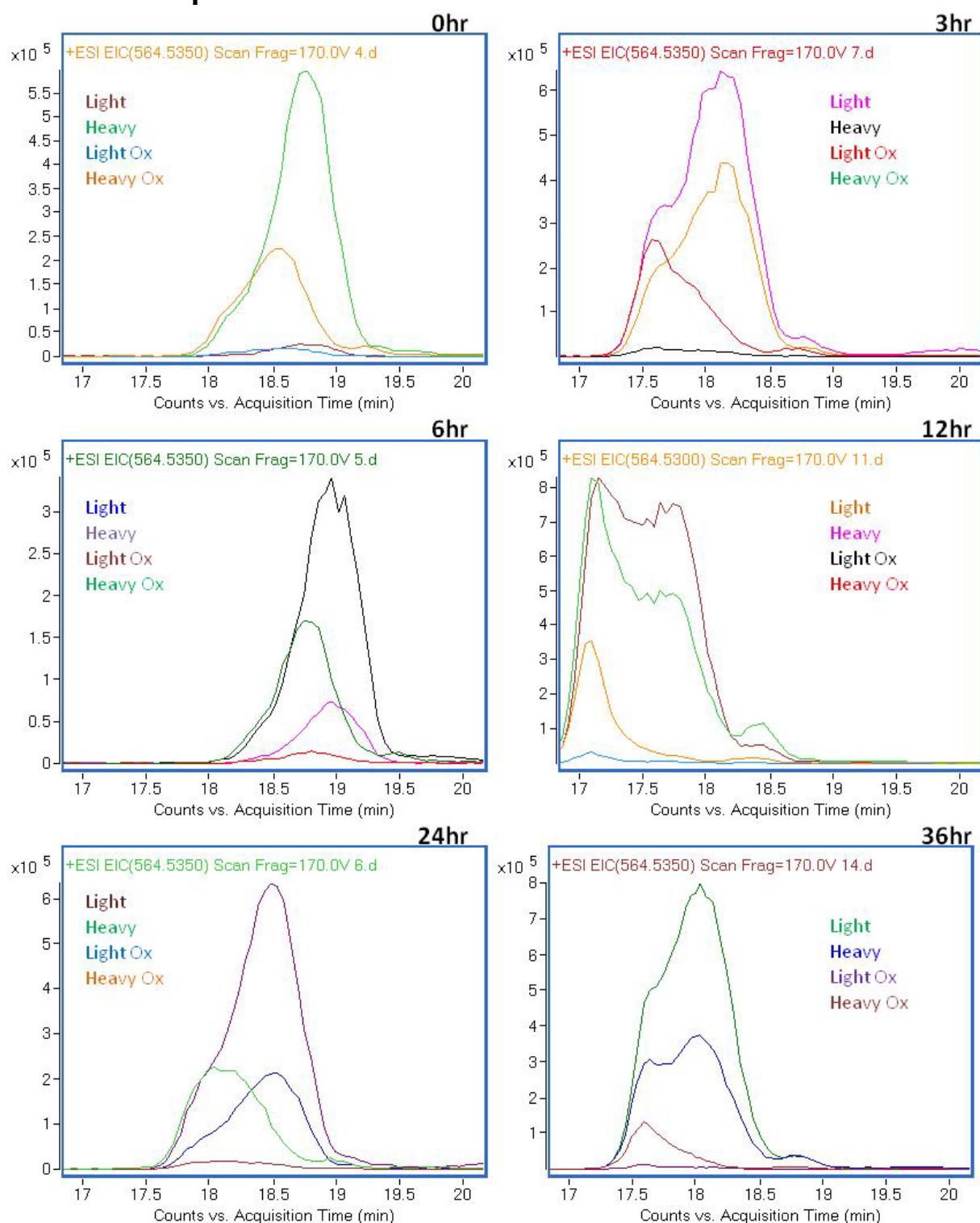
	b+	b++	b+++	y+	y++	y+++		
Y	1	164.07	82.54	55.36			23	Y
D	2	279.10	140.05	93.70	2358.20	1179.60	786.74	22
C	3	439.13	220.07	147.05	2243.17	1122.09	748.39	21
E	4	568.17	284.59	190.06	2083.14	1042.07	695.05	20
V	5	667.24	334.12	223.08	1954.10	977.55	652.04	19
N	6	797.28	399.14	266.43	1855.03	928.02	619.01	18
V	7	896.35	448.68	299.46	1724.99	863.00	575.67	17
P	8	993.40	497.20	331.80	1625.92	813.46	542.65	16
V	9	1092.47	546.74	364.83	1528.87	764.94	510.29	15
L	10	1205.55	603.28	402.52	1429.80	715.40	477.27	14
G	11	1262.57	631.79	421.53	1316.72	658.86	439.58	13
S	12	1349.60	675.31	460.54	1259.70	630.35	420.57	12
S	13	1436.64	718.82	479.55	1172.66	586.84	391.56	11
T	14	1537.68	769.35	513.23	1085.63	543.32	362.55	10
L	15	1650.77	825.89	560.93	984.58	492.80	328.87	9
L	16	1763.85	882.43	588.62	871.50	436.25	291.17	8
Q	17	1891.91	946.46	631.31	758.42	379.71	253.48	7
G	18	1948.93	974.97	660.32	630.36	315.68	210.79	6
G	19	2005.95	1003.48	669.32	573.34	287.17	191.78	5
D	20	2120.98	1080.99	707.67	516.31	258.66	172.78	4
L	21	2234.06	1117.54	746.36	401.29	201.15	134.43	3
L	22	2347.15	1174.08	783.05	288.20	144.61	96.74	2
R	23				175.12	88.06	59.04	1

**Figure S23:** Annotated spectrum of hydroxylated HIF2-CAD peptide on Agilent platform.



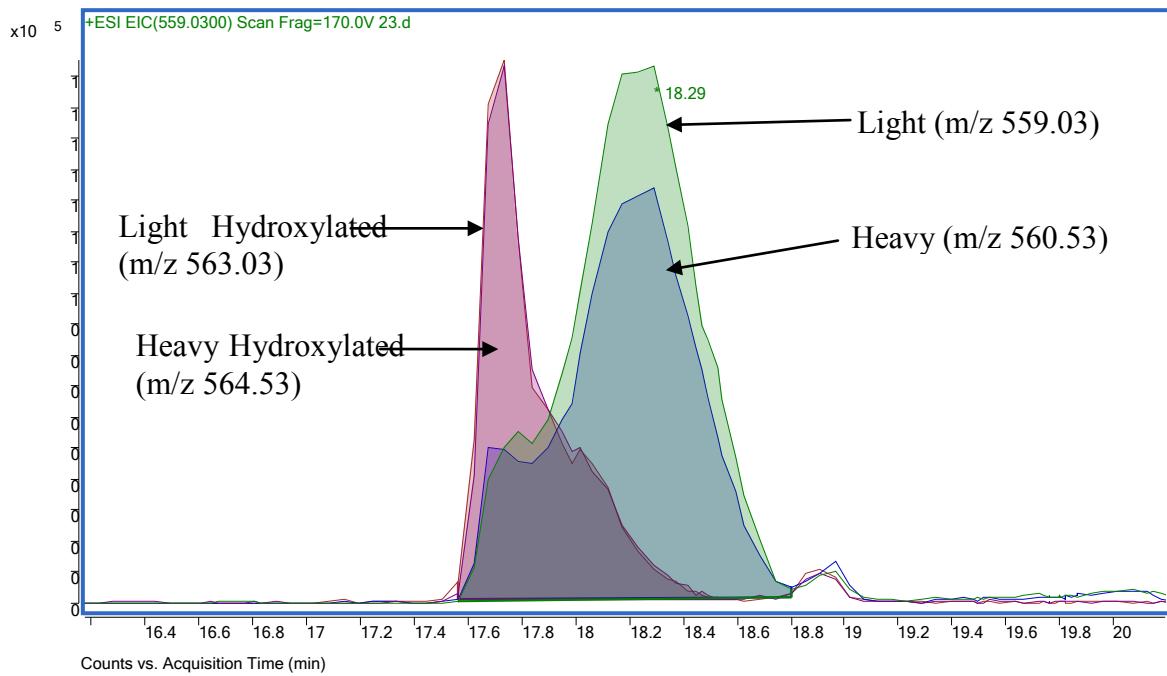
**Figure S24:** Extracted ion chromatograms showing retention times of HIF2-CAD peptides on Agilent platform.

## Section B – Experimental Results

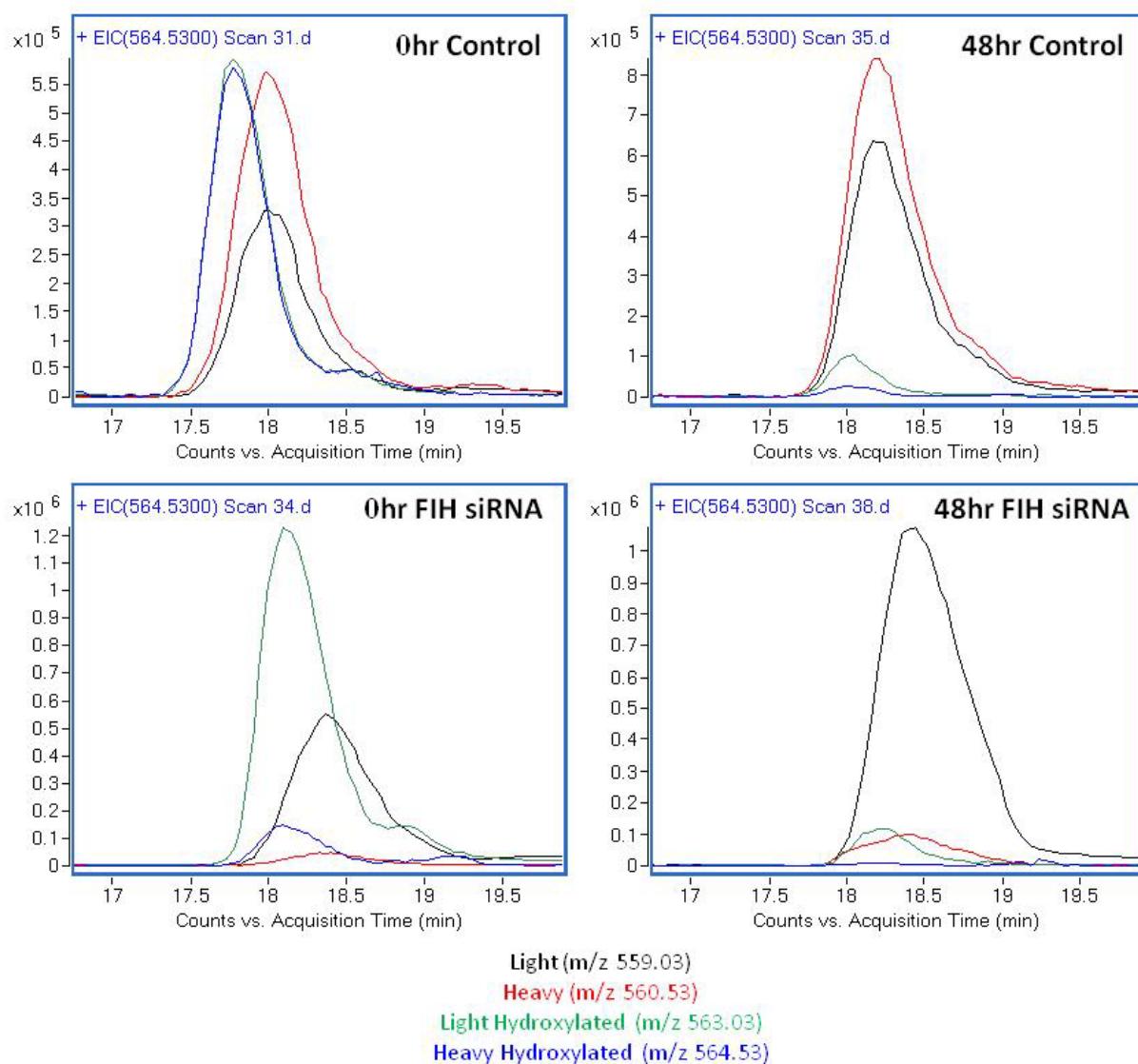


**Figure S25:** Supplement to Figure 2B. Extracted ion chromatograms for the Rabankyrin-5 Asn-485 AAGAGNEAAALFLATNGAHVNHR peptide in a SILAC chase experiment over 36 h following the addition of “light” media and DMOG. Chromatograms show

increasing proportion of the newly synthesised light peptide versus heavy labelled peptide over time. Percentage hydroxylation of heavy peptide remains constant indicating a lack of reversal of hydroxylation (see Figure 2B). Hydroxylation of newly synthesised “light” peptide is suppressed by DMOG. Data from Agilent platform.



**Figure S26:** Rabankyrin-5 Asn-485 hydroxylation, light-chase control (-DMOG), 24 h. Lack of DMOG treatment results in hydroxylation of newly synthesised light peptides. Absence of light hydroxylation in Figure S25 confirms efficacy of DMOG treatment.



**Fig. S27:** Supplement to Figure 5B. 0hr and 48hr time points in a SILAC chase experiment following treatment with a control siRNA or siRNA specific to FIH. Extracted ion chromatograms show the Rabankyrin-5 Asn-485 peptide AAGAGNEAAALFLATNGAHVNHR. Knock-down of FIH by the specific siRNA suppresses hydroxylation, as shown by the lack of hydroxylated peptide in the lower chromatograms.