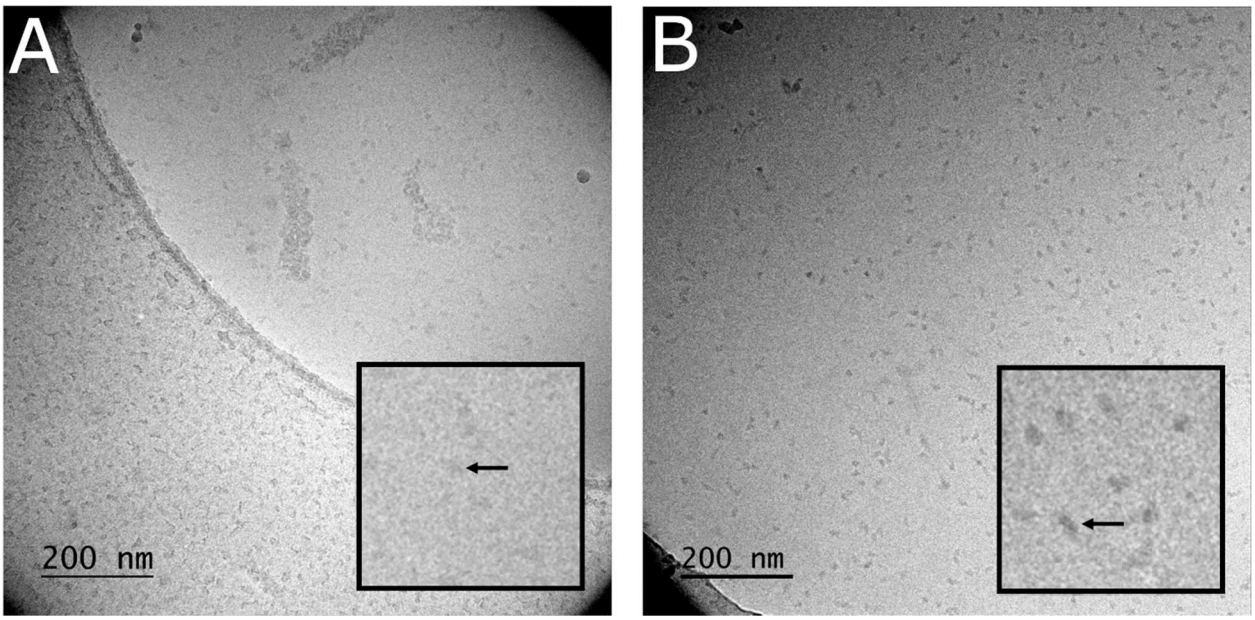
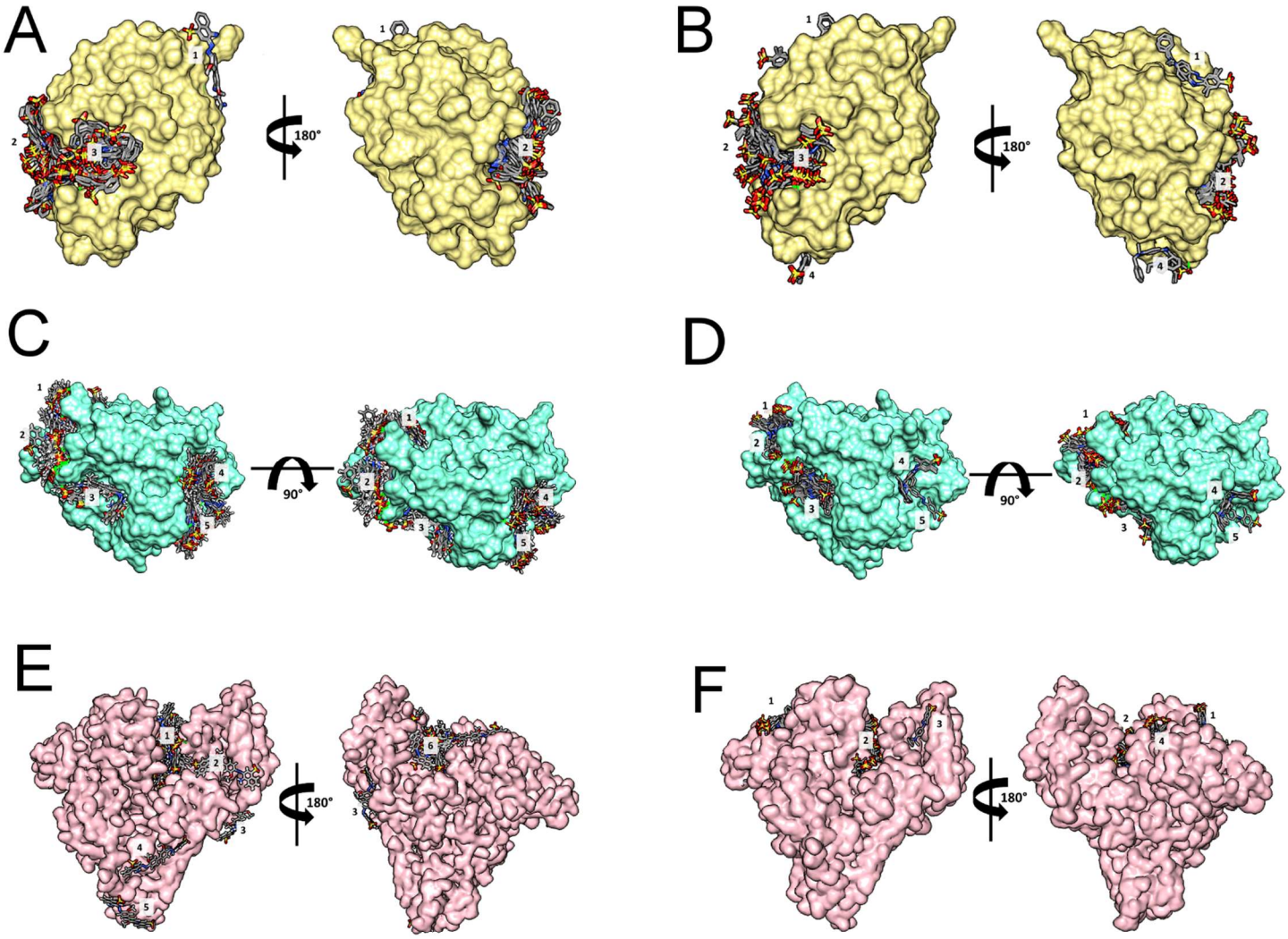
Supplementary Materials

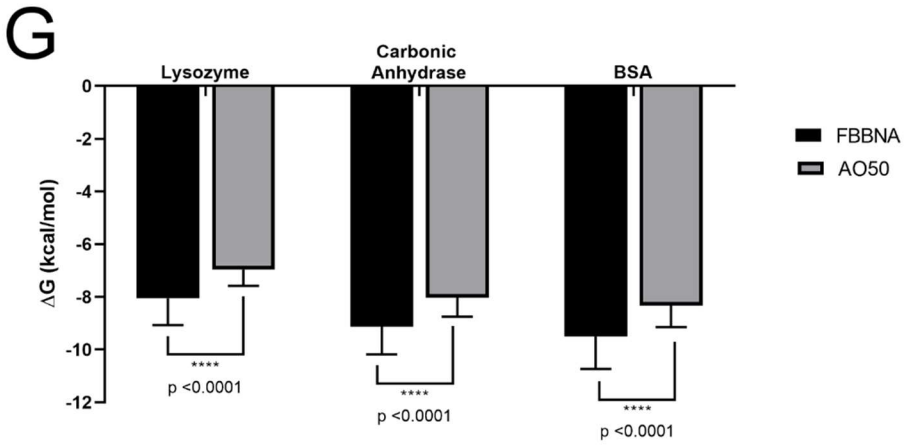


Supplementary Figure 1. Treatment with small molecule metallodyes increases contrast and reduces aggregation of thyroglobulin particles. A) Electron micrograph of thyroglobulin, 5 µM. Protein has a high affinity for the carbon grid, and forms some aggregates in the ice layer. Curve indicates edge of a hole in the carbon grid. Inset shows 300% zoom image of particles; arrow indicates a particle. B) Electron micrograph of thyroglobulin, 5 µM, treated with 1 mg/mL DB199 dye. Protein formed fewer aggregates and had more contrast than the untreated thyroglobulin. Inset shows 300% zoom image of particles; arrow indicates a particle.

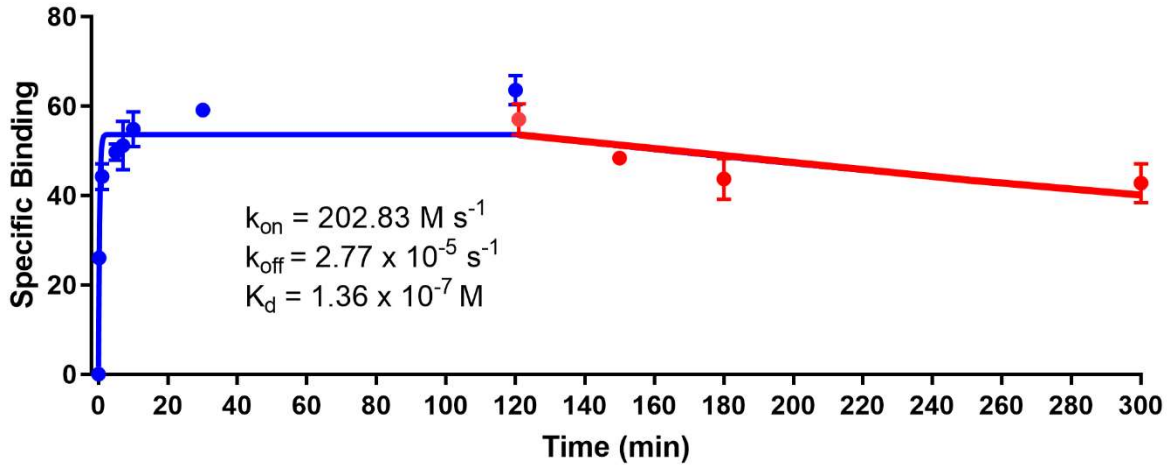
Supplementary Figure 2: Amino acid composition of standard six proteins. Tyrosine, lysine, arginine, and lysine + arginine content for each of the six standard proteins used in this study is examined.

Protein Name	Amino Acids	# Tyr	# Lys	# Arg	# Lys + Arg	% Tyr	% Lys	% Arg	% Lys + Arg
Lysozyme (LY)	147	3	6	12	18	2.04%	4.08%	8.16%	12.24%
Carbonic anhydrase (CA)	260	8	18	9	27	3.08%	11.25%	3.46%	10.38%
Serum albumin (BSA)	607	21	60	26	86	3.46%	9.88%	4.28%	14.17%
Catalase (CT)	527	19	28	32	60	3.61%	5.31%	6.07%	11.39%
Apoferritin (AF)	357	13	22	19	41	3.64%	6.16%	5.32%	11.48%
Thyroglobulin (TG)	2769	72	74	181	255	2.60%	2.67%	6.54%	9.21%
Average of six proteins						3.07%	6.55%	5.63%	11.47%
Average protein composition, vertebrates						3.3%	7.2%	4.2%	11.4%


A
180°
B
180°
C
90°
D
90°
E
180°
F
180°


G
Lysozyme
Carbonic Anhydrase
BSA
ΔG (kcal/mol)
0
-2
-4
-6
-8
-10
-12
****
p <0.0001
****
p <0.0001
****
p <0.0001
FBBNA
AO50

Supplementary Figure 3. Docking of FBBNA-PINK and AO50 with hen egg white lysozyme (6QWY), bovine carbonic anhydrase (1V9E), and bovine serum albumin (3V03) reveals similar docking positions but different binding affinities. Proteins are represented using a space-filling model, and unique regions of binding are marked with numbers to aid in localization. A) 257 docking positions of FBBNA-PINK with lysozyme. Docked positions were segregated in 38 clusters, covering 3 unique regions of lysozyme. B) 253 docking positions of AO50 with lysozyme. Docked positions were segregated into 37 clusters covering 4 unique regions of lysozyme. C) 257 docking positions of FBBNA-PINK with carbonic anhydrase. Docked positions were segregated in 35 clusters, covering 5 unique regions of carbonic anhydrase. D) 252 docking positions of AO50 with carbonic anhydrase. Docked positions were segregated into 37 clusters covering 5 unique regions of carbonic anhydrase. E) 257 docking positions of FBBNA-PINK with bovine serum albumin. Docked positions were segregated in 31 clusters, covering 6 unique regions of BSA. F) 257 docking positions of AO50 with BSA. Docked positions were segregated into 40 clusters covering 4 unique regions of BSA. G) Predicted ΔG values for each docked position of FBBNA or AO50 with lysozyme, carbonic anhydrase, and BSA. ΔG values for FBBNA binding to each protein were significantly less negative ($p<0.0001$) than for AO50 binding by unpaired Student's t-test.



Supplementary Figure 4. Association and dissociation kinetics of FBBNA binding to thyroglobulin reveal a rapid on-rate and a slow off-rate which makes it suitable for protein painting experiments. Dissociation of FBBNA from thyroglobulin was measured and compared to the association of FBBNA to thyroglobulin by curve-fitting kinetics data to the association then dissociation model in GraphPad Prism, with HotNM set to 258,881 (concentration of FBBNA in nM) and time0 set to 120 (time at which dissociation experiments begin and association experiments end).

Supplementary Figure 5. Two individual hotspots on ZO-1 were identified when complexed with YAP2. Hotspots are identified as the tryptic cleavage sites blocked by dye treatment but protected during complexation with a protein binding partner. This leads to an identification pattern for hotspots where hotspot peptides are present in unpainted samples, absent in painted samples due to blockage of the tryptic cleavage site by dye coverage, and present in the dyed complexed samples where complexation protects the tryptic cleavage site from dye coverage. A) Peptide DNPHFQSGETSIVISDVLK from ZO-1, following tryptic cleavage at R42, is identified by MS/MS in the unpainted sample 11292018_Amanda_3. B) Peptide TAGGDRADFWR from ZO-1, following tryptic cleavage at K592, is identified by MS/MS in the unpainted sample 11292018_Amanda_3. C) All ZO-1 peptides identified within 1% FDR in the ZO-1 painted sample 11292018_Amanda_7; neither the DNPHFQSGETSIVISDVLK nor the TAGGDRADFWR peptides were identified in the sample, indicating blockage of R42 and K592 by the dye treatment. D) Peptide DNPHFQSGETSIVISDVLK from ZO-1, following tryptic cleavage at R42, is identified by MS/MS in the painted complexed sample 11292018_Amanda_10. E) Peptide TAGGDRADFWR from ZO-1, following tryptic cleavage at K592, is identified by MS/MS in the painted complexed sample 11292018_Amanda_10.

A. R42 of unpainted ZO-1 sample 11292018_Amanda_3 is identified by the presence of peptide DNPHFQSGETSIVISDVLK tandem mass spectrum matched via Sequest.

Peptide Summary

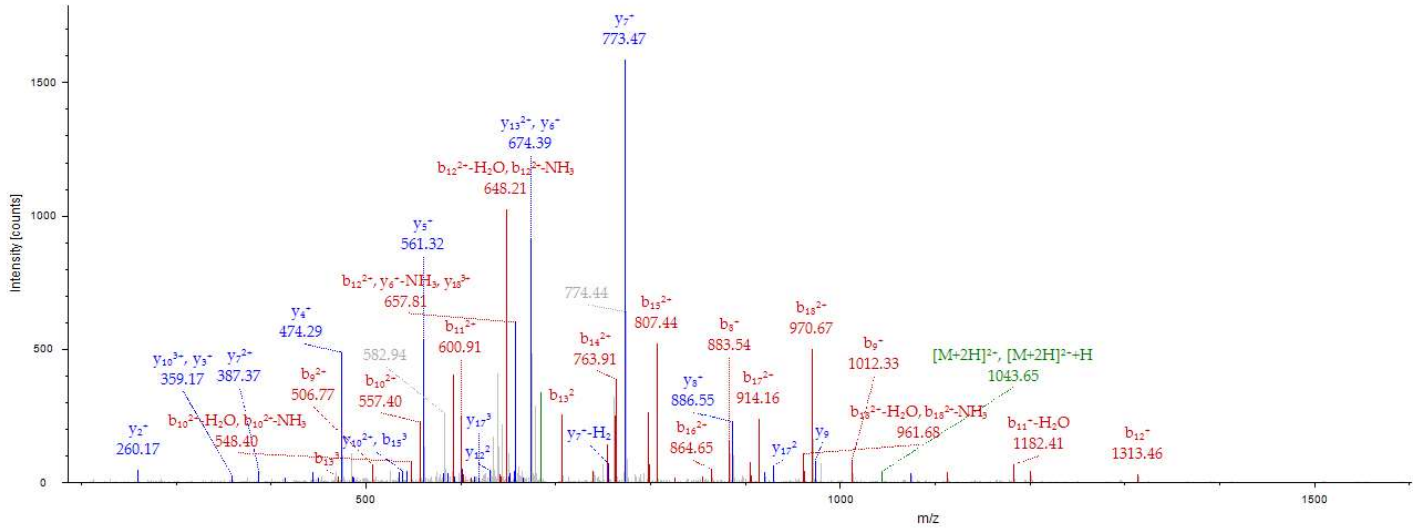
Sequence: DNPHFQSGETSIVISDVLK, Charge: +3, Monoisotopic m/z: 696.02032 Da (+0.43 mmu/+0.62 ppm), MH+: 2086.04642 Da, RT: 16.7727 min,
Identified with: Sequest HT (v1.17); XCorr:4.35, Percolator q-Value:0, Percolator PEP:0.00562, Ions matched by search engine: 0/0
Fragment match tolerance used for search: 0.6 Da
Fragments used for search: b; b-H_2O; b-NH_3; y; y-H_2O; y-NH_3

#1	b^+	b^{2+}	b^{3+}	Seq.	y^+	y^{2+}	y^{3+}	#2
1	116.03422	58.52075	39.34959	D				19
2	230.07715	115.54221	77.36390	N	1971.01818	986.01273	657.67758	18
3	327.12991	164.06859	109.71482	P	1856.97525	928.99127	619.66327	17
4	464.18882	232.59805	155.40113	H	1759.92249	880.46488	587.31235	16
5	611.25724	306.13226	204.42393	F	1622.86358	811.93543	541.62604	15
6	739.31581	370.16155	247.11012	Q	1475.79516	738.40122	492.60324	14
7	826.34784	413.67756	276.12080	S	1347.73659	674.37193	449.91705	13
8	883.36931	442.18829	295.12795	G	1260.70456	630.85592	420.90637	12
9	1012.41190	506.70959	338.14215	E	1203.68309	602.34519	401.89922	11
10	1113.45958	557.23343	371.82471	T	1074.64050	537.82389	358.88502	10
11	1200.49161	600.74944	400.83539	S	973.59282	487.30005	325.20246	9
12	1313.57567	657.29147	438.53007	I	886.56080	443.78404	296.19178	8
13	1412.64408	706.82568	471.55288	V	773.47673	387.24200	258.49709	7
14	1525.72815	763.36771	509.24757	I	674.40832	337.70780	225.47429	6
15	1612.76018	806.88373	538.25824	S	561.32425	281.16576	187.77960	5
16	1727.78712	864.39720	576.60056	D	474.29223	237.64975	158.76893	4
17	1826.85553	913.93140	609.62336	V	359.26528	180.13628	120.42661	3
18	1939.93960	970.47344	647.31805	L	260.19687	130.60207	87.40381	2
19				K	147.11280	74.06004	49.70912	1

#1	b-H_2O^+	b-H_2O^{2+}	b-H_2O^{3+}	b-NH_3^+	b-NH_3^{2+}	b-NH_3^{3+}	Seq.	y-H_2O^+	y-H_2O^{2+}	y-H_2O^{3+}	y-NH_3^+	y-NH_3^{2+}	y-NH_3^{3+}	#2
1	98.02365	49.51547	33.34607				D							19
2	212.06658	106.53693	71.36038	213.05060	107.02894	71.68838	N	1953.00762	977.00745	651.67406	1953.99163	977.49945	652.00206	18
3	309.11935	155.06331	103.71130	310.10336	155.55532	104.03930	P	1838.96469	919.98598	613.65975	1839.94870	920.47799	613.98775	17
4	446.17826	223.59277	149.39760	447.16227	224.08478	149.72561	H	1741.91193	871.45960	581.30883	1742.89594	871.95161	581.63683	16
5	593.24667	297.12697	198.42041	594.23069	297.61898	198.74841	F	1604.85301	802.93015	535.62252	1605.83703	803.42215	535.95053	15
6	721.30525	361.15626	241.10660	722.28926	361.64827	241.43461	Q	1457.78460	729.39594	486.59972	1458.76862	729.88795	486.92772	14
7	808.33728	404.67228	270.11728	809.32129	405.16428	270.44528	S	1329.72602	665.36665	443.91353	1330.71004	665.85866	444.24153	13
8	865.35874	433.18301	289.12443	866.34276	433.67502	289.45244	G	1242.69399	621.85064	414.90285	1243.67801	622.34264	415.23085	12
9	994.40133	497.70431	332.13863	995.38535	498.19631	332.46663	E	1185.67253	593.33990	395.89569	1186.65655	593.83191	396.22370	11
10	1095.44901	548.22814	365.82119	1096.43303	548.72015	366.14919	T	1056.62994	528.81861	352.88150	1057.61395	529.31061	353.20950	10
11	1182.48104	591.74416	394.83186	1183.46506	592.23617	395.15987	S	955.58226	478.29477	319.19894	956.56627	478.78678	319.52694	9
12	1295.56510	648.28619	432.52655	1296.54912	648.77820	432.85456	I	868.55023	434.77875	290.18826	869.53425	435.27076	290.51627	8
13	1394.63352	697.82040	465.54936	1395.61753	698.31241	465.87736	V	755.46617	378.23672	252.49357	756.45018	378.72873	252.82158	7
14	1507.71758	754.36243	503.24405	1508.70160	754.85444	503.57205	I	656.39775	328.70251	219.47077	657.38177	329.19452	219.79877	6
15	1594.74961	797.87844	532.25472	1595.73363	798.37045	532.58273	S	543.31369	272.16048	181.77608	544.29770	272.65249	182.10409	5
16	1709.77655	855.39192	570.59704	1710.76057	855.88392	570.92504	D	456.28166	228.64447	152.76540	457.26568	229.13648	153.09341	4
17	1808.84497	904.92612	603.61984	1809.82898	905.41813	603.94785	V				342.23873	171.62300	114.75110	3
18	1921.92903	961.46815	641.31453	1922.91305	961.96016	641.64253	L				243.17032	122.08880	81.72829	2
19							K				130.08626	65.54677	44.03360	1

Fragment Spectrum

11292018_Amanda_3.raw #3250 RT: 16.7727 min
ITMS, 696.3547@cid35.00, z=+3, Mono m/z=696.02032 Da, MH+=2086.04642 Da, Match Tol.=0.6 Da



B. K592 of unpainted ZO-1 sample 11292018_Amanda_3 is identified by the presence of peptide TAGGDRADFWR tandem mass spectrum matched via Sequest.

Peptide Summary

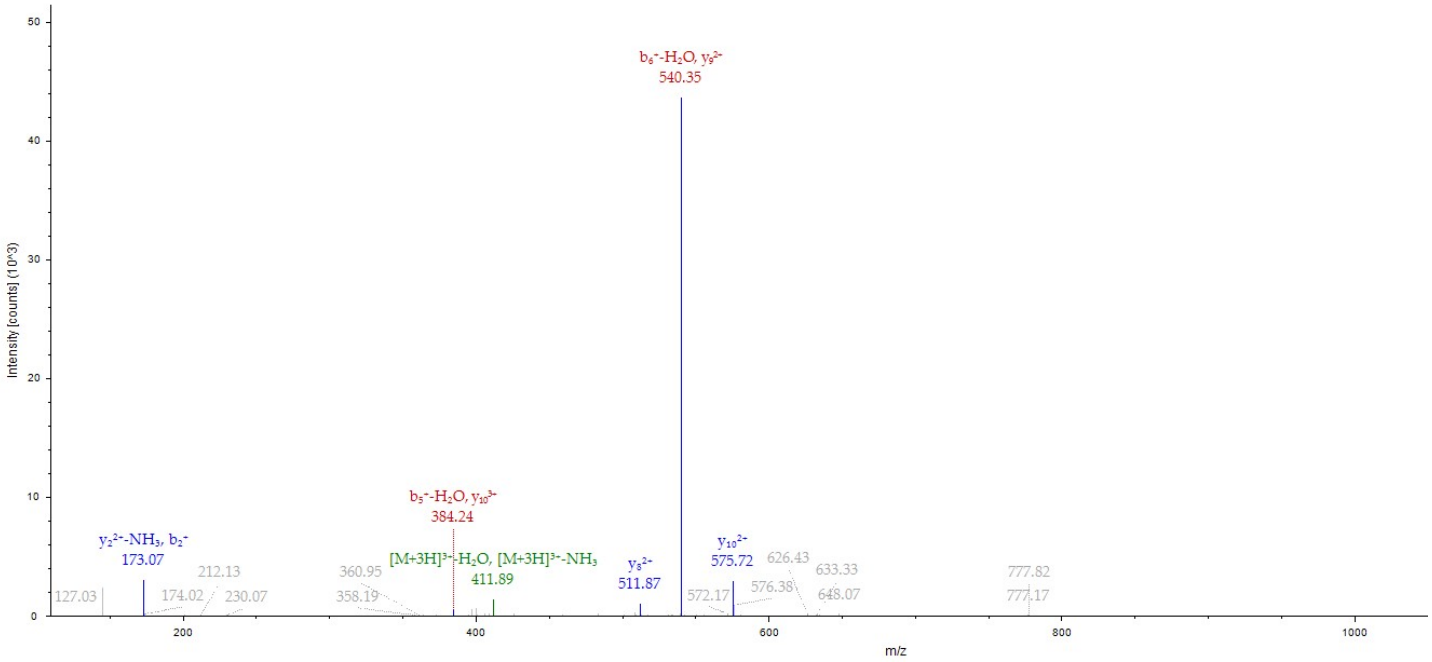
Sequence: TAGGDRADFWR, Charge: +3, Monoisotopic m/z: 417.86713 Da (+0.11 mmu/+0.25 ppm), MH+: 1251.58683 Da, RT: 10.7403 min,
Identified with: Sequest HT (v1.17); XCorr:1.00, Percolator q-Value:0, Percolator PEP:0.108, Ions matched by search engine: 0/0
Fragment match tolerance used for search: 0.6 Da
Fragments used for search: b; b-H_2O; b-NH_3; y; y-H_2O; y-NH_3

#1	b^+	b^{2+}	b^{3+}	Seq.	y^+	y^{2+}	y^{3+}	#2
1	102.05496	51.53112	34.68984	T				11
2	173.09207	87.04967	58.36887	A	1150.53883	575.77305	384.18446	10
3	230.11353	115.56040	77.37603	G	1079.50172	540.25450	360.50542	9
4	287.13500	144.07114	96.38318	G	1022.48025	511.74376	341.49827	8
5	402.16194	201.58461	134.72550	D	965.45879	483.23303	322.49111	7
6	558.26305	279.63516	186.75920	R	850.43185	425.71956	284.14880	6
7	629.30016	315.15372	210.43824	A	694.33074	347.66901	232.11510	5
8	744.32711	372.66719	248.78055	D	623.29362	312.15045	208.43606	4
9	891.39552	446.20140	297.80336	F	508.26668	254.63698	170.09374	3
10	1077.47483	539.24106	359.82980	W	361.19827	181.10277	121.07094	2
11				R	175.11895	88.06311	59.04450	1

#1	b-H_2O^+	b-H_2O^{2+}	b-H_2O^{3+}	b-NH_3^+	b-NH_3^{2+}	b-NH_3^{3+}	Seq.	y-H_2O^+	y-H_2O^{2+}	y-H_2O^{3+}	y-NH_3^+	y-NH_3^{2+}	y-NH_3^{3+}	#2
1	84.04439	42.52583	28.68631				T							11
2	155.08150	78.04439	52.36535				A	1132.52827	566.76777	378.18094	1133.51228	567.25978	378.50894	10
3	212.10297	106.55512	71.37251				G	1061.49115	531.24921	354.50190	1062.47517	531.74122	354.82991	9
4	269.12443	135.06585	90.37966				G	1004.46969	502.73848	335.49475	1005.45370	503.23049	335.82275	8
5	384.15137	192.57933	128.72198				D	947.44822	474.22775	316.48759	948.43224	474.71976	316.81560	7
6	540.25249	270.62988	180.75568	541.23650	271.12189	181.08368	R	832.42128	416.71428	278.14528	833.40530	417.20629	278.47328	6
7	611.28960	306.14844	204.43472	612.27361	306.64045	204.76272	A	676.32017	338.66372	226.11157	677.30419	339.15573	226.43958	5
8	726.31654	363.66191	242.77703	727.30056	364.15392	243.10504	D	605.28306	303.14517	202.43254	606.26707	303.63717	202.76054	4
9	873.38496	437.19612	291.79984	874.36897	437.68812	292.12784	F				491.24013	246.12370	164.41823	3
10	1059.46427	530.23577	353.82627	1060.44828	530.72778	354.15428	W				344.17172	172.58950	115.39542	2
11							R				158.09240	79.54984	53.36899	1

Fragment Spectrum

11292018_Amanda_3.raw #2137 RT: 10.7403 min
ITMS, 417.8671@cid35.00, z=+3, Mono m/z=417.86713 Da, MH+=1251.58683 Da, Match Tol.=0.6 Da



C. Painted ZO-1 sample 11292018_Amanda_7 does not contain peptides DNPHFQSGETSIVISDVLK and TAGGDRADFWR of ZO-1 within the ZO-1-derived peptide set as identified by Sequest

Confidence	Sequence	Modifications	Quality PEP	Theo. MH+ [Da]	XCorr A Sequest HT
High	LEEPTPAPSTSYSPQADSLR		2.25E-05	2146.03	5.84
High	DLEQPTYRYESSSYTDQFSR		0.000128	2472.095	5.83
High	EAIQQQQNQLVWVSEGK		0.000292	1985.009	5.74
High	LAGGNDVGIFVAGVLEDSPAAK		0.000294	2100.097	5.73
High	LAGGNDVGIFVAGVLEDSPAAKEGLEEGDQILR		0.00098	3339.707	5.47
High	SNHYDPEEDEEYYR		0.004996	1845.72	5.28
High	LAREEPDIYQIAK		0.006509	1545.827	5.16
High	NRAEQLASVQYTLPK		0.025228	1717.923	4.97
High	EISQDSLAARDGNIQEGDVVLK		0.02479	2357.194	4.89
High	AEQLASVQYTLPK		0.004091	1447.779	4.67
High	IVESDVGDSFYIR		0.001315	1499.738	4.67
High	INGTVTENMSLTDAK	1xOxidation [M9]	0.004809	1609.774	4.61
High	EAGFLRPVTIFGPIADVAR		0.101178	2029.123	4.35
High	YESSSYTDQFSR		0.001785	1469.618	4.23
High	APGFGFGIAISGGR		0.000598	1306.69	4.18
High	GGPAEGQLQENDR		0.004767	1370.63	4.18
High	GIFNSNGGVLSSIETGVSIIIPQGAIPEGVEQEIYFK		0.040913	3863.011	4.08
High	VNNVDFTNIIR		0.021356	1304.696	4.06
High	INGTVTENMSLTDAK		0.001	1593.779	4.01
High	TVEEVTVERNEK		0.004176	1432.728	3.98
High	SFPDKAPVNGTEQTQK		0.021734	1746.866	3.86
High	EDLSAQPVQTK		0.009045	1215.622	3.85
High	GEEVTILAQK		0.047704	1087.599	3.83
High	AIPVSPSAVEEDEDEDGHTVVATAR		0.000531	2594.222	3.68
High	YQINNISTVPK		0.014297	1276.69	3.6
High	TVEEVTVER		0.071982	1061.547	3.58
High	IDSPGFKPASQQK		0.129717	1402.733	3.54
High	SREDLSAQPVQTK		0.130076	1458.755	3.53
High	SYEQVPPQGFTSR		0.00119	1495.718	3.51
High	EDLSAQPVQTKFPAYER		0.019618	1978.987	3.36
High	DQEPSLSSHVDPTK		0.008706	1539.729	3.31
High	AEASSPVPYLSPETNPASSTSAVNHNVNLTNVR		0.024862	3423.678	3.26
High	EDTAQAAFYPQK		0.040559	1368.643	3.26
High	VVDTLYNGK		0.013924	1008.536	3.26
High	DGNIQEGDVVLK		0.011473	1286.659	3.24
High	GKPPEADGVDR		0.008161	1140.564	3.23
High	EGLEEGDQILR		0.049104	1258.627	3.16
High	DNSILPPLDK		0.175179	1111.599	3.1
High	LGSWLAIR		0.063823	915.5411	3.09
High	EISQDSLAAR		0.012455	1089.553	2.89
High	MRLCPESR	1xCarbamidomethyl [C4]	0.031127	1048.503	2.87
High	LASHIFVK		0.001754	914.5458	2.72

High	SVASSQPAKPTK		0.009342	1200.658	2.69
High	ISKPGAVSTPVK		0.008732	1183.705	2.67
High	DLEQPTYR		0.195479	1021.495	2.58
High	KNEEYGLR		0.085155	1008.511	2.52
High	RSVASSQPAKPTK		0.312427	1356.759	2.51
High	TPSTEAAHIMLR	1xOxidation [M10]	0.007495	1342.678	2.49
High	HSPQQPSNGSLR		0.022379	1307.645	2.42
High	FLKPVELR		0.075347	1001.614	2.32
High	TPSTEAAHIMLR		0.015205	1326.683	2.21
High	DNSILPPLDKEK		0.277303	1368.737	2.15
High	QYFEQYSR		0.091148	1120.506	2.12
High	DPYPEEMMR		0.240421	1167.481	2.11
High	EEPDIYQIAK		0.462834	1205.605	2.06
High	SVASSQPAKPTKVTLVK		0.006066	1741.022	2.01
High	MVVQRDER		0.060433	1032.525	1.98
High	GDSVGLR		0.40714	703.3733	1.85
High	FPAYER		0.270398	782.3832	1.83
High	NEEYGLR		0.113505	880.4159	1.8
High	ADGATSDDLDLHDDR		0.101178	1615.683	1.77
High	QASRDLEQPTYR		0.084914	1463.724	1.72
High	QLSYFDR		0.291539	928.4523	1.53
High	TVTPAYNR		0.249877	921.4789	1.45
High	QIIDQDK		0.216669	859.452	1.43
High	GIIPNKNR		0.026358	911.5421	1.4
High	GIIPNK		0.255698	641.3981	1.29
High	FEEPAPLSYDSRPR		0.033283	1663.807	1.14
High	GKPPEADGVDRSFGEK		0.459853	1688.824	0.72

D. R42 of painted ZO-1/YAP2 complex sample 11292018_Amanda_10 is identified by the presence of peptide DNPHFQSGETSIVISDVLK tandem mass spectrum matched via Sequest

Peptide Summary

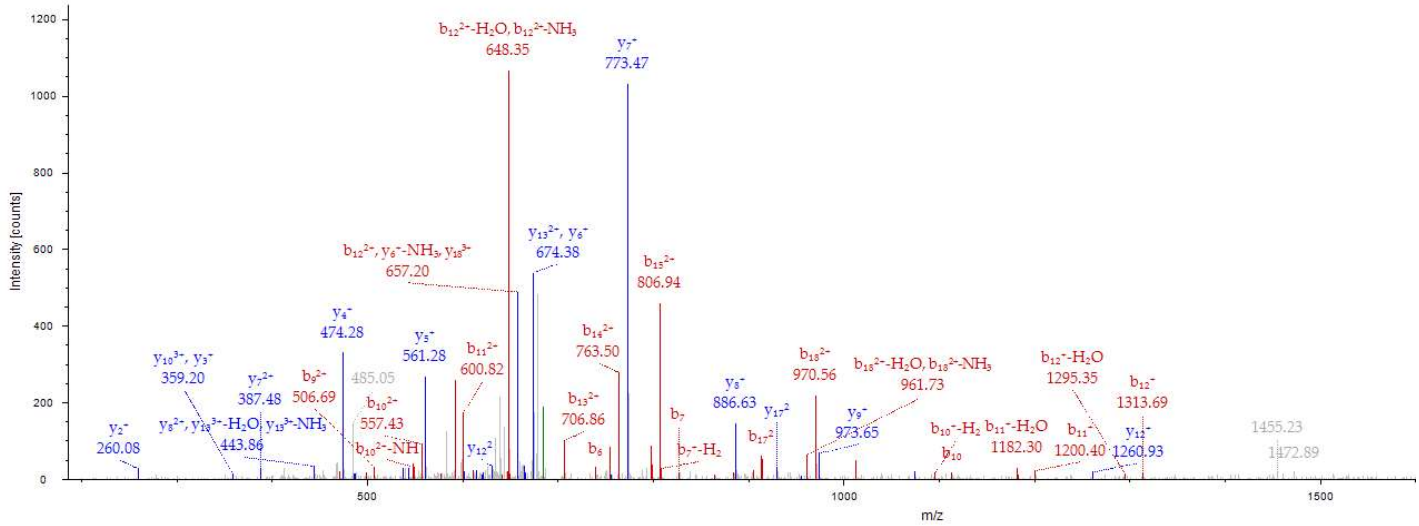
Sequence: DNPHFQSGETSIVISDVLK, Charge: +3, Monoisotopic m/z: 696.02008 Da (+0.19 mmu/+0.27 ppm), MH+: 2086.04569 Da, RT: 16.6581 min,
Identified with: Sequest HT (v1.17); XCorr:4.60, Percolator q-Value:0, Percolator PEP:0.00161, Ions matched by search engine: 0/0
Fragment match tolerance used for search: 0.6 Da
Fragments used for search: b; b-H_2O; b-NH_3; y; y-H_2O; y-NH_3

#1	b^+	b^{2+}	b^{3+}	Seq.	y^+	y^{2+}	y^{3+}	#2
1	116.03422	58.52075	39.34959	D				19
2	230.07715	115.54221	77.36390	N	1971.01818	986.01273	657.67758	18
3	327.12991	164.06859	109.71482	P	1856.97525	928.99127	619.66327	17
4	464.18882	232.59805	155.40113	H	1759.92249	880.46488	587.31235	16
5	611.25724	306.13226	204.42393	F	1622.86358	811.93543	541.62604	15
6	739.31581	370.16155	247.11012	Q	1475.79516	738.40122	492.60324	14
7	826.34784	413.67756	276.12080	S	1347.73659	674.37193	449.91705	13
8	883.36931	442.18829	295.12795	G	1260.70456	630.85592	420.90637	12
9	1012.41190	506.70959	338.14215	E	1203.68309	602.34519	401.89922	11
10	1113.45958	557.23343	371.82471	T	1074.64050	537.82389	358.88502	10
11	1200.49161	600.74944	400.83539	S	973.59282	487.30005	325.20246	9
12	1313.57567	657.29147	438.53007	I	886.56080	443.78404	296.19178	8
13	1412.64408	706.82568	471.55288	V	773.47673	387.24200	258.49709	7
14	1525.72815	763.36771	509.24757	I	674.40832	337.70780	225.47429	6
15	1612.76018	806.88373	538.25824	S	561.32425	281.16576	187.77960	5
16	1727.78712	864.39720	576.60056	D	474.29223	237.64975	158.76893	4
17	1826.85553	913.93140	609.62336	V	359.26528	180.13628	120.42661	3
18	1939.93960	970.47344	647.31805	L	260.19687	130.60207	87.40381	2
19				K	147.11280	74.06004	49.70912	1

#1	b-H_2O^+	b-H_2O^{2+}	b-H_2O^{3+}	b-NH_3^+	b-NH_3^{2+}	b-NH_3^{3+}	Seq.	y-H_2O^+	y-H_2O^{2+}	y-H_2O^{3+}	y-NH_3^+	y-NH_3^{2+}	y-NH_3^{3+}	#2
1	98.02365	49.51547	33.34607				D							19
2	212.06658	106.53693	71.36038	213.05060	107.02894	71.68838	N	1953.00762	977.00745	651.67406	1953.99163	977.49945	652.00206	18
3	309.11935	155.06331	103.71130	310.10336	155.55532	104.03930	P	1838.96469	919.98598	613.65975	1839.94870	920.47799	613.98775	17
4	446.17826	223.59277	149.39760	447.16227	224.08478	149.72561	H	1741.91193	871.45960	581.30883	1742.89594	871.95161	581.63683	16
5	593.24667	297.12697	198.42041	594.23069	297.61898	198.74841	F	1604.85301	802.93015	535.62252	1605.83703	803.42215	535.95053	15
6	721.30525	361.15626	241.10660	722.28926	361.64827	241.43461	Q	1457.78460	729.39594	486.59972	1458.76862	729.88795	486.92772	14
7	808.33728	404.67228	270.11728	809.32129	405.16428	270.44528	S	1329.72602	665.36665	443.91353	1330.71004	665.85866	444.24153	13
8	865.35874	433.18301	289.12443	866.34276	433.67502	289.45244	G	1242.69399	621.85064	414.90285	1243.67801	622.34264	415.23085	12
9	994.40133	497.70431	332.13863	995.38535	498.19631	332.46663	E	1185.67253	593.33990	395.89569	1186.65655	593.83191	396.22370	11
10	1095.44901	548.22814	365.82119	1096.43303	548.72015	366.14919	T	1056.62994	528.81861	352.88150	1057.61395	529.31061	353.20950	10
11	1182.48104	591.74416	394.83186	1183.46506	592.23617	395.15987	S	955.58226	478.29477	319.19894	956.56627	478.78678	319.52694	9
12	1295.56510	648.28619	432.52655	1296.54912	648.77820	432.85456	I	868.55023	434.77875	290.18826	869.53425	435.27076	290.51627	8
13	1394.63352	697.82040	465.54936	1395.61753	698.31241	465.87736	V	755.46617	378.23672	252.49357	756.45018	378.72873	252.82158	7
14	1507.71758	754.36243	503.24405	1508.70160	754.85444	503.57205	I	656.39775	328.70251	219.47077	657.38177	329.19452	219.79877	6
15	1594.74961	797.87844	532.25472	1595.73363	798.37045	532.58273	S	543.31369	272.16048	181.77608	544.29770	272.65249	182.10409	5
16	1709.77655	855.39192	570.59704	1710.76057	855.88392	570.92504	D	456.28166	228.64447	152.76540	457.26568	229.13648	153.09341	4
17	1808.84497	904.92612	603.61984	1809.82898	905.41813	603.94785	V				342.23873	171.62300	114.75110	3
18	1921.92903	961.46815	641.31453	1922.91305	961.96016	641.64253	L				243.17032	122.08880	81.72829	2
19							K				130.08626	65.54677	44.03360	1

Fragment Spectrum

11292018_Amanda_10.raw #3286 RT: 16.6581 min
ITMS, 696.0201@cid35.00, z=+3, Mono m/z=696.02008 Da, MH+=2086.04569 Da, Match Tol.=0.6 Da



E. K592 of painted ZO-1/YAP2 complex sample 11292018_Amanda_10 is identified by the presence of peptide TAGGDRADFWR tandem mass spectrum matched via Sequest

Peptide Summary

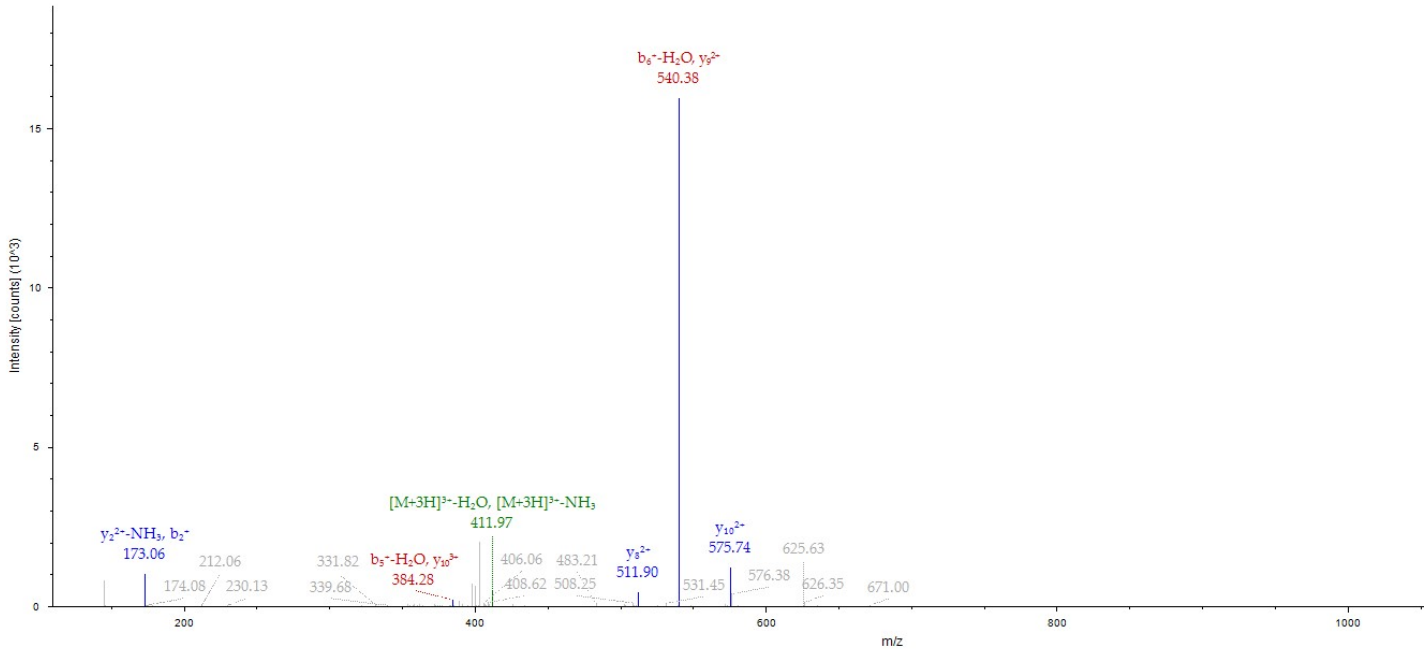
Sequence: TAGGDRADFWR, Charge: +3, Monoisotopic m/z: 417.86691 Da (-0.11 mmu/-0.26 ppm), MH+: 1251.58619 Da, RT: 10.6433 min,
Identified with: Sequest HT (v1.17); XCorr:1.06, Percolator q-Value:0.00322, Percolator PEP:0.17, Ions matched by search engine: 0/0
Fragment match tolerance used for search: 0.6 Da
Fragments used for search: b; b-H_2O; b-NH_3; y; y-H_2O; y-NH_3

#1	b^+	b^{2+}	b^{3+}	Seq.	y^+	y^{2+}	y^{3+}	#2
1	102.05496	51.53112	34.68984	T				11
2	173.09207	87.04967	58.36887	A	1150.53883	575.77305	384.18446	10
3	230.11353	115.56040	77.37603	G	1079.50172	540.25450	360.50542	9
4	287.13500	144.07114	96.38318	G	1022.48025	511.74376	341.49827	8
5	402.16194	201.58461	134.72550	D	965.45879	483.23303	322.49111	7
6	558.26305	279.63516	186.75920	R	850.43185	425.71956	284.14880	6
7	629.30016	315.15372	210.43824	A	694.33074	347.66901	232.11510	5
8	744.32711	372.66719	248.78055	D	623.29362	312.15045	208.43606	4
9	891.39552	446.20140	297.80336	F	508.26668	254.63698	170.09374	3
10	1077.47483	539.24106	359.82980	W	361.19827	181.10277	121.07094	2
11				R	175.11895	88.06311	59.04450	1

#1	b-H_2O^+	b-H_2O^{2+}	b-H_2O^{3+}	b-NH_3^+	b-NH_3^{2+}	b-NH_3^{3+}	Seq.	y-H_2O^+	y-H_2O^{2+}	y-H_2O^{3+}	y-NH_3^+	y-NH_3^{2+}	y-NH_3^{3+}	#2
1	84.04439	42.52583	28.68631				T							11
2	155.08150	78.04439	52.36535				A	1132.52827	566.76777	378.18094	1133.51228	567.25978	378.50894	10
3	212.10297	106.55512	71.37251				G	1061.49115	531.24921	354.50190	1062.47517	531.74122	354.82991	9
4	269.12443	135.06585	90.37966				G	1004.46969	502.73848	335.49475	1005.45370	503.23049	335.82275	8
5	384.15137	192.57933	128.72198				D	947.44822	474.22775	316.48759	948.43224	474.71976	316.81560	7
6	540.25249	270.62988	180.75568	541.23650	271.12189	181.08368	R	832.42128	416.71428	278.14528	833.40530	417.20629	278.47328	6
7	611.28960	306.14844	204.43472	612.27361	306.64045	204.76272	A	676.32017	338.66372	226.11157	677.30419	339.15573	226.43958	5
8	726.31654	363.66191	242.77703	727.30056	364.15392	243.10504	D	605.28306	303.14517	202.43254	606.26707	303.63717	202.76054	4
9	873.38496	437.19612	291.79984	874.36897	437.68812	292.12784	F				491.24013	246.12370	164.41823	3
10	1059.46427	530.23577	353.82627	1060.44828	530.72778	354.15428	W				344.17172	172.58950	115.39542	2
11							R				158.09240	79.54984	53.36899	1

Fragment Spectrum

11292018_Amanda_10.raw #2182 RT: 10.6433 min
ITMS, 417.8669@cid35.00, z=+3, Mono m/z=417.86691 Da, MH+=1251.58619 Da, Match Tol.=0.6 Da



Supplementary Figure 6: Control peptides in ZO-1 do not change in the presence of dye coverage or complexation with YAP2. A) Control peptide NRAEQLASVQYTLPK is present in the unpainted ZO-1 sample 11292018_Amanda_3. B) Control peptide APGFGFGIAISGGR is present in the unpainted ZO-1 sample 11292018_Amanda_3. C) Control peptide NRAEQLASVQYTLPK is present in the painted ZO-1 sample 11292018_Amanda_7. D) Control peptide APGFGFGIAISGGR is present in the painted ZO-1 sample 11292018_Amanda_7. E) Control peptide NRAEQLASVQYTLPK is present in the painted ZO-1/YAP2 complex sample 11292018_Amanda_10. F) Control peptide APGFGFGIAISGGR is present in the painted ZO-1/YAP2 complex sample 11292018_Amanda_10.

A. Control peptide NRAEQLASVQYTLPK is present in the unpainted ZO-1 sample 11292018_Amanda_3.

Peptide Summary

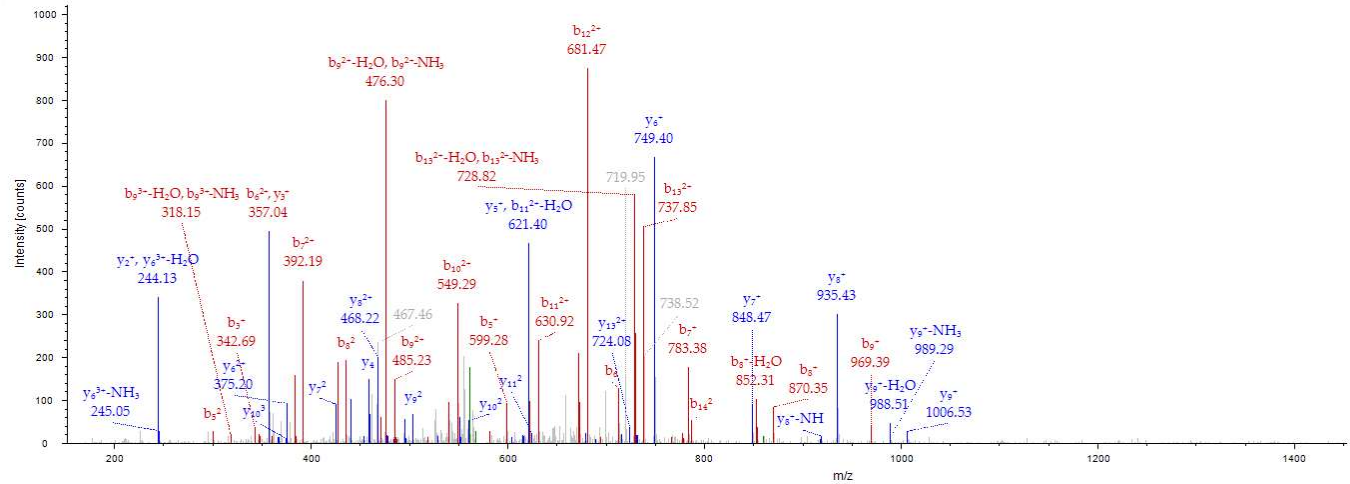
Sequence: NRAEQLASVQYTLPK, Charge: +3, Monoisotopic m/z: 573.31329 Da (+0.72 mmu/+1.26 ppm), MH+: 1717.92533 Da, RT: 12.4821 min,
Identified with: Sequest HT (v1.17); XCorr:3.88, Percolator q-Value:0, Percolator PEP:0.06, Ions matched by search engine: 0/0
Fragment match tolerance used for search: 0.6 Da
Fragments used for search: b; b-H_2O; b-NH_3; y; y-H_2O; y-NH_3

#1	b^+	b^{2+}	b^{3+}	Seq.	y^+	y^{2+}	y^{3+}	#2
1	115.05020	58.02874	39.02159	N				15
2	271.15131	136.07930	91.05529	R	1603.88023	802.44375	535.29826	14
3	342.18843	171.59785	114.73433	A	1447.77912	724.39320	483.26456	13
4	471.23102	236.11915	157.74852	E	1376.74201	688.87464	459.58552	12
5	599.28960	300.14844	200.43472	Q	1247.69941	624.35335	416.57132	11
6	712.37366	356.69047	238.12941	L	1119.64084	560.32406	373.88513	10
7	783.41078	392.20903	261.80844	A	1006.55677	503.78202	336.19044	9
8	870.44281	435.72504	290.81912	S	935.51966	468.26347	312.51140	8
9	969.51122	485.25925	323.84192	V	848.48763	424.74745	283.50073	7
10	1097.56980	549.28854	366.52812	Q	749.41922	375.21325	250.47792	6
11	1260.63312	630.82020	420.88256	Y	621.36064	311.18396	207.79173	5
12	1361.68080	681.34404	454.56512	T	458.29731	229.65229	153.43729	4
13	1474.76487	737.88607	492.25981	L	357.24963	179.12845	119.75473	3
14	1571.81763	786.41245	524.61073	P	244.16557	122.58642	82.06004	2
15				K	147.11280	74.06004	49.70912	1

#1	b-H_2O^+	b-H_2O^{2+}	b-H_2O^{3+}	b-NH_3^+	b-NH_3^{2+}	b-NH_3^{3+}	Seq.	y-H_2O^+	y-H_2O^{2+}	y-H_2O^{3+}	y-NH_3^+	y-NH_3^{2+}	y-NH_3^{3+}	#2
1				98.02365	49.51547	33.34607	N							15
2				254.12477	127.56602	85.37977	R	1585.86967	793.43847	529.29474	1586.85368	793.93048	529.62275	14
3				325.16188	163.08458	109.05881	A	1429.76856	715.38792	477.26104	1430.75257	715.87992	477.58904	13
4	453.22046	227.11387	151.74500	454.20447	227.60587	152.07301	E	1358.73144	679.86936	453.58200	1359.71546	680.36137	453.91000	12
5	581.27903	291.14316	194.43120	582.26305	291.63516	194.75920	Q	1229.68885	615.34806	410.56780	1230.67286	615.84007	410.89581	11
6	694.36310	347.68519	232.12588	695.34711	348.17720	232.45389	L	1101.63027	551.31877	367.88161	1102.61429	551.81078	368.20961	10
7	765.40021	383.20374	255.80492	766.38423	383.69575	256.13293	A	988.54621	494.77674	330.18692	989.53022	495.26875	330.51493	9
8	852.43224	426.71976	284.81560	853.41626	427.21177	285.14360	S	917.50909	459.25819	306.50788	918.49311	459.75019	306.83589	8
9	951.50065	476.25397	317.83840	952.48467	476.74597	318.16641	V	830.47707	415.74217	277.49721	831.46108	416.23418	277.82521	7
10	1079.55923	540.28325	360.52460	1080.54325	540.77526	360.85260	Q	731.40865	366.20796	244.47440	732.39267	366.69997	244.80241	6
11	1242.62256	621.81492	414.87904	1243.60658	622.30693	415.20704	Y	603.35007	302.17868	201.78821	604.33409	302.67068	202.11621	5
12	1343.67024	672.33876	448.56160	1344.65425	672.83077	448.88960	T	440.28675	220.64701	147.43377	441.27076	221.13902	147.76177	4
13	1456.75430	728.88079	486.25629	1457.73832	729.37280	486.58429	L				340.22308	170.61518	114.07921	3
14	1553.80707	777.40717	518.60721	1554.79108	777.89918	518.93521	P				227.13902	114.07315	76.38452	2
15							K				130.08626	65.54677	44.03360	1

Fragment Spectrum

11292018_Amanda_3.raw #2317 RT: 12.4821 min
ITMS, 573.3133@cid35.00, z=+3, Mono m/z=573.31329 Da, MH+=1717.92533 Da, Match Tol.=0.6 Da



B. Control peptide APGFGFGIAISGGR is present in the unpainted ZO-1 sample 11292018_Amanda_3.

Peptide Summary

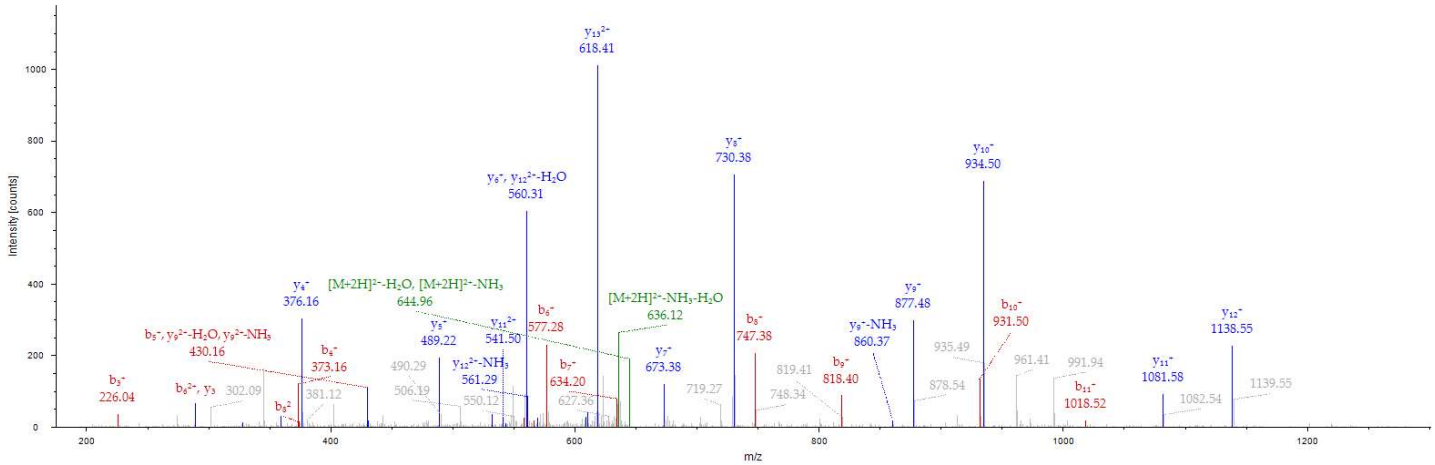
Sequence: APGFGFGIAISGGR, Charge: +2, Monoisotopic m/z: 653.84943 Da (+0.67 mmu/+1.02 ppm), MH+: 1306.69158 Da, RT: 17.0113 min,
Identified with: Sequest HT (v1.17); XCorr:4.26, Percolator q-Value:0, Percolator PEP:0.0173, Ions matched by search engine: 0/0
Fragment match tolerance used for search: 0.6 Da
Fragments used for search: b; b-H_2O; y; y-H_2O; y-NH_3

#1	b^+	b^{2+}	Seq.	y^+	y^{2+}	#2
1	72.04439	36.52583	A			14
2	169.09715	85.05222	P	1235.65313	618.33020	13
3	226.11862	113.56295	G	1138.60037	569.80382	12
4	373.18703	187.09715	F	1081.57890	541.29309	11
5	430.20850	215.60789	G	934.51049	467.75888	10
6	577.27691	289.14209	F	877.48903	439.24815	9
7	634.29837	317.65282	G	730.42061	365.71394	8
8	747.38244	374.19486	I	673.39915	337.20321	7
9	818.41955	409.71341	A	560.31509	280.66118	6
10	931.50361	466.25545	I	489.27797	245.14262	5
11	1018.53564	509.77146	S	376.19391	188.60059	4
12	1075.55711	538.28219	G	289.16188	145.08458	3
13	1132.57857	566.79292	G	232.14042	116.57385	2
14			R	175.11895	88.06311	1

#1	b-H_2O^+	b-H_2O^{2+}	Seq.	y-H_2O^+	y-H_2O^{2+}	y-NH_3^+	y-NH_3^{2+}	#2
1			A					14
2			P	1217.64257	609.32492	1218.62658	609.81693	13
3			G	1120.58980	560.79854	1121.57382	561.29055	12
4			F	1063.56834	532.28781	1064.55236	532.77982	11
5			G	916.49993	458.75360	917.48394	459.24561	10
6			F	859.47846	430.24287	860.46248	430.73488	9
7			G	712.41005	356.70866	713.39406	357.20067	8
8			I	655.38858	328.19793	656.37260	328.68994	7
9			A	542.30452	271.65590	543.28854	272.14791	6
10			I	471.26741	236.13734	472.25142	236.62935	5
11	1000.52508	500.76618	S	358.18334	179.59531	359.16736	180.08732	4
12	1057.54654	529.27691	G			272.13533	136.57130	3
13	1114.56801	557.78764	G			215.11387	108.06057	2
14			R			158.09240	79.54984	1

Fragment Spectrum

11292018_Amanda_3.raw #3126 RT: 17.0113 min
ITMS, 653.8494@cid35.00, z=+2, Mono m/z=653.84943 Da, MH+=1306.69158 Da, Match Tol.=0.6 Da



C. Control peptide NRAEQLASVQYTLPK is present in the painted ZO-1 sample 11292018_Amanda_7.

Peptide Summary

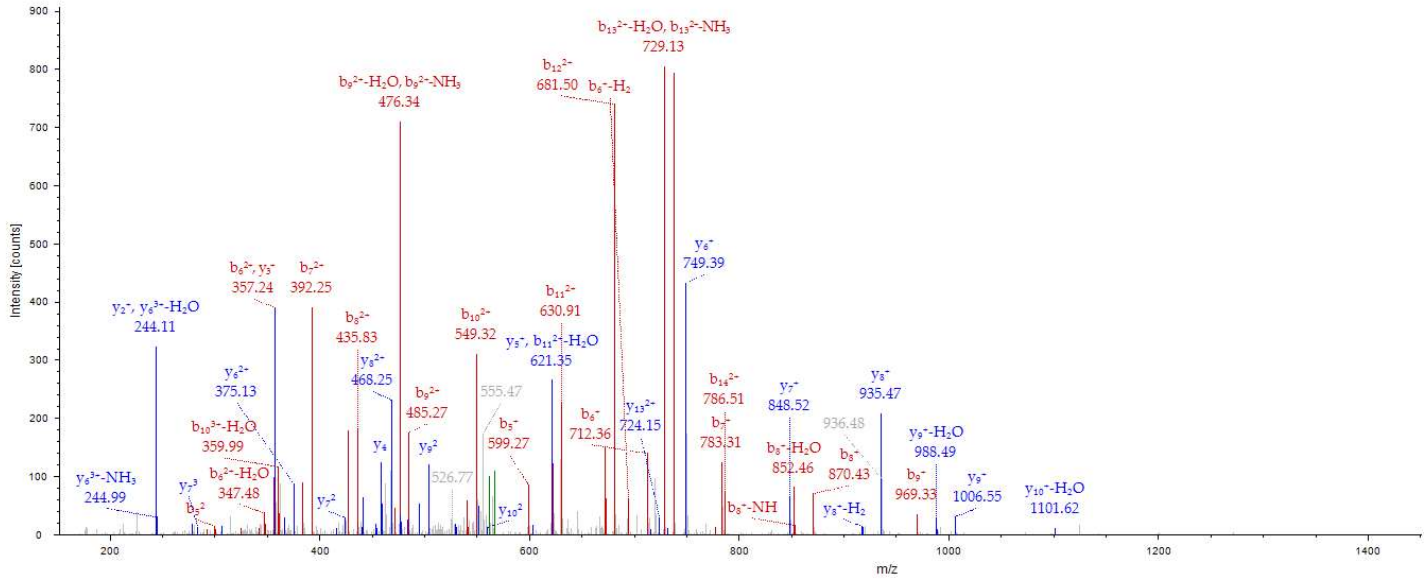
Sequence: NRAEQLASVQYTLPK, Charge: +3, Monoisotopic m/z: 573.31287 Da (+0.3 mmu/+0.52 ppm), MH+: 1717.92405 Da, RT: 12.6669 min,
Identified with: Sequest HT (v1.17); XCorr:4.97, Percolator q-Value:0, Percolator PEP:0.00873, Ions matched by search engine: 0/0
Fragment match tolerance used for search: 0.6 Da
Fragments used for search: b; b-H_2O; b-NH_3; y; y-H_2O; y-NH_3

#1	b^+	b^{2+}	b^{3+}	Seq.	y^+	y^{2+}	y^{3+}	#2
1	115.05020	58.02874	39.02159	N				15
2	271.15131	136.07930	91.05529	R	1603.88023	802.44375	535.29826	14
3	342.18843	171.59785	114.73433	A	1447.77912	724.39320	483.26456	13
4	471.23102	236.11915	157.74852	E	1376.74201	688.87464	459.58552	12
5	599.28960	300.14844	200.43472	Q	1247.69941	624.35335	416.57132	11
6	712.37366	356.69047	238.12941	L	1119.64084	560.32406	373.88513	10
7	783.41078	392.20903	261.80844	A	1006.55677	503.78202	336.19044	9
8	870.44281	435.72504	290.81912	S	935.51966	468.26347	312.51140	8
9	969.51122	485.25925	323.84192	V	848.48763	424.74745	283.50073	7
10	1097.56980	549.28854	366.52812	Q	749.41922	375.21325	250.47792	6
11	1260.63312	630.82020	420.88256	Y	621.36064	311.18396	207.79173	5
12	1361.68080	681.34404	454.56512	T	458.29731	229.65229	153.43729	4
13	1474.76487	737.88607	492.25981	L	357.24963	179.12845	119.75473	3
14	1571.81763	786.41245	524.61073	P	244.16557	122.58642	82.06004	2
15				K	147.11280	74.06004	49.70912	1

#1	b-H_2O^+	b-H_2O^{2+}	b-H_2O^{3+}	b-NH_3^+	b-NH_3^{2+}	b-NH_3^{3+}	Seq.	y-H_2O^+	y-H_2O^{2+}	y-H_2O^{3+}	y-NH_3^+	y-NH_3^{2+}	y-NH_3^{3+}	#2
1				98.02365	49.51547	33.34607	N							15
2				254.12477	127.56602	85.37977	R	1585.86967	793.43847	529.29474	1586.85368	793.93048	529.62275	14
3				325.16188	163.08458	109.05881	A	1429.76856	715.38792	477.26104	1430.75257	715.87992	477.58904	13
4	453.22046	227.11387	151.74500	454.20447	227.60587	152.07301	E	1358.73144	679.86936	453.58200	1359.71546	680.36137	453.91000	12
5	581.27903	291.14316	194.43120	582.26305	291.63516	194.75920	Q	1229.68885	615.34806	410.56780	1230.67286	615.84007	410.89581	11
6	694.36310	347.68519	232.12588	695.34711	348.17720	232.45389	L	1101.63027	551.31877	367.88161	1102.61429	551.81078	368.20961	10
7	765.40021	383.20374	255.80492	766.38423	383.69575	256.13293	A	988.54621	494.77674	330.18692	989.53022	495.26875	330.51493	9
8	852.43224	426.71976	284.81560	853.41626	427.21177	285.14360	S	917.50909	459.25819	306.50788	918.49311	459.75019	306.83589	8
9	951.50065	476.25397	317.83840	952.48467	476.74597	318.16641	V	830.47707	415.74217	277.49721	831.46108	416.23418	277.82521	7
10	1079.55923	540.28325	360.52460	1080.54325	540.77526	360.85260	Q	731.40865	366.20796	244.47440	732.39267	366.69997	244.80241	6
11	1242.62256	621.81492	414.87904	1243.60658	622.30693	415.20704	Y	603.35007	302.17868	201.78821	604.33409	302.67068	202.11621	5
12	1343.67024	672.33876	448.56160	1344.65425	672.83077	448.88960	T	440.28675	220.64701	147.43377	441.27076	221.13902	147.76177	4
13	1456.75430	728.88079	486.25629	1457.73832	729.37280	486.58429	L				340.22308	170.61518	114.07921	3
14	1553.80707	777.40717	518.60721	1554.79108	777.89918	518.93521	P				227.13902	114.07315	76.38452	2
15							K				130.08626	65.54677	44.03360	1

Fragment Spectrum

11292018_Amanda_7.raw #2347 RT: 12.6669 min
ITMS, 573.6472@cid35.00, z=+3, Mono m/z=573.31287 Da, MH+=1717.92405 Da, Match Tol.=0.6 Da



D. Control peptide APGFGFGIAISGGR is present in the painted ZO-1 sample 11292018_Amanda_7.

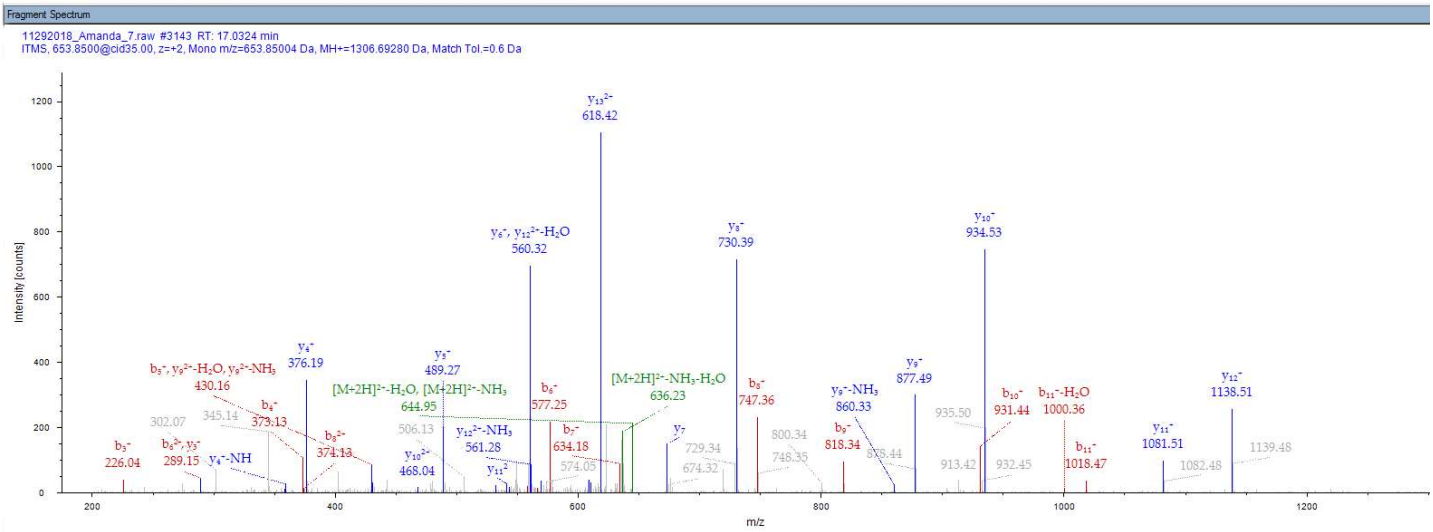
Peptide Summary

Sequence: APGFGFGIAISGGR, Charge: +2, Monoisotopic m/z: 653.85004 Da (+1.28 mmu/+1.95 ppm), MH+: 1306.69280 Da, RT: 17.0324 min,
Identified with: Sequest HT (v1.17); XCorr:4.18, Percolator q-Value:0, Percolator PEP:9.43e-05, Ions matched by search engine: 0/0
Fragment match tolerance used for search: 0.6 Da
Fragments used for search: b; b-H_2O; y; y-H_2O; y-NH_3

#1	b^+	b^{2+}	Seq.	y^+	y^{2+}	#2
1	72.04439	36.52583	A			14
2	169.09715	85.05222	P	1235.65313	618.33020	13
3	226.11862	113.56295	G	1138.60037	569.80382	12
4	373.18703	187.09715	F	1081.57890	541.29309	11
5	430.20850	215.60789	G	934.51049	467.75888	10
6	577.27691	289.14209	F	877.48903	439.24815	9
7	634.29837	317.65282	G	730.42061	365.71394	8
8	747.38244	374.19486	I	673.39915	337.20321	7
9	818.41955	409.71341	A	560.31509	280.66118	6
10	931.50361	466.25545	I	489.27797	245.14262	5
11	1018.53564	509.77146	S	376.19391	188.60059	4
12	1075.55711	538.28219	G	289.16188	145.08458	3
13	1132.57857	566.79292	G	232.14042	116.57385	2
14			R	175.11895	88.06311	1

#1	b-H_2O^+	b-H_2O^{2+}	Seq.	y-H_2O^+	y-H_2O^{2+}	y-NH_3^+	y-NH_3^{2+}	#2
1			A					14
2			P	1217.64257	609.32492	1218.62658	609.81693	13
3			G	1120.58980	560.79854	1121.57382	561.29055	12
4			F	1063.56834	532.28781	1064.55236	532.77982	11
5			G	916.49993	458.75360	917.48394	459.24561	10
6			F	859.47846	430.24287	860.46248	430.73488	9
7			G	712.41005	356.70866	713.39406	357.20067	8
8			I	655.38858	328.19793	656.37260	328.68994	7
9			A	542.30452	271.65590	543.28854	272.14791	6
10			I	471.26741	236.13734	472.25142	236.62935	5
11	1000.52508	500.76618	S	358.18334	179.59531	359.16736	180.08732	4
12	1057.54654	529.27691	G			272.13533	136.57130	3
13	1114.56801	557.78764	G			215.11387	108.06057	2
14			R			158.09240	79.54984	1

Fragment Spectrum



E. Control peptide NRAEQLASVQYTLPK is present in the painted ZO-1/YAP2 complex sample 11292018_Amanda_10.

Peptide Summary

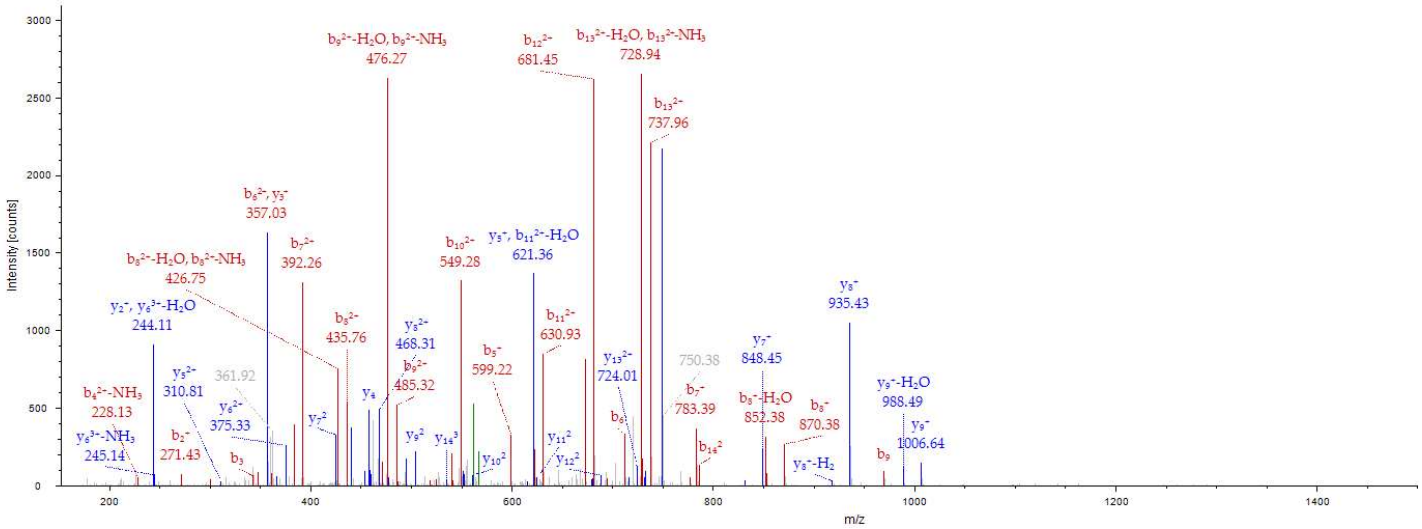
Sequence: NRAEQLASVQYTLPK, Charge: +3, Monoisotopic m/z: 573.31323 Da (+0.66 mmu/+1.16 ppm), MH+: 1717.92514 Da, RT: 12.6017 min,
Identified with: Sequest HT (v1.17); XCorr:4.87, Percolator q-Value:0, Percolator PEP:0.000743, Ions matched by search engine: 0/0
Fragment match tolerance used for search: 0.6 Da
Fragments used for search: b; b-H_2O; b-NH_3; y; y-H_2O; y-NH_3

#1	b^+	b^{2+}	b^{3+}	Seq.	y^+	y^{2+}	y^{3+}	#2
1	115.05020	58.02874	39.02159	N				15
2	271.15131	136.07930	91.05529	R	1603.88023	802.44375	535.29826	14
3	342.18843	171.59785	114.73433	A	1447.77912	724.39320	483.26456	13
4	471.23102	236.11915	157.74852	E	1376.74201	688.87464	459.58552	12
5	599.28960	300.14844	200.43472	Q	1247.69941	624.35335	416.57132	11
6	712.37366	356.69047	238.12941	L	1119.64084	560.32406	373.88513	10
7	783.41078	392.20903	261.80844	A	1006.55677	503.78202	336.19044	9
8	870.44281	435.72504	290.81912	S	935.51966	468.26347	312.51140	8
9	969.51122	485.25925	323.84192	V	848.48763	424.74745	283.50073	7
10	1097.56980	549.28854	366.52812	Q	749.41922	375.21325	250.47792	6
11	1260.63312	630.82020	420.88256	Y	621.36064	311.18396	207.79173	5
12	1361.68080	681.34404	454.56512	T	458.29731	229.65229	153.43729	4
13	1474.76487	737.88607	492.25981	L	357.24963	179.12845	119.75473	3
14	1571.81763	786.41245	524.61073	P	244.16557	122.58642	82.06004	2
15				K	147.11280	74.06004	49.70912	1

#1	b-H_2O^+	b-H_2O^{2+}	b-H_2O^{3+}	b-NH_3^+	b-NH_3^{2+}	b-NH_3^{3+}	Seq.	y-H_2O^+	y-H_2O^{2+}	y-H_2O^{3+}	y-NH_3^+	y-NH_3^{2+}	y-NH_3^{3+}	#2
1				98.02365	49.51547	33.34607	N							15
2				254.12477	127.56602	85.37977	R	1585.86967	793.43847	529.29474	1586.85368	793.93048	529.62275	14
3				325.16188	163.08458	109.05881	A	1429.76856	715.38792	477.26104	1430.75257	715.87992	477.58904	13
4	453.22046	227.11387	151.74500	454.20447	227.60587	152.07301	E	1358.73144	679.86936	453.58200	1359.71546	680.36137	453.91000	12
5	581.27903	291.14316	194.43120	582.26305	291.63516	194.75920	Q	1229.68885	615.34806	410.56780	1230.67286	615.84007	410.89581	11
6	694.36310	347.68519	232.12588	695.34711	348.17720	232.45389	L	1101.63027	551.31877	367.88161	1102.61429	551.81078	368.20961	10
7	765.40021	383.20374	255.80492	766.38423	383.69575	256.13293	A	988.54621	494.77674	330.18692	989.53022	495.26875	330.51493	9
8	852.43224	426.71976	284.81560	853.41626	427.21177	285.14360	S	917.50909	459.25819	306.50788	918.49311	459.75019	306.83589	8
9	951.50065	476.25397	317.83840	952.48467	476.74587	318.16641	V	830.47707	415.74217	277.49721	831.46108	416.23418	277.82521	7
10	1079.55923	540.28325	360.52460	1080.54325	540.77526	360.85260	Q	731.40865	366.20796	244.47440	732.39267	366.69997	244.80241	6
11	1242.62256	621.81492	414.87904	1243.60658	622.30693	415.20704	Y	603.35007	302.17868	201.78821	604.33409	302.67068	202.11621	5
12	1343.67024	672.33876	448.56160	1344.65425	672.83077	448.88960	T	440.28675	220.64701	147.43377	441.27076	221.13902	147.76177	4
13	1456.75430	728.88079	486.25629	1457.73832	729.37280	486.58429	L				340.22308	170.61518	114.07921	3
14	1553.80707	777.40717	518.60721	1554.79108	777.89918	518.93521	P				227.13902	114.07315	76.38452	2
15							K				130.08626	65.54677	44.03360	1

Fragment Spectrum

11292018_Amanda_10.raw #2523 RT: 12.6017 min
ITMS, 573.3132@cid35.00, z=+3, Mono m/z=573.31323 Da, MH+=1717.92514 Da, Match Tol.=0.6 Da



F. Control peptide APGFGFGIAISGGR is present in the painted ZO-1/YAP2 complex sample 11292018_Amanda_10.

Peptide Summary

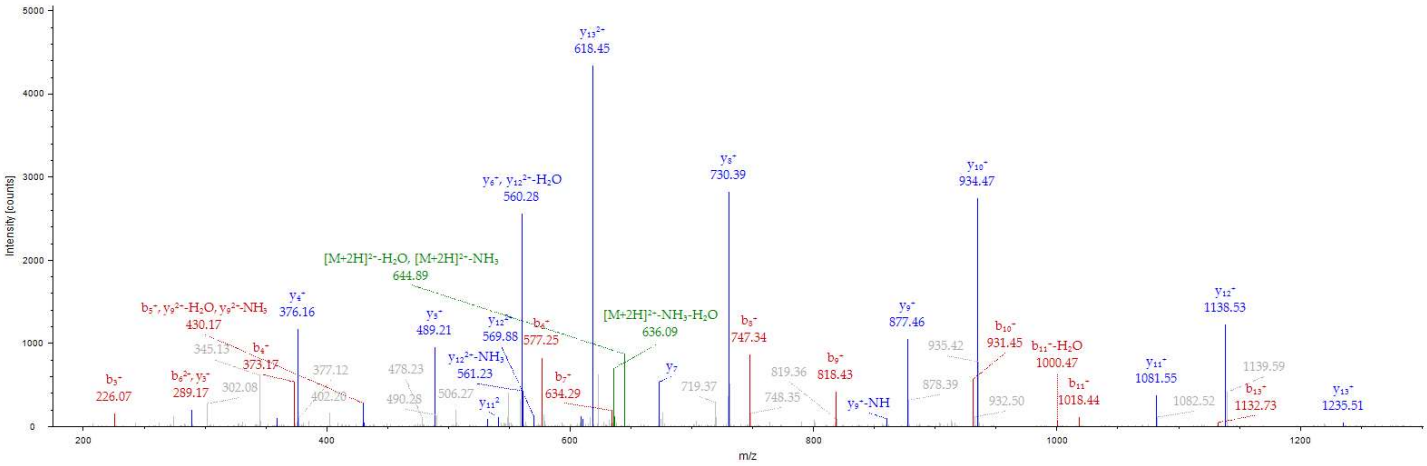
Sequence: APGFGFGIAISGGR, Charge: +2, Monoisotopic m/z: 653.84857 Da (-0.19 mmu/-0.29 ppm), MH+: 1306.68987 Da, RT: 17.1244 min,
Identified with: Sequest HT (v1.17); XCorr:4.40, Percolator q-Value:0, Percolator PEP:0.00226, Ions matched by search engine: 0/0
Fragment match tolerance used for search: 0.6 Da
Fragments used for search: b; b-H_2O; y; y-H_2O; y-NH_3

#1	b^+	b^{2+}	Seq.	y^+	y^{2+}	#2
1	72.04439	36.52583	A			14
2	169.09715	85.05222	P	1235.65313	618.33020	13
3	226.11862	113.56295	G	1138.60037	569.80382	12
4	373.18703	187.09715	F	1081.57890	541.29309	11
5	430.20850	215.60789	G	934.51049	467.75888	10
6	577.27691	289.14209	F	877.48903	439.24815	9
7	634.29837	317.65282	G	730.42061	365.71394	8
8	747.38244	374.19486	I	673.39915	337.20321	7
9	818.41955	409.71341	A	560.31509	280.66118	6
10	931.50361	466.25545	I	489.27797	245.14262	5
11	1018.53564	509.77146	S	376.19391	188.60059	4
12	1075.55711	538.28219	G	289.16188	145.08458	3
13	1132.57857	566.79292	G	232.14042	116.57385	2
14			R	175.11895	88.06311	1

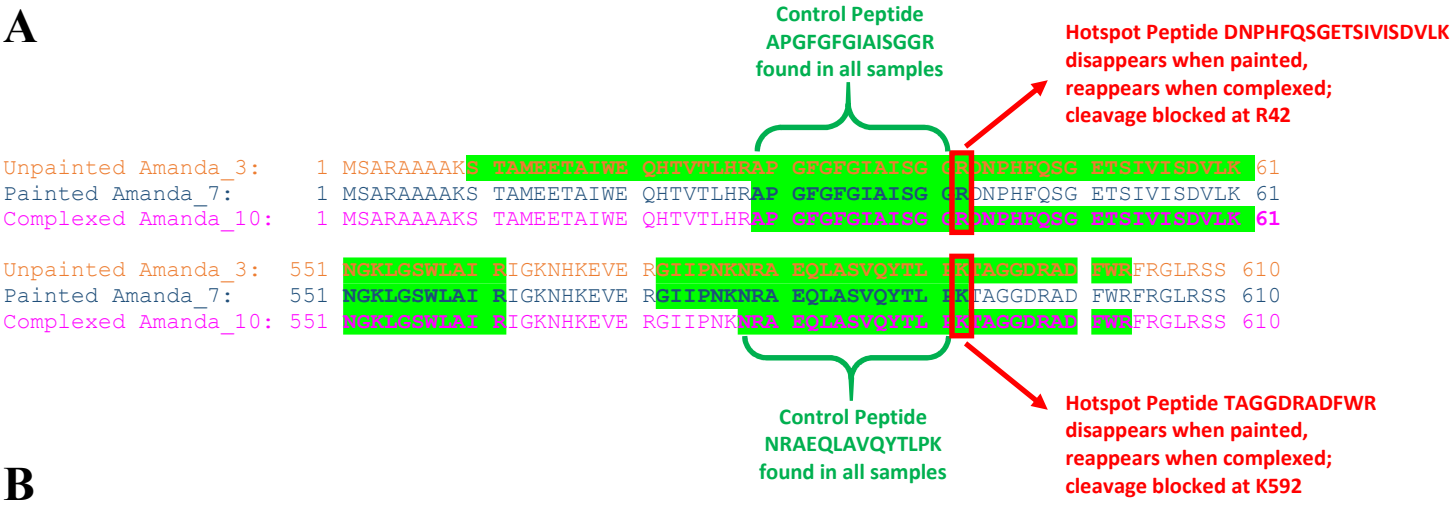
#1	b-H_2O^+	b-H_2O^{2+}	Seq.	y-H_2O^+	y-H_2O^{2+}	y-NH_3^+	y-NH_3^{2+}	#2
1			A					14
2			P	1217.64257	609.32492	1218.62658	609.81693	13
3			G	1120.58980	560.79854	1121.57382	561.29055	12
4			F	1063.56834	532.28781	1064.55236	532.77982	11
5			G	916.49993	458.75360	917.48394	459.24561	10
6			F	859.47846	430.24287	860.46248	430.73488	9
7			G	712.41005	356.70866	713.39406	357.20067	8
8			I	655.38858	328.19793	656.37260	328.68994	7
9			A	542.30452	271.65590	543.28854	272.14791	6
10			I	471.26741	236.13734	472.25142	236.62935	5
11	1000.52508	500.76618	S	358.18334	179.59531	359.16736	180.08732	4
12	1057.54654	529.27691	G			272.13533	136.57130	3
13	1114.56801	557.78764	G			215.11387	108.06057	2
14			R			158.09240	79.54984	1

Fragment Spectrum

11292018_Amanda_10.raw #3306 RT: 17.1244 min
ITMS, 653.8486@cid35.00, z=+2, Mono m/z=653.84857 Da, MH+=1306.68987 Da, Match Tol.=0.6 Da



A



B

R42 Interaction Site (Peptide DNPHFQSGETSIVISDVLK)

Fisher's Exact Test, Freeman-Halton Extension: p = 0.001*

	Unpainted Samples	Painted Samples	Complexed Samples
Peptide Present	5	0	5
Peptide Absent	0	6	1

K592 Interaction Site (Peptide TAGGDRADFWR)

Fisher's Exact Test, Freeman-Halton Extension: p = 0.006*

	Unpainted Samples	Painted Samples	Complexed Samples
Peptide Present	4	0	5
Peptide Absent	1	6	1

Supplementary Figure 7: ZO-1 hotspots for its interaction with YAP2 are determined by differential analysis of unpainted ZO-1, painted ZO-1, and painted ZO-1/YAP2 complex. A) To determine the presence of ZO-1 hotspots for an independent mass spectrometry experiment, the sequence of ZO-1 for an unpainted sample, a painted sample, and a complexed sample are aligned, and peptides identified in each sample are highlighted in green, using the trials depicted in Supplementary Figures 4 and 5. Peptides which are present in the unpainted sample, absent in the painted sample, and return in the complexed sample are considered indicative of regions that are solvent-accessible in the painted sample, but not solvent-accessible in the complexed sample, such as DNPHFQSGETSIVISDVLK. The missed tryptic cleavage site, such as R42 for the peptide DNPHFQSGETSIVISDVLK, is the identified hotspot. Experiments were completed with 2 technical replicates per trial for three independent trials, resulting in 6 samples per condition. One unpainted trial was excluded from the analysis due to loss of sample volume prior to mass spectrometry. Significant difference between subgroups was evaluated by Fisher's exact test with the Freeman-Halton expansion for *m x n* tables. B) Hotspots were considered statistically significant when a peptide was present in 5 out of 6 unpainted samples, absent in 5 out of 6 painted samples, and present in 5 out of 6 painted complexed samples (p = 0.03 via Fisher's exact test with the Freeman-Halton extension; greater than 95% confidence to reject the null hypothesis that presence or absence of a peptide is group-independent). Hotspots R42 (p = 0.001) and K592 (p = 0.006) were statistically significant.

Supplementary Figure 8. Sequence Alignment of ZO-1 from 11 diverse species shows hotpots identified via protein painting are found in evolutionarily conserved regions. *Homo sapiens* ZO-1 is shown in red, and hotspots identified in complex with YAP2 are highlighted in yellow.

```
ZO-1 Clustal Alignment

Species represented:
Homo sapiens (Human; NP_003248.3)
Aliuropoda melanoleuca (Giant Panda; XP_011230579)
Odobenus rosmarus divergens (walrus, XP_004413034.1)
Balaenoptera acutorostrata scammoni (Minke whale, XP_007167555.1)
Melopsittacus undulates (parakeet, XP_012983641.1)
Struthio camelus australis (ostrich, XP_009679778.1)
Pogona vitticeps (central bearded dragon lizard, XP_020640389.1)
Python bivittatus (Burmese python, XP_007424590.2)
Callorhinchus milii (elephant shark, XP_007907131.1)
Salmo salar (Atlantic salmon, XP_013982297.1)
Hippocampus comes (tiger tail seahorse, XP_019722858.1)

S.salar      ---------- ---------- ----mkyqky itvmqmamgv tainrdncip pkrqmwvtpt  36
H.comes      ---------- ---------- ----mkyqky itvmqmamgv tasn-kdclpt krqlwvtps  35
C.milii      ---------- ---------- ---------- ---------- ---------- ---------  0
B.scammoni   ---------- ---------- ---------- ---------- ---------- ---------  0
H.sapiens    ---------- ---------- ---------- ---------- ---------- ---------  0
A.melanoleuca ---------- ---------- ---------- ---------- ---------- --------- 0
O.divergens   ---------- ---------- ---------- ---------- ---------- --------- 0
M.undulatus   ---------- ---------- ----mkyqky ltvlqmaigv tasnrgslmpl krklwvtps 36
S.australis   ---------- ---------- ---------- ---------- ---------- --------- 0
P.vitticeps   mqrlerlcrw rrrgrsrdrg gdgkmkyqky ltvlqmaigv tasnrgslmpl krklwvtps 60
P.bivittatus  ---------- ---------- ---------- ---------- ---------- --------- 0


S.salar       dglsgigatg igtaassaai teaqdsaeaa aedaaaaraa aragamcgsga iggcsvmfa 96
H.comes       dgetspsgvp ---------- -es------- ---------- -sdgp-igatg gagamamaa 65
C.milii       ---------- ---------- ---------- ---------- ---------- --------- 0
B.scammoni    ---------- ---------- ---------- ---------- ---------- --------- 0
H.sapiens     ---------- ---------- ---------- ---------- ---------- --------- 0
A.melanoleuca ---------- ---------- ---------- ---------- ---------- --------- 0
O.divergens   ---------- ---------- ---------- ---------- ---------- --------- 0
M.undulatus   senpn----- ---------- ---------- ---------- ---g------- --------- 42
S.australis   ---------- ---------- ---------- ---------- ---------- --------- 0
P.vitticeps   senps----- ---------- ---------- ---------- ---g------- --------- 66
P.bivittatus  ---------- ---------- ---------- ---------- ---------- --------- 0


S.salar       tstlslpmtq gkpslrrikg rihrsksldsidlldsnsaa meetviweqht vtlhrapgf 156
H.comes       tstlslpmsq gkpslrrikg rihrsksldsidlldsnsaa meetviweqht vtlhraagf 125
C.milii       ---------- ---------- --------ms arsaggkntv meetviweqht vtlhrapgf 32
B.scammoni    ---------- ---------- ---------- ---------- meetaiweqht vtlhrapgf 20
H.sapiens     ---------- ---------- --------ms araaaaksta meetaiweqht vtlhrapgf 32
A.melanoleuca ---------- ---------- ---mrqnqsq wntvedknta meetaiweqht vtlhrapgf 37
O.divergens   ---------- ---------- --------ms araaaaknta meetaiweqht vtlhrapgf 32
M.undulatus   ---atcsvsq gkpslrrikg rihrsksldsidfceftstt meetaiweqht vtlhrapgf 99
S.australis   ---------- ---------- ---------- ---------- meetaiweqht vtlhrapgf 20
P.vitticeps   ---sncsisq gkpslrrikg rihrsksldsldfcefnstt meetaiweqht vtlhrapgf 123
P.bivittatus  ---------- ---------- ---------- ---------- meetaiweqqt vtlhrapgf 20
                                                         *****.****:******* **

S.salar        gfgiaisggr dnphfqsget sivisdvlkg gpaegllsendrvvmvnavsmdnvehayav  216
H.comes        gfgiaisggr dnphfqsget sivisdvlkg gpaegllqendrvvmvnavsmdnvehayav  185
C.milii        gfgiaisggk dnphfqsget sivisdvlkg gpaegllqendrvvmvnavsmdnvehafav  92
B.scammoni     gfgiaisggr dnphfqsget sivisdvlkg gpaegqlqendrvamvngvsmdnvehafav  80
H.sapiens      gfgiaisggR DNPHFQSGET SIVISDVLkg gpaegqlqendrvamvngvsmdnvehafav  92
A.melanoleuca  gfgiaisggr dnphfqsget sivisdvlkg gpaegqlqendrvamvngvsmdnvehafav  97
O.divergens    gfgiaisggr dnphfqsget sivisdvlkg gpaegqlqendrvamvngvsmdnvehafav  92
M.undulatus    gfgiaisggr dnphfqsget sivisdvlkg gpaegllqendrvamvngvsmdnvehafav  159
S.australis    gfgiaisggr dnphfqsget sivisdvlkg gpaegllqendrvamvngvsmdnvehafav  80
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P.vitticeps     gfgiaisggr dnphfqsget sivisdvlkg gpaegllqendrvamvngvsmdnvehafav  183
P.bivittatus    gfgiaisggr dnphfqsget sivisdvlkg gpaegqlqendrvtmvngvsmdnvehafav  80
                ************:************************ *.*****.***.*********:**

S.salar         qqlrksgknakitirrkrrvqipvshpgdre-tmseheeedtdedd-gyeqhsgrsgpts     274
H.comes         qqlrksgknakitirrkrkvqipvsrpgdre-tmseheeedsdeed-gyehhsgragqsa     243
C.milii         qqlrksgknakitirrkrkvqmpvmrpepdrrpisepeddsyedeeelydrksgrsgpsp     152
B.scammoni      qqlrksgknakitirrkkkvqipvsrpdpe--tvseneedsydee--vhdrrsgrgglv-     135
H.sapiens       qqlrksgknakitirrkkkvqipvsrpdpe--pvsdneedsydee--ihdprsgrsgvv-     147
A.melanoleuca   qqlrksgknakitirrkkkvqipvsrpdpe--pvsgneedsydee--vhdprssrgglv-     152
O.divergens     qqlrksgknakitirrkkkvqipvsrpdpe--pvseneedsydee--vhdprssrgglv-     147
M.undulatus     qqlrksgknakitirrmkkiqipvarpepe--pvseneddsydee--irdprssrsgls-     214
S.australis     qqlrksgknakitirrmkkiqipvarpepe--pvseneedsydee--irdprssrggps-     135
P.vitticeps     qqlrksgknakitirrmkkiqipitrtepd--pvsendedsyeed--mhdprgsrgaas-     238
P.bivittatus    qqlrksgknaritirrmkktqipvtrtdpd--pvsendddsyeee--lhdprsshgat--     134
                **********:***** :: *:*: :       :*  :::. :::    : :..:..

S.salar         aygaasggtasrrtndrerersnssrrehsasrersisphsg-rsqgsstpsrpakvtlv     333
H.comes         -yagasggtsssrrhd--rersssgrrdhsasrersvsprsdrrsqassapsrpskvtlv     300
C.milii         ----------------ysgrrsersigrradsrerslsprsdrrsvtsnlppkpikvtlv     196
B.scammoni      -----------------nrrsekswardrsasrerslsprsdrrsvassqparptkvtlv     178
H.sapiens       -----------------nrrsekiwprdrsasrerslsprsdrrsvassqpakptkvtlv     190
A.melanoleuca   -----------------srrsekswardrsasrerslsprsdrrsvassqpakptkvtlv     195
O.divergens     -----------------srrsdkswardrsasrerslsprsdrrslassqpakptkvtlv     190
M.undulatus     ----------------anrrhekswvrdrsasrerslsprsdrrsvtssqpakptkvtlv     258
S.australis     ----------------asrrhekswvrdrsasrerslsprsdrrsvtssqpakptkvtlv     179
P.vitticeps     ----------------lsrrpekswvrdrsasrerslsprsdrrsvgsgqpakptkvtlv     282
P.bivittatus    -----------------srrhekswtrdrsasrersasprsdrrsvassqpakptkvtlv     177
                                  . ..     :: ***** **:*. **  *. * :* *****

S.salar         ksrkneeyglrlashifvkdispeslaardgniqegdvvlkingtvtenlslidakklie     393
H.comes         ksrkneeyglrlashifvkdispeslaardgniqegdvvlkingtvtenlslidakklie     360
C.milii         ksrkneeyglrlashifvkeispeglaakdgnihegdvvlkingtvtenmslgdakklie     256
B.scammoni      ksrkneeyglrlashifvkeisqdslaardgniqegdvvlkingtvtenmsltdaktlie     238
H.sapiens       ksrkneeyglrlashifvkeisqdslaardgniqegdvvlkingtvtenmsltdaktlie     250
A.melanoleuca   ksrkneeyglrlashifvkeisqdslaardgniqegdvvlkingtvtenmsltdaktlie     255
O.divergens     ksrkneeyglrlashifvkeisqdslaardgniqegdvvlkingtvtenmsltdaktlie     250
M.undulatus     ksrkneeyglrlashifvkeisqdslaardgniqegdvvlkingtvtenmsladaktlie     318
S.australis     ksrkneenvlrlashifvkeisqdslaardgniqegdvvlkingtvtenmsladaktlie     239
P.vitticeps     ksrkneeyglrlashifvkeisqdslaardgniqegdvvlkingtvtenmsltdaktlie     342
P.bivittatus    ksrkneeyglrlashifvkeisqdslaakdgniqegdvvlkingtvtenmsltdaktlie     237
                *******  **********:** :.***:****:***************:** ***.***

S.salar         rskgklkmvvqrddratllnipdmddsipsgnnsdrddiseihsltsdhstrshdra-rg     452
H.comes         rskgklkmvvqrddratllnipdlddsipsannsdrddiseihsltsdhsnrsrg-----     415
C.milii         rskgklkmvvqrdekatllnvpdlddsipsanaserddiseihslasdhsnrsnerprh-     315
B.scammoni      rskgklkmvvqrderatllnvpdlsdsihsanaserddiseiqslasdhsgrshdrpprh     298
H.sapiens       rskgklkmvvqrderatllnvpdlsdsihsanaserddiseiqslasdhsgrshdrpprr     310
A.melanoleuca   rskgklkmvvqrderatllnvpdlsdsihsanaserddiseiqslasdhsgrshdrpprh     315
O.divergens     rskgklkmvvqrderatllnvpdlsdsihsanaserddiseiqslasdhsgrshdrpprh     310
M.undulatus     rskgklkmvvqrderatllnvpdlsdsihsanaserddiseiqslasdhsnrshdrp-rr     377
S.australis     rskgklkmvvqrderatllnvpdlsdsihsanaserddiseiqslasdhsnrshdrp-rr     298
P.vitticeps     rskgklkmvvqrderatllnvpdlsdsihsanaserddiseiqslasdhsnrshdrp-rr     401
P.bivittatus    rskgklkmvvqrderatllnvpdlsdsihsanaserddiseiqslasdhsnrshdkp-rr     296
                **************::*****:**:.*** *.* *:*******:**:**** **.

S.salar         srsrspt-rsepsdpsrhsprqisngshrsrdee---riskpgvmstpvkgsqealiqai     508
H.comes         -rgrspd-rnepsdhlrlsprqitngshrsrdde---r-skpgvmstpvkssddgvlsqg     469
C.milii         -rsrspdkrsdws---dhspqpisngsqrphedditvqgtkpgamstpvksteeafvak-     370
B. scammoni     srsrspdqrsepsdhsrqspqqpsngslrsreee---riskpgavstpvkhaddh-tpkt     354
H.sapiens       srsrspdqrsepsdhsrhspqqpsngslrsrdee---riskpgavstpvkhaddh-tpkt     366
A.melanoleuca   srsrspdqrsepsdhsrhspqqpssgslrsreee---riskpgavstplkhaddh-tpkt     371
O.divergens     srsrspdqrsepsdhsrhspqqpssgslrsreee---riskpgavstpvkhvddh-tpkt     366
M.undulatus     srsrspdqrsepsdhsrhspqqpsngslrskede---ritkpgavstpvknaddi--skt     432
S.australis     srsrspdqrsepsdhsrhspqqpsngslrsrede---ritkpgavstpvknadda--skt     353
P.vitticeps     srsrspdqrsepsdhsrhspqqpsngshrsrdee---riakpgavstpvknteelpvsks     458
P.bivittatus    srsrspdqrsepsdhtrhspqqpnngshwnrdde---rivkpgavstpvksteeaslpkv     353
                 *.***  *.: *     **:  ..**   ::::   :  ***.:***:*  ::
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S.salar         s-dqplare-dklpplpepkpvyaqpgqpdvdlpvspsdapvpsaghddsilrpsmklvk     566
H.comes         s-dqsssrddkqlpplpepkpvyaqpgqpdvdlpvspsdapvpsaahddsilrpsmklvk     528
C.milii         ---vaeervekqapplpepkpvyaqpgqpdvdlpvspsdapvpnashddgmlrptmklvk     427
B.scammoni      veevivernekqtpnlpepkpvyaqvgqpdvdlpvspsdgilpnathedgilrpsmklvk     414
H.sapiens       veevtvernekqtpslpepkpvyaqvgqpdvdlpvspsdgvlpnsthedgilrpsmklvk     426
A.melanoleuca   veevvvernekqaptlpepkpvyaqggqpdvdlpvspsdgvlpnsthedgilrpsmklvk     431
O.divergens     veevvvernekqaptlpepkpvyaqvgqpdvdlpvspsdgvlpnsthedgilrpsmklvk     426
M.undulatus     meevaveraekqtpplpepkpvyaqggqpdvdlpvspsdgplpnsthedgmlrpsmklvk     492
S.australis     vedvaaertekqtpplpepkpvyaqggqpdvdlpvspsdgplpnsthedgmlrpsmklvk     413
P.vitticeps     veesvtersdkqipplpepkpvyaqagqpdvdlpvspsdgplpnsthedgmlrpsmklvk     518
P.bivittatus    veesvaerndkqtpplpepkpvyaqagqpdvdlpvsptdgplpnsthedgmlrpsmklvk     413
                       *  .: * ********** ***********:*. :*.: *:*.:***:*****

S.salar         fkkgesvglrlaggndvgifvagvledspaakegleegdqilrvnnvdfaniireeavlf     626
H.comes         fkkgesvglrlaggndvgifvagvleespaakegleegdqilrvnnvdfaniireeavlf     588
C.milii         frkgesvglrlaggndvgifvagvledspaakegleegdqilrvnsvdfaniireeavlf     487
B. scammoni     frkgdsvglrlaggndvgifvagvledspaakegleegdqilrvnnvdftniireeavlf     474
H.sapiens       frkgdsvglrlaggndvgifvagvledspaakegleegdqilrvnnvdftniireeavlf     486
A.melanoleuca   frkgdsvglrlaggndvgifvagvledspaakegleegdqilrvnnvdftniireeavlf     491
O.divergens     frkgdsvglrlaggndvgifvagvledspaakegleegdqilrvnnvdftniireeavlf     486
M.undulatus     frkgdsvglrlaggndvgifvagvledspaakegleegdqilrvnnvdftniireeavlf     552
S.australis     fkkgdsvglrlaggndvgifvagvledspaakegleegdqilrvnnvdftniireeavlf     473
P.vitticeps     fkkgdsvglrlaggndvgifvagvledspaakegleegdqilrvnnvdftniireeavlf     578
P.bivittatus    fkkgdsvglrlaggndvgifvagvledspaakegleegdqilrvnnvdftniireeavlf     473
                *:**:**********************:*****************.***:***********

S.salar         lldlprgeevtilaqkkkdvyrrivesdvgdsfyirthfeyekespyglsfnkgevfrvv     686
H.comes         lldlprgeevtilaqkkkdvyrrivesdvgdsfyirthfeyekespyglsfnkgevfrvv     648
C.milii         lldlpkgeevtilaqqkkdvyrrivesdvgdsfyirthfdyekespyglsfnrgevfrvv     547
B. scammoni     lldlpkgeevtilaqkkkdvyrrivesdvgdsfyirthfeyekespyglsfnkgevfrvv     534
H.sapiens       lldlpkgeevtilaqkkkdvyrrivesdvgdsfyirthfeyekespyglsfnkgevfrvv     546
A.melanoleuca   lldlpkgeevtilaqkkkdvyrrivesdvgdsfyirthfeyekespyglsfnkgevfrvv     551
O.divergens     lldlpkgeevtilaqkkkdvyrrivesdvgdsfyirthfeyekespyglsfnkgevfrvv     546
M.undulatus     lldlpkgeevtilaqkkkdvyrrivesdvgdsfyirthfeyekespyglsfnkgevfrvv     612
S.australis     lldlpkgeevtilaqkkkdvyrrivesdvgdsfyirthfeyekespyglsfnkgevfrvv     533
P.vitticeps     lldlpkgeevtilaqkkkdvyrrivesdvgdsfyirthfeyekespyglsfnkgevfrvv     638
P.bivittatus    lldlpkgeevtilaqkkkdvyrrivesdvgdsfyirthfeyekespyglsfnkgevfrvv     533
                *****:*********:************************:************:*******

S.salar         dtlyngklgswlairigknhqevergiipnknraeqlssvqytlpktpggdradfwrfrg     746
H.comes         dtlyngklgswlairigknhqevergiipnknraeqlssvqytlpktpggdradfwrfrg     708
C.milii         dtlyngklgswlairigknhkevergiipnrnraeqlssvqytlpktaggdradfwrfrg     607
B. scammoni     dtlyngklgswlairigknhkevergiipnknraeqlasvqytlpktaggdradfwrfrg     594
H.sapiens       dtlyngklgswlairigknhkevergiipnknraeqlasvqytlpKTAGGDRADFWRfrg     606
A.melanoleuca   dtlyngklgswlairigknhkevergiipnknraeqlasvqytlpktaggdradfwrfrg     611
O.divergens     dtlyngklgswlairigknhkevergiipnknraeqlasvqytlpktaggdradfwrfrg     606
M.undulatus     dtlyngklgswlairigknhkevergiipnknraeqlasvqytlpktaggdradfwrfrg     672
S.australis     dtlyngklgswlairigknhkevergiipnknraeqlasvqytlpktaggdradfwrfrg     593
P.vitticeps     dtlyngklgswlairigknhkevergiipnknraeqlasvqytlpktaggdradfwrfrg     698
P.bivittatus    dtlyngklgswlairigknhkevergiipnknraeqlasvqytlpktaggdradfwrfrg     593
                ********************:*********:******:********* ************


S.salar         lrtskrnlrksredlsaqpvqtkfpayervvlreagflrpvvifgsiadvareklareep     806
H.comes         lrsskrnlrksredlsaqpvqtkfpayervvlreagflrpvvifgpiadvareklareep     768
C.milii         lrsskrnlrksredlsaqpvhtkfpayervvlreagflrpvvifgpiadvareklareep     667
B. scammoni     lrsskrnlrksredlsaqpvqtkfpayervvlreagflrpvtifgpiadvareklareep     654
H.sapiens       lrsskrnlrksredlsaqpvqtkfpayervvlreagflrpvtifgpiadvareklareep     666
A.melanoleuca   lrsskrnlrksredlsaqpvqtkfpayervvlreagflrpvtifgpiadvareklareep     671
O.divergens     lrsskrnlrksredlsaqpvqtkfpayervvlreagflrpvtifgpiadvareklareep     666
M.undulatus     lrsskrnlrksredlsaqpvqtkfpayervvlreagflrpvtifgpiadvareklareep     732
S.australis     lrsskrnlrksredlsaqpvqtkfpayervvlreagflrpvtifgpiadvareklareep     653
P.vitticeps     lrsskrnlrksredlsaqpvqtkfpayervvlreagflrpvtifgpiadvareklareep     758
P.bivittatus    lrsskrnlrksredlsaqpvqtkfpayervvlreagflrpvtifgpiadvareklareep     653
                **:*****************:********************.*** **************

S.salar         dlfelaksepkdagkdqrssgiirlhtikqiidrdrhavlditpnavdrlnyaqwypivv     866
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H.comes         difelakseprdagtdqkssgiirlhtikqiidrdkhavlditpnavdrlnyaqwypivv     828
C.milii         dqyevarseprdagtdqrssgiirlhtikqvidrdkhavlditpnavdrlnyaqwypivv     727
B.scammoni      diyqiakseprdagtdqrssgiirlhtikqiidqdkhalldvtpnavdrlnyaqwypivv     714
H.sapiens       diyqiakseprdagtdqrssgiirlhtikqiidqdkhalldvtpnavdrlnyaqwypivv     726
A.melanoleuca   diyqiakseprdagtdqrssgiirlhtikqiidqdkhalldvtpnavdrlnyaqwypivv     731
O.divergens     diyqiakseprdagtdqrssgiirlhtikqiidqdkhalldvtpnavdrlnyaqwypivv     726
M.undulatus     difqiakseprdagtdqrssgiirlhtikqiidrdkhalldvtpnavdrlnyaqwypivv     792
S.australis     difqiakseprdagtdqrssgiirlhtikqiidrdkhalldvtpnavdrlnyaqwypivv     713
P.vitticeps     diyqiakseprdagtdqrssgiirlhtikqiidkdkhalldvtpnavdrlnyaqwypivv     818
P.bivittatus    diyqiakseprdagtdqrssgiirlhtikqiidrdkhalldvtpnavdrlnyaqwypivv     713
                * :::*:***:***.**:************:**:*:**:**:******************

S.salar         flnpdskqgvktmrtrlcpesrksarklyeralklrknnhhlftttinmnnmndgwygal     926
H.comes         flnpdtkqgvknmrtrlcpesrksarklyeralklrknnhhlftttinlnnmndgwfgal     888
C.milii         flnpdtkqgvktmrqrlcpesrksarklferamklrknnhhlftstinlnsmnegwyggl     787
B.scammoni      flnpdskqgvktmrmrlcpesrksarklyershklrknnhhlftttinlnsmndgwygal     774
H.sapiens       flnpdskqgvktmrmrlcpesrksarklyershklrknnhhlftttinlnsmndgwygal     786
A.melanoleuca   flnpdskqgvktmrmrlcpesrksarklyershklrknnhhlftttinlnsmndgwygal     791
O.divergens     flnpdskqgvktmrmrlcpesrksarklyershklrknnhhlftttinlnsmndgwygal     786
M.undulatus     flnpdskqgvktmrmrlcpesrksarklyerahklrknnhhlftttinlnsmnegwygal     852
S.australis     flnpdskqgvktmrmrlcpesrksarklyerahklrknnhhlftttinlnsmnegwygal     773
P.vitticeps     flnpdskqgvktmrmrlcpesrksarklyerahklrknnhhlftatinlnsmndgwygal     878
P.bivitattus    flnpdskqgvktmrtrlcpesrksarklyerahklrknnhhlftatinlnsmndgwygal     773
                *****:*****.** **************:**: ***********:***:*.**:**:*.*

S.salar         ketiqqqqnqlvwvsegkadgapdddldlhddrlsylsapgseysmystdsrhtsdyedt     986
H.comes         keiiqqqqnqlvwvsegkadgaaeddldihddrlsylsapgseysmystdsrhtsdyddt     948
C.milii         kesvqqqqnqlvwvsegkadgtaeddldlhddrlsylsapgseysmystdsrhtsdyddt     847
B.scammoni      keaiqqqqnqlvwvsegkadgatsddldlhddrlsylsapgseysmystdsrhtsdyedt     834
H.sapiens       keaiqqqqnqlvwvsegkadgatsddldlhddrlsylsapgseysmystdsrhtsdyedt     846
A.melanoleuca   keaiqqqqnqlvwvsegkadgatsddldlhddrlsylsapgseysmystdsrhtsdyedt     851
O.divergens     keaiqqqqnqlvwvsegkadgatsddldlhddrlsylsapgseysmystdsrhtsdyedt     846
M.undulatus     keaiqqqqnqlvwvsegkadgatsedldlhddrlsylsapgseysmystdsrhtsdyedt     912
S.australis     keaiqqqqnqlvwvsegkadgatsddldlhddrlsylsapgseysmystdsrhtsdyedt     833
P.vitticeps     keaiqqqqnqlvwvsegkadgatsddldlhddrlsylsapgseysmystdsrhtsdyedt     938
P.bivitattus    keaiqqqqnqlvwvsegkadgttsddldlhddrlsylsapgseysmystdsrhtsdyedt     833
                ** :******************: .:***:******************************:**

S.salar         dteggaytdqeldetlndevglptepaitrssepvredppviqe---plryggyqhtvqp     1043
H.comes         dteggaytdqeldetlnddvgpptgpaitrssepvredppviqe---ppgyagyqhalqs     1005
C.milii         dteggaytdqeldetlnddig-ptesaitrssepvledsslihpeplpqtyptypv----     902
B.scammoni      dteggaytdqeldetlndevgtppesaitrssepvredssgmhhdh--qtyspyspqaqp     892
H.sapiens       dteggaytdqeldetlndevgtppesaitrssepvredssgmhhen--qtyppyspqaqp     904
A.melanoleuca   dteggaytdqeldetlndevgtppesaitrssepvredssgmhhen--qtyppyspqaqp     909
O.divergens     dteggaytdqeldetlndevgtppesaitrssepvredssgmhhen--qtyppyspqaqp     904
M.undulatus     dteggaytdqeldetlndevgtppesaitrssepvredssgmhhdt--qtystyasqaqp     970
S.australis     dteggaytdqeldetlndevgtppesaitrssepvredssgmhhet--qtyppyvsqaqp     891
P.vitticeps     dteggaytdqeldetlndevgtppesaitrssepvredssgvhhen--qtyppyaaqaqp     996
P.bivitattus    dteggaytdqeldetlndevgtppesaitrssepvredssgvhqen--qayppygsqgqp     891
                *******************::* *   ********* **   ::       *  *

S.salar         dplnridpagfkapvaqqnekaeavpatmpilpqqpeqpvllaeteaaptavnntvngva     1103
H.comes         dplnridpagfkapapqqkaeataipsnp-------qlpeplaetv------tpavktvg     1052
C.milii         -qparidspiykppvnqptadilp--ai----ptspesnpvvav----pstvnsrvd---     948
B.scammoni      qpvhridspglkta-sqqkaeass--pg-pylspetn---pass----tsavnpnvn---     938
H.sapiens       qpihridspgfkpa-sqqkaeass--pv-pylspetn---pass----tsavnhnvn---     950
A.melanoleuca   qpihridspgfkta-sqqkaeass--pv-pylspetn---sass----tsavnhnvt---     955
O.divergens     qpvhridspgfkta-sqqkaeass--pv-pylspetn---pass----tsavnhnvp---     950
M.undulatus     qpnlrvdssgfktt-tqpkaeasp--av-pylspspesnpatss----asavnanvn---     1019
S.australis     qpnlridssgfktaasqakaeasp--av-pylspspesnpatss----tstvnpnvn---     941
P.vitticeps     qpnhriespgfkpsaaqpkadasp--aa-pylspspetnpaass----tsavhpsvs---     1046
P.bivitattus    qpnhriespgfksstaqpkadasp-----pylspspdtapptss----tsaanpcvs---     939
                    *::    *    * . .               :     :            *

S.salar         glshglslgpiaapqskpnpgpgpeadrlrmptpdlaqp----lapaptlepeplqsgpp     1159
H.comes         gpspgeapg-----ahhrlpspnpeagslkrptpelape---------svtsetrqsgla     1098
C.milii         -ltnvrleei----appshnisepqpdpysnqpspeprrvihsvsperlrdtep-aaahh     1002
B.scammoni      -ltnvrleep----tpapsasdspqadslraprteaahi--------mlrgqep-slssh     984
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H.sapiens       -ltnvrleep----tpapstsyspqadslrtpsteaahi--------mlrdqep-slssh     996
A.melanoleuca   -ltnvrlegp----tpapstsyspqadslrtpsteaahi--------mlrdqep-slpsh     1001
O.divergens     -ltnvrlegp----tpvpstsyspqadslrtpsteaahi--------mlrdqep-slpsh     996
M.undulatus     -ltnvrleep----taapynsyqpqagplrtsstegahi--------vlreqessslsph     1066
S.australis     -ltnvrleep----tpapynsyqpqagplrtsstegahi--------vlrdqepsslsph     988
P.vitticeps     -lnsirleep----glapynsyppqagplrtpsaeaphl--------mlrdqeppslsph     1093
P.bivitattus    -ltnvrleep----gaapynsyqpqvgpsrmpgpeaphv--------llrdqelaslpph     986
                             .        *: .                          *

S.salar         ssepqmykkdpyleepvrvnhggvkps--------pamgppmtyesqppyqdqhpyrdyd     1211
H.comes         gseskmfqkdpyspdstgrpgpglkp--------------gnynpqqgyhpdqqpyrdyd     1144
C.milii         aepskmykkdsynd-evarqnydlkqpvgnlasrldne--rqsyerqqhrdyerqqhfee     1059
B.scammoni      vdpakvyrqdpype-evmrqnhvlkqpavahpgqrldkepslsyepqppyaerqasrdle     1043
H.sapiens       vdptkvyrkdpype-emmrqnhvlkqpavshpghrpdkepnltyepqlpyvekqasrdle     1055
A.melanoleuca   vdpakvyrkdpype-emmrqnhvlkqpavghpgqrpdkepnlsyesqppyvekqanrdle     1060
O.divergens     vdpakvyrkdpype-emmrqnhvlkqppaghpgqrpdkdpnlsyesqppyvekqacrdle     1055
M.undulatus     idpakiyrkdpyvneeasrqsyvlkqptinhpvqrqerdpnliyesqaqyaekqpsrdye     1126
S.australis     idpakvyrkdpyvteeasrqsyilkqpainhpvqrqerdpnliyesqaqyaekqpsreye     1048
P.vitticeps     idpakmyrkdpyineeppranyvlkq-----paqrqekdsnltyepqpqytekqanrdye     1148
P.bivitattus    idstkmyrkdpfvneepprqnyslkq-----pvqrqekdpnltyelqgqypekpahrdye     1041
                 .  :::::* :           :*                     *     .   :  :

S.salar         hppnryd---gggylepkyrnfdsqlhheknvphyd-dqwppynqqtsgppphq-qqhpp     1266
H.comes         hppsrfdvsgggyvepkyrnydsppf-ensvphydqqqwnpygqttst---------an     1194
C.milii         qplyryes--aakyeeqppqrygagsqyeetpppyn-dkwiqynekpphtyh----hehp     1112
B.scammoni      qpayryds--s-nyadqfprnydhrlryddripaye-eqwsyyddkqpyq-prpsfdnqh     1098
H.sapiens       qptyryes--s-sytdqfsrnyehrlryedrvpmye-eqwsyyddkqpyp-srppfdnqh     1110
A.melanoleuca   qptyryds--s-sytdqfsrnydhrlryeeriptye-eqwsyyddkqpyq-prtpfdnqh     1115
O.divergens     qptyryds--s-sytdqrsrnydhrlcyeervptye-eqwsyyddkqpyq-prpsfdnqh     1110
M.undulatus     qssyryds--a-nyvdqfprgydprlhydervppye-ehwayydekppynqtrtayenqp     1182
S.australis     qsayryds--t-nyvdqfsrgydprlhyddrvppye-ehwayydekqsyq-prtaydsqp     1103
P.vitticeps     playryds--t-nyvdhysrnydprlhyqedvppyd-dhwayydekppyq-prppydgqp     1203
P.bivitattus    playryds--s-syadpfphgydprlpyeervppye-dhwmyydekqpyq-prppyesqp     1096
                    *::      * :   : :      :.  * *: ::*  *.:

S.salar         gpgydprlsyedg-perdysppqlrydet--plgyngr-prygkpagp---gpirydepp     1319
H.comes         sqgydqrlpysdg-ldpqytpp-lrfdepppqqgfdgr-prygkptvp---gparyddpp     1248
C.milii         prdhepkyldd-----ypdhsyqpryeets-htgyenraprfdkplqdykltqphysdqa     1166
B.scammoni      srdldsrqhpeesse-rgyf---prfeept-plsyesr-prydpplrt---stlrheeqp     1149
H.sapiens       sqdldsrqhpeesse-rgyf---prfeepa-plsydsr-pryeqapra---salrheeqp     1161
A.melanoleuca   prdldsrqhpeesse-rgyy---prfeepa-plsydsr-prydqpprt---stlrheeqp     1166
O.divergens     prdldsrqhpeesse-rgyy---prfeepa-tlsydsr-prydqpprt---stlrheeqp     1161
M.undulatus     prdldsrqntdeste-rsyypaqprfeepp-tmsydgr-pryehapknfslpqvryedqh     1239
S.australis     prdmdsrqnteeste-rsyypaqprfeepp-pmsydgr-pryehapknfslpqvryedqh     1160
P.vitticeps     prdfdprpnaeeste-rgyfpaqprfeepp-pvaydnr-pryehgpknfnltqlryeeqn     1260
P.bivitattus    prdfdprpsaeesaarssyfpaqprfeesp-pvgydsr-pryepapkafglsqlryeeqp     1154
                  . : :   .             *::*     .::.* **:            ::.:

S.salar         psvpggyaplrydqeph--pyppatrspeppkqyyqgepaqrpgpaynqappq-hrgykp     1376
H.comes         plspdl----hynqdsqltshspaarspsgq-----------rptynqgqtqhvkgykp     1292
C.milii         lhgyd--prphyeyeaq--afhpptsrspe-shyydsqg-----rtydqgppqghns-lp     1215
B.scammoni      apgfd--vhgryrpdaq--pysstgpkasepkqyfdqyp-----rsyeqvppqsfts-ka     1199
H.sapiens       apgyd--thgrlrpeaq--phpsagpkpaeskqyfeqys-----rsyeqvppqgfts-ra     1211
A.melanoleuca   apgyd--mhnryrpeaq--sys-vgpkatepkqyfdqyp-----rsyeqvppqgfts-ka     1215
O.divergens     tpgyd--vhnryraeaq--syssagpkasepkqyfdqyp-----rsyeqvpsqgfts-ka     1211
M.undulatus     tvgyd--thgrykpeaq--pyqsaisrspepkqyfdphv-----raydqgppqaysa-ka     1289
S.australis     tsgyd--thgrykpeaq--pypsavsrspeskqyfdphm-----rgyeqgppqaysa-ka     1210
P.vitticeps     pagye--mhgryktesq--afssavprspepkhyfesqp-----rgyepippqgfga-ka     1310
P.bivitattus    ppgye--vhgryksepq--ayssavprspepkhyfeaqp-----rgheqgpppgcsa-ka     1204
                          :   : :   .                          ::        .

S.salar         ppqqyepimsfdapvpapkpqpealrpspgdtvittapnplpppp-------------rv     1423
H.comes         qqydsapvnsdnsltsppksevttfspl--------dtsklntar-------------ke     1331
C.milii         --rhyeqsfsteasfpl-ppppqa----kpvepfhpvskvlpppppvhmhmeleegeeee     1268
B.scammoni      gpghyeplhgaalv-pp-----qh----kpevppa-stkplppppa------------la     1236
H.sapiens       --ghfeplhgaaav-pplipssqh----kpealps-ntkplppppt------------qt     1251
A.melanoleuca   --ghyeplhgaavv-pplipasqh----kpevlps-ntkplppppt------------lt     1255
O.divergens     --ghyeplhgaavv-pplipasqh----kpevlps-ntkplppppt------------lt     1251
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```
M.undulatus     --gqyepshstsgvslppppssqt----kpevlps-nskplptpps------------la     1330
S.australis     --gqfepshstsgislppppssqt----kpevlps-nskplptpps------------la     1251
P.vitticeps     --gqydpphnpsgvlps--lpsqs----kpeglpstsnkplptqp--------------a     1348
P.bivitattus    --gpyepshsaagphpa--lpsqs----kpevt-tsnskplptpp--------------a     1241
                       .                              . *

S.salar         eleddpairaqsvlsrvkmfenkrsvsvdrakeagdtvgl-rsadlptkpg---acipka     1479
H.comes         lheddpamqpqsvltrvkmfenkrsvsvdrardtadpsgn-kaadlplkag---giipka     1387
C.milii         eeeedpamkpqsvltrvklfeskkstlsk-nkeptegss-mrppdvapkpvlvpvtaskp     1326
B.scammoni      eeeedpamkpqsvltrvkmfenkrsasvenkkdenhnsgf-kppevtskpagapvlgpka     1295
H.sapiens       eeeedpamkpqsvltrvkmfenkrsasletkkdvndtgsf-kppevaskpsgapiigpkp     1310
A.melanoleuca   eeeedpamkpqsvltrvkmfenkrsasleskkdenhtaaf-kppevaskpsgapiigpka     1314
O.divergens     eeeedpamkpqsvltrvkmfenkrsasleskkdenhtagf-kppevaskppgapiigpka     1310
M.undulatus     eedddpamkpqsvrsrvkiferrrspslekmkdpsdtsav-kppelapkptlatasgpks     1389
S.australis     eeeedpamkpqsvrsrvkiferrrspslekmkdpndtsav-kppelapkptlatvsgpkp     1310
P.vitticeps     eeeddpamkpqsvltrvkmfenkrssslekikdsnelpvv-kppelapkpslnpvsgpkp     1407
P.bivitattus    eeeddpamkpqsvltrvkmfenkrssslekikesndlsavkhppelapkpnvapvtgpka     1301
                  ::***:: *** :***:** ::*   .  ::  .     :  ::  *         *

S.salar         ns---lsnldqeksfrvpepqkprevsdddivrsnhynpdedeeyyrkqlsyfdrrslda     1536
H.comes         ns---lsnldsdksfrvpepqkpqskaaddivrsnhydpdededyyrkqlsyfdrlqtgp     1444
C.milii         aiqplleqekpphlykgpepqkpqvklpqemprvnhydpdedeedyrkqlsyfgrrsydn     1386
B.scammoni      paqnqli-ehdrtlhripepqkpqmkppedivrsnhydpeedeeyyrkqlsyfdrrsfes     1354
H.sapiens       tsqnqfs-ehdktlyripepqkpqlkppedivrsnhydpeedeeyyrkqlsyfdrrsfen     1369
A.melanoleuca   tpqnqfs-ehdktlyripepqkpqmkppedivrsnhydpeedeeyyrkqlsyfdrrsfen     1373
O.divergens     tpqnqfs-ehdktlyripepqkpqmkppedivrsnhydpeedeeyyrkqlsyfdrrsfdn     1369
M.undulatus     tsqshy--ehdkttyrapepqrpqvkppedivrsnhydpeedeeyyrkqlsyfdrrsfen     1447
S.australis     tsqsqy--ehdkatyrapepqrpqakppedivrsnhydpeedeeyyrkqlsyfdrrsfen     1368
P.vitticeps     vsqnqy--ehdkpayrapepqrpqakppedivranhydpeedeeyyrkqlsyfdrrsfen     1465
P.bivitattus    ssqnqc--ereksayrapepqrpqakppedivranhydpeedeeyyrkqlsyfdrrnydn     1359
                             .: ****:*:    ::: * ***:*:***: ********.* .

S.salar         vkappqttpviatkpaaqpqahpgynypr-------------------------aesvek     1571
H.comes         akp--------------qaqaqtmhtfsr-------------------------tesvek     1465
C.milii         kppa----a-sfsepakpa----qpnytgyvpr-----------------stpaefdhvk     1420
B.scammoni      kpsahvpas-hlsepakpq-----pqfssysskflgsytssdylersamwgksaeaeavd     1408
H.sapiens       kppahiaas-hlsepakpahsqnqsnfssyss-----------------kgkppeadgvd     1411
A.melanoleuca   kpsthipag-hlsepakpvhsqnqpnfssyss-----------------kgkspeadgmd     1415
O.divergens     kpsthipag-hlsepakplhsqnqpnfssyss-----------------kgkspeadgmd     1411
M.undulatus     kpsaqvpas-hhseptkpihsqnqlnftnys-kflgsytsydylkrnikwgkttdseste     1505
S.australis     kpsaqvpas-hhseptkpmhsqnqlnftnys-kllgsytsydylkrnikwgkttdaesmd     1426
P.vitticeps     kpampvpss-hhaepakpvhlhnqlnysnysskflgsytsygylkrnikwgksidmetld     1524
P.bivitattus    kpatqapgs-hhpeqgkavplhnqlnytnyssk-----------------gksadmepld     1401
                                             :                            .  .

S.salar         vspverryepvpqvtpaappai--l-----------------------------------     1594
H.comes         pnaaekryepvpqltpslppat--l-----------------------------------     1488
C.milii         ptivdkrydpspqlsap----qsrygppsqpslnlpvpplvhpkpsplevssvprelpsa     1476
B.scammoni      rpfgekrydplqatpppppplp-aqysqaaqpgpstalslhshtkgtlgegnsmsldfqss     1467
H.sapiens       rsfgekryepiqatpppppplp-sqyaqpsqpvtsas--lhihskgahgegnsvsldfqns     1468
A.melanoleuca   rafgekryepvqatpppppplp-sqyaqpaqsstsssslalhthakgahgegnsvsldfqns     1474
O.divergens     rsfgekryepvqatpppppplp-sqyaqpaqpstsssslalhmhakgahgegssvsldfqns     1470
M.undulatus     rsvgekryepvpqvtspspasavqytqp-qsinspvlslpahhkpalsevnsv-------     1557
S.australis     rpvgekryepipqvttpppassvqytqp-qsinspvlslpahhkpalsevnsv-------     1478
P.vitticeps     ravgekryetapqvipppppappvqytqp-qsinnpivslqpks--tltevnsv-sdfqns     1580
P.bivitattus    rglgdkrydpppqatp--pvpsvqytqp-qsinnpivslqpks--vla------edfqns     1450
                    ::**:

S.salar         --pkpt-------spedphdspkadtvttnylpqksyp--ekspvngtamkd-----qpk     1638
H.comes         --pkps-------teaqpp--sredtvhsnflphksfp--ekspmngtseklpktvtsgg     1535
C.milii         --shpkseppqskpqafrpntrdeivhpnfypqksfpvperapvngmeqsh-k------     1527
B.scammoni      lvskpdpp-psqnkpaayrpanredaaqsafypqksfp--dkapangaeqtq-k------     1517
H.sapiens       lvskpdpp-psqnkpatfrppnredtaqaafypqksfp--dkapvngteqtq-k------     1518
A.melanoleuca   lvskpdpp-psqnkpatfrppnredtvqstfypqksfp--dkapangaeqtq-k------     1524
O.divergens     lvskpdpp-psqnkpatfrppnredtvqstfypqksfp--dkapvngaeqtq-k------     1520
M.undulatus     ----sdps-ppqnkpavfrs-sredtvqstfypqksfp--dkgpingteqiq-k------     1602
S.australis     ----sdpppppqnkpaifrs-sredtvqstfypqksfp--dkgpingteqiq-k------     1524
P.vitticeps     tlpkpeps-lpqnkplafrsssredtvqstfypqksfp--dkgpvngteqip-k------     1630
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P.bivitattus    tlskpeppppqnkpaafrsssredtvqstyypqksfp--dkgpingteplp-k------     1501
                             .        : : .   : *:**:*  ::.* **

S.salar         apantsynryv--pkpytnsakpferkfdspkfnhnllpnnktdlap--aikapanstap     1694
H.comes         appassynryv--pkpfttsarpferkfespkfnhnllpndkpdmat--kgq----sasp     1587
C.milii         -q-pavynrfa--pkpytsaarpferkfespkfnhnllptegqpp---------------     1568
B.scammoni      -titpaynrft--pkpytssarpferkfespkfshnlltsetahkpdlss-kaptspktl     1573
H.sapiens       -tvtpaynrft--pkpytssarpferkfespkfnhnllpsetahkpdlss-ktptspktl     1574
A.melanoleuca   -tvtpaynrft--pkpytssarpferkfespkfnhnllpsetahkpdlss-kapaspktl     1580
O.divergens     -tvtpaynrft--pkpytssarpferkfespkfnhnllpsetaqkpdlss-kapaspqtl     1576
M.undulatus     -tvtpsynrft--tkpytsaarpferkfespkfnhnllpneaqhkpelps-kspnspqpi     1658
S.australis     -tvtpsynrft--tkpytsaarpferkfespkfnhnllpneaqhkpelps-kspnspqpi     1580
P.vitticeps     -tvtpaynrft--tkpytsaarpferkfespkfnhnllpdenqhkpelps-ksqnspqpl     1686
P.bivitattus    -t-tpaynrfttttkpytsaarpferkfespkfnhnllpeetlqkpeissskpqpspqpi     1559
                      ***:.   **:*.:*:******:****.****  :

S.salar         akpqispqptdldsgldtftrtmdnrskyqhnninavpkaipvspsaledd-dedeghtv     1753
H.comes         vkps-qsqttdhdsgldtftrtmdhrskyqhnninavpkaipvsptaldddededeghtv     1646
C.milii         -tphspsqppdfdsgtdtfprqtdhrakylanninaipkaipvspsaledd-edddshtv     1626
B.scammoni      vkahgstqppefdsgvetfsvhtdnkpkyqvnsistgpkavpvspsaveed-ededghtv     1632
H.sapiens       vkshslaqppefdsgvetfsihae-kpkyqinnistvpkaipvspsaveed-ededghtv     1632
A.melanoleuca   akahsatqppefdsgvetfsihad-kpkyqmnnistvpkaipvspsaveed-ededghtv     1638
O.divergens     akahgaaqppefdsgvetfslhad-kpkyqmnnistvpkavpvspsaveed-ededghtv     1634
M.undulatus     lkahsssqppefdsgldtfavqvd-kpkyqpnnvnavpkaipvspsalede-eeedghtv     1716
S.australis     lkahsssqppefdsgmdtftvqad-kpkyqpnninavpkaipvspsalede-eeedghtv     1638
P.vitticeps     lkahsssq-pefdsavdtfsiqad-klkyqpnninavpkaipvspsaledd-deedghtv     1743
P.bivitattus    lkahssaq-pefesnldsfsgqad-klkyqpnnisavpkaipvspsaledd-deeeghtv     1616
                 .     *  : :*  ::*    : : **  *.:.: ***:****:*:::: ::::.***

S.salar         vatargvfncnggvlssietgvsiiipqgaipegveqeiyfkvcrdnsilppldkekget     1813
H.comes         vatargvfnsnggvlssietgvsiiipqgaipegieqeiyfkvcrdnsilppldkekget     1706
C.milii         vatargifdsnggvlssvetgvsiiipkgaipegveqeiyfkvcrdnsilppldkekget     1686
B.scammoni      vatargvfnsnggvlssietgvsiiipqgaipegveqeiyfkvcrdnsilppldkekget     1692
H.sapiens       vatargifnsnggvlssietgvsiiipqgaipegveqeiyfkvcrdnsilppldkekget     1692
A.melanoleuca   vatargvfnsnggvlssietgvsiiipqgaipegieqeiyfkvcrdnsilppldkekget     1698
O.divergens     vatargvfnsnggvlssietgvsiiipqgaipegieqeiyfkvcrdnsilppldkekget     1694
M.undulatus     vatargvfnsnggvlssietgvsiiipqgaipegieqeiyfkvcrdnsilppldkekget     1776
S.australis     vatargvfnsnggvlssietgvsiiipqgaipegieqeiyfkvcrdnsilppldkekget     1698
P.vitticeps     vatargvfnsnggvlssietgvsiiipqgaipegieqeiyfkvcrdnsilppldkekget     1803
P.bivitattus    iatargvfnsnggvlssietgvsiiipqgaipegieqeiyfkvcrdnsilppldkekget     1676
                :*****:*:.*******:*********:******:*************************

S.salar         llsplvmcgphglkflkpvelrlphcasmtpdgwsfalkssdsssgdpkswqnqslpgdp     1873
H.comes         llsplvmcgphglkflkpvelrlphc--------------------dpknwqnkslpgdp     1746
C.milii         llsplvmcgphglkflkpvelrlphcasmtpdg-------------dpkswqnkslpsds     1733
B.scammoni      llsplvmcgphglkflkpvelrlphcasmtpdg-------------dpktwqnkclpgdp     1739
H.sapiens       llsplvmcgphglkflkpvelrlphc--------------------dpktwqnkclpgdp     1732
A.melanoleuca   llsplvmcgphglkflkpvelrlphc--------------------dpktwqnkclpgdp     1738
O.divergens     llsplvmcgphglkflkpvelrlphcasmtpdgwsfalkssdsssgdpktwqnkclpgdp     1754
M.undulatus     llsplvmcgphglkflkpvelrlphcasmtpdgwsfalkssdsssgepktwqnkslpgdp     1836
S.australis     llsplvmcgphglkflkpvelrlphcasmtpdgwsfalkssdsssgdpktwqnkslpgdp     1758
P.vitticeps     llsplvmcgphglkflkpvelrlphcasmtpdgwsfalkssdsssgdpktwqnkslpgdp     1863
P.bivitattus    llsplvmcgphglkflkpvelrlphcasmtpdgwsfalkssdsssgdpktwqnkslpgdp     1736
                **************************                    :**.***:.**.*

S.salar         nylvgancvsvlidhf   1889
H.comes         nylvgancvsvlidhf   1762
C.milii         nyhvgancvsvlidhf   1749
B.scammoni      nylvgancvsvlidhf   1755
H.sapiens       nylvgancvsvlidhf   1748
A.melanoleuca   nylvgancvsvlidhf   1754
O.divergens     nylvgancvsvlidhf   1770
M.undulatus     nylvgancvsvlidhf   1852
S.australis     nylvgancvsvlidhf   1774
P.vitticeps     nylvgancvsvlidhf   1879
P.bivitattus    nylvgancvsvlidhf   1752
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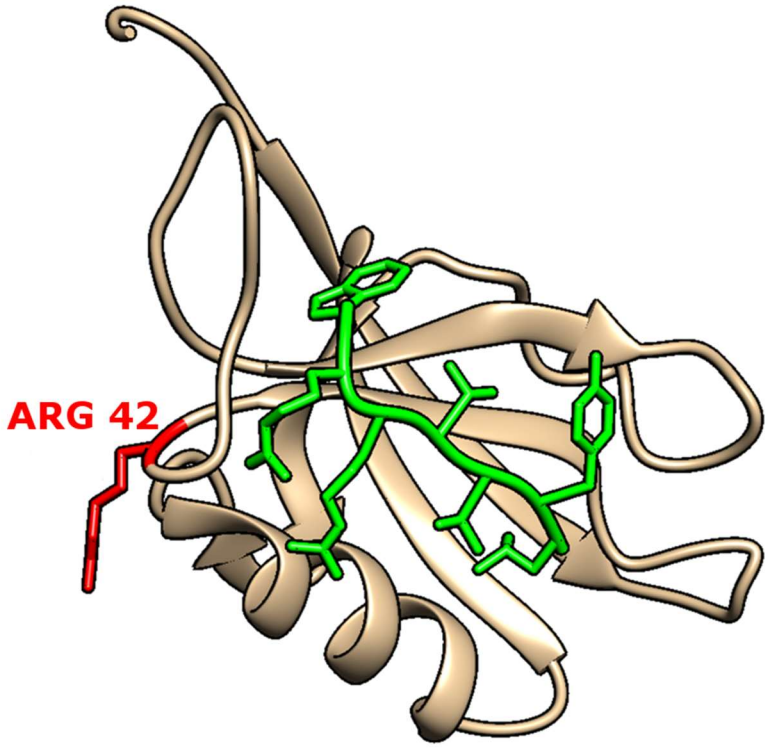


Supplementary Figure 9. Hotspot R42 of ZO-1 is located in close proximity to the binding cavity of the first PDZ domain. The heptapeptide WRRTTYL, shown in green, was crystalized in the binding cleft of the first PDZ-domain (brown) of ZO-1 (PDB 2H2B). Identified hotspot R42 to located near the binding site. The YAP2 C-terminal pentapeptide FLTWL is predicted to bind in this binding cleft and interact with R42.

Supplementary Figure 10. Three individual hotspots on YAP2 were identified when complexed with ZO-1.

Hotspots are identified as the tryptic cleavage sites blocked by dye treatment but protected during complexation with a protein binding partner. This leads to an identification pattern for hotspots where hotspot peptides are present in unpainted samples, absent in painted samples due to blockage of the tryptic cleavage site by dye coverage, and present in the dyed complexed samples where complexation protects the tryptic cleavage site from dye coverage. A) Peptide KLPDSFFKPPEPK from YAP2, following tryptic cleavage at R89, is identified by MS/MS in the unpainted sample 11292018_Amanda_1. B) Peptide QSSFEIPDDVPLPAGWEMAK from YAP2, following tryptic cleavage at R161, is identified by MS/MS in the unpainted sample 11292018_Amanda_1. C) Peptide YFLNHIDQTTTWQDPRK from YAP2, following tryptic cleavage at R187, is identified by MS/MS in the unpainted sample 11292018_Amanda_1. D) All YAP2 peptides identified within 1% FDR in the ZO-1 painted sample 11292018_Amanda_5; neither the KLPDSFFKPPEPK, QSSFEIPDDVPLPAGWEMAK, nor the YFLNHIDQTTTWQDPRK peptides were identified in the sample, indicating blockage of R89, R161, and R189 by the dye treatment. E) Peptide KLPDSFFKPPEPK from YAP2, following tryptic cleavage at R89, is identified by MS/MS in the painted complexed sample 11292018_Amanda_10. F) Peptide QSSFEIPDDVPLPAGWEMAK from YAP2, following tryptic cleavage at R161, is identified by MS/MS in the painted complexed sample 11292018_Amanda_10. G) Peptide YFLNHIDQTTTWQDPRK from YAP2, following tryptic cleavage at K592, is identified by MS/MS in the painted complexed sample 11292018_Amanda_10.

A. R89 of unpainted YAP2 sample 11292018_Amanda_1 is identified by the presence of peptide KLPDSFFKPPEPK tandem mass spectrum matched via Sequest

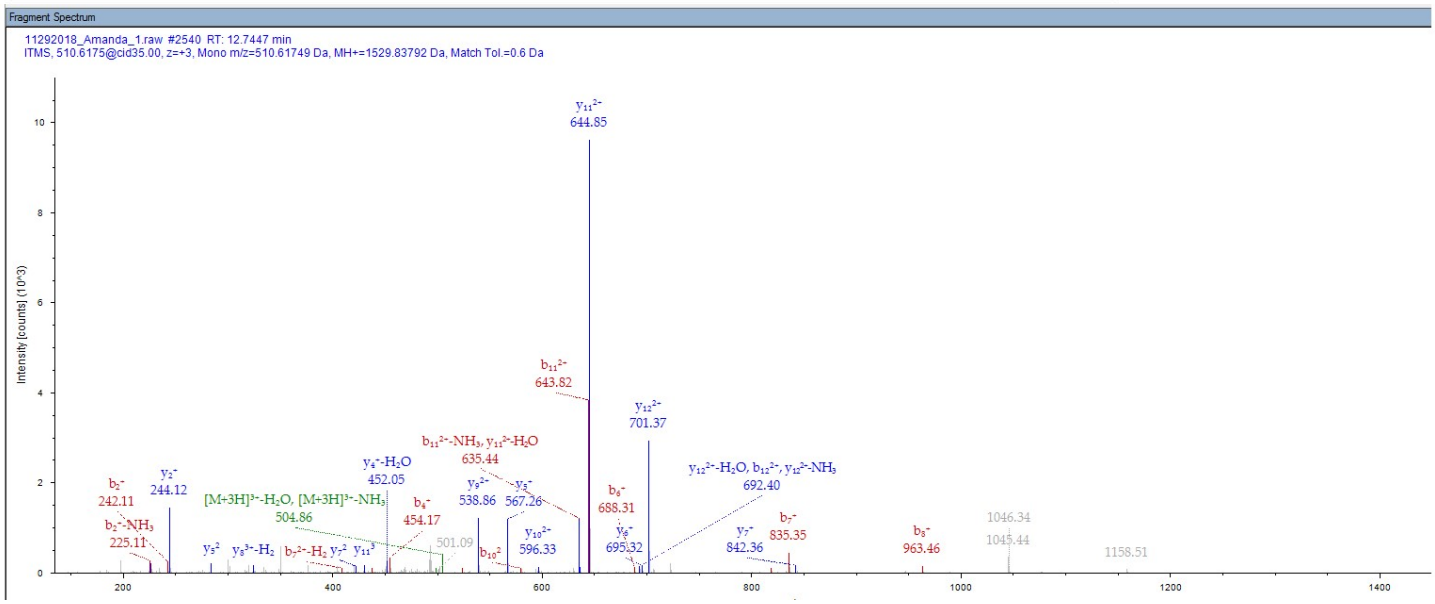
Peptide Summary

Sequence: KLPDSFFKPPEPK, Charge: +3, Monoisotopic m/z: 510.61749 Da (+0.56 mmu/+1.1 ppm), MH+: 1529.83792 Da, RT: 12.7447 min,
Identified with: Sequest HT (v1.17); XCorr:3.28, Percolator q-Value:0, Percolator PEP:0.153, Ions matched by search engine: 0/0
Fragment match tolerance used for search: 0.6 Da
Fragments used for search: b; b-H_2O; b-NH_3; y; y-H_2O; y-NH_3

#1	b^+	b^{2+}	b^{3+}	Seq.	y^+	y^{2+}	y^{3+}	#2
1	129.10224	65.05476	43.70560	K				13
2	242.18630	121.59679	81.40029	L	1401.74128	701.37428	467.91861	12
3	339.23907	170.12317	113.75121	P	1288.65721	644.83225	430.22392	11
4	454.26601	227.63664	152.09352	D	1191.60445	596.30586	397.87300	10
5	541.29804	271.15266	181.10420	S	1076.57751	538.79239	359.53069	9
6	688.36645	344.68686	230.12700	F	989.54548	495.27638	330.52001	8
7	835.43487	418.22107	279.14981	F	842.47707	421.74217	281.49721	7
8	963.52983	482.26855	321.84813	K	695.40865	348.20796	232.47440	6
9	1060.58259	530.79493	354.19905	P	567.31369	284.16048	189.77608	5
10	1157.63536	579.32132	386.54997	P	470.26092	235.63410	157.42516	4
11	1286.67795	643.84261	429.56417	E	373.20816	187.10772	125.07424	3
12	1383.73071	692.36900	461.91509	P	244.16557	122.58642	82.06004	2
13				K	147.11280	74.06004	49.70912	1

#1	b-H_2O^+	b-H_2O^{2+}	b-H_2O^{3+}	b-NH_3^+	b-NH_3^{2+}	b-NH_3^{3+}	Seq.	y-H_2O^+	y-H_2O^{2+}	y-H_2O^{3+}	y-NH_3^+	y-NH_3^{2+}	y-NH_3^{3+}	#2
1				112.07569	56.54148	38.03008	K							13
2				225.15975	113.08352	75.72477	L	1383.73071	692.36900	461.91509	1384.71473	692.86100	462.24309	12
3				322.21252	161.60990	108.07569	P	1270.64665	635.82696	424.22040	1271.63067	636.31897	424.54841	11
4	436.25545	218.63136	146.09000	437.23946	219.12337	146.41800	D	1173.59389	587.30058	391.86948	1174.57790	587.79259	392.19748	10
5	523.28747	262.14738	175.10068	524.27149	262.63938	175.42868	S	1058.56694	529.78711	353.52717	1059.55096	530.27912	353.85517	9
6	670.35589	335.68158	224.12348	671.33990	336.17359	224.45149	F	971.53491	486.27110	324.51649	972.51893	486.76310	324.84449	8
7	817.42430	409.21579	273.14628	818.40832	409.70780	273.47429	F	824.46650	412.73689	275.49368	825.45052	413.22890	275.82169	7
8	945.51926	473.26327	315.84461	946.50328	473.75528	316.17261	K	677.39809	339.20268	226.47088	678.38210	339.69469	226.79889	6
9	1042.57203	521.78965	348.19553	1043.55604	522.28166	348.52353	P	549.30312	275.15520	183.77256	550.28714	275.64721	184.10056	5
10	1139.62479	570.31603	380.54645	1140.60881	570.80804	380.87445	P	452.25036	226.62882	151.42164	453.23438	227.12083	151.74964	4
11	1268.66739	634.83733	423.56065	1269.65140	635.32934	423.88865	E	355.19760	178.10244	119.07072	356.18161	178.59444	119.39872	3
12	1365.72015	683.36371	455.91157	1366.70416	683.85572	456.23957	P				227.13902	114.07315	76.38452	2
13							K				130.08626	65.54677	44.03360	1

Fragment Spectrum



B. R161 of unpainted YAP2 sample 11292018_Amanda_1 is identified by the presence of peptide QSSFEIPDDVPLPAGWEMAK tandem mass spectrum matched via Sequest

Peptide Summary

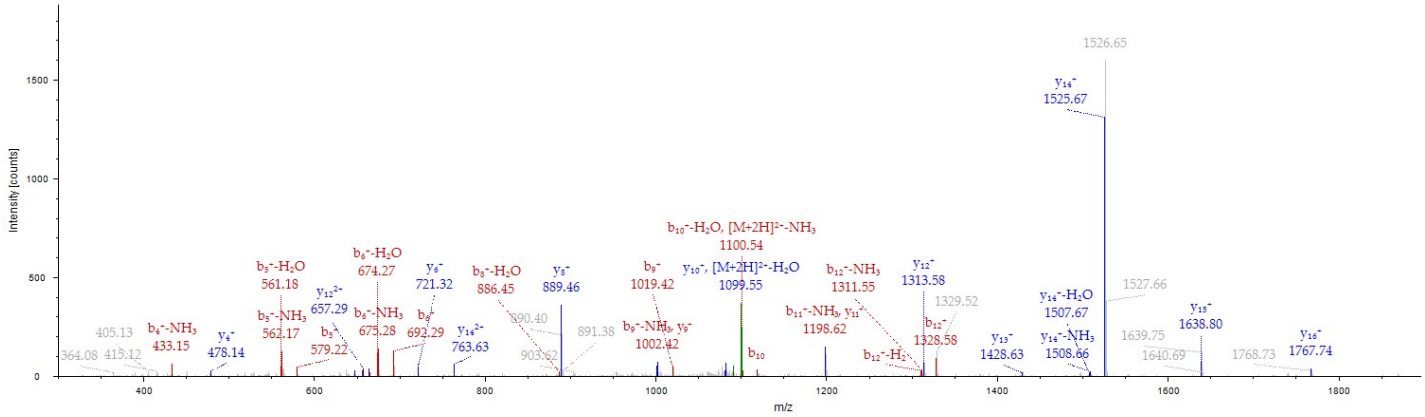
Sequence: QSSFEIPDDVPLPAGWEMAK, Charge: +2, Monoisotopic m/z: 1109.03113 Da (+0.87 mmu/+0.78 ppm), MH+: 2217.05498 Da, RT: 20.3265 min,
Identified with: Sequest HT (v1.17); XCorr:4.67, Percolator q-Value:0, Percolator PEP:0.00214, Ions matched by search engine: 0/0
Fragment match tolerance used for search: 0.6 Da
Fragments used for search: b; b-H_2O; b-NH_3; y; y-H_2O; y-NH_3

#1	b^+	b^{2+}	Seq.	y^+	y^{2+}	#2
1	129.06585	65.03657	Q			20
2	216.09788	108.55258	S	2088.99467	1045.00097	19
3	303.12991	152.06859	S	2001.96264	1001.48496	18
4	450.19832	225.60280	F	1914.93061	957.96894	17
5	579.24092	290.12410	E	1767.86220	884.43474	16
6	692.32498	346.66613	I	1638.81960	819.91344	15
7	789.37775	395.19251	P	1525.73554	763.37141	14
8	904.40469	452.70598	D	1428.68278	714.84503	13
9	1019.43163	510.21945	D	1313.65583	657.33156	12
10	1118.50005	559.75366	V	1198.62889	599.81808	11
11	1215.55281	608.28004	P	1099.56048	550.28388	10
12	1328.63687	664.82207	L	1002.50771	501.75750	9
13	1425.68964	713.34846	P	889.42365	445.21546	8
14	1496.72675	748.86701	A	792.37089	396.68908	7
15	1553.74821	777.37775	G	721.33377	361.17052	6
16	1739.82753	870.41740	W	664.31231	332.65979	5
17	1868.87012	934.93870	E	478.23300	239.62014	4
18	1999.91060	1000.45894	M	349.19040	175.09884	3
19	2070.94772	1035.97750	A	218.14992	109.57860	2
20			K	147.11280	74.06004	1

#1	b-H_2O^+	b-H_2O^{2+}	b-NH_3^+	b-NH_3^{2+}	Seq.	y-H_2O^+	y-H_2O^{2+}	y-NH_3^+	y-NH_3^{2+}	#2
1			112.03930	56.52329	Q					20
2	198.08732	99.54730	199.07133	100.03930	S	2070.98410	1035.99569	2071.96812	1036.48770	19
3	285.11935	143.06331	286.10336	143.55532	S	1983.95208	992.47968	1984.93609	992.97168	18
4	432.18776	216.59752	433.17178	217.08953	F	1896.92005	948.96366	1897.90406	949.45567	17
5	561.23035	281.11881	562.21437	281.61082	E	1749.85163	875.42945	1750.83565	875.92146	16
6	674.31442	337.66085	675.29843	338.15285	I	1620.80904	810.90816	1621.79306	811.40017	15
7	771.36718	386.18723	772.35120	386.67924	P	1507.72498	754.36613	1508.70899	754.85813	14
8	886.39412	443.70070	887.37814	444.19271	D	1410.67221	705.83974	1411.65623	706.33175	13
9	1001.42107	501.21417	1002.40508	501.70618	D	1295.64527	648.32627	1296.62929	648.81828	12
10	1100.48948	550.74838	1101.47350	551.24039	V	1180.61833	590.81280	1181.60234	591.30481	11
11	1197.54224	599.27476	1198.52626	599.76677	P	1081.54991	541.27859	1082.53393	541.77060	10
12	1310.62631	655.81679	1311.61032	656.30880	L	984.49715	492.75221	985.48116	493.24422	9
13	1407.67907	704.34317	1408.66309	704.83518	P	871.41309	436.21018	872.39710	436.70219	8
14	1478.71619	739.86173	1479.70020	740.35374	A	774.36032	387.68380	775.34434	388.17581	7
15	1535.73765	768.37246	1536.72167	768.86447	G	703.32321	352.16524	704.30722	352.65725	6
16	1721.81696	861.41212	1722.80098	861.90413	W	646.30174	323.65451	647.28576	324.14652	5
17	1850.85956	925.93342	1851.84357	926.42542	E	460.22243	230.61485	461.20645	231.10686	4
18	1981.90004	991.45366	1982.88406	991.94567	M			332.16385	166.58557	3
19	2052.93715	1026.97222	2053.92117	1027.46422	A			201.12337	101.06532	2
20					K			130.08626	65.54677	1

Fragment Spectrum

11292018_Amanda_1.raw #4091 RT: 20.3265 min
ITMS, 1109.5333@cid35.00, z=+2, Mono m/z=1109.03113 Da, MH+=2217.05498 Da, Match Tol.=0.6 Da



C. R187 of unpainted YAP2 sample 11292018_Amanda_1 is identified by the presence of peptide YFLNHIDQTTTWQDPRK tandem mass spectrum matched via Sequest

Peptide Summary

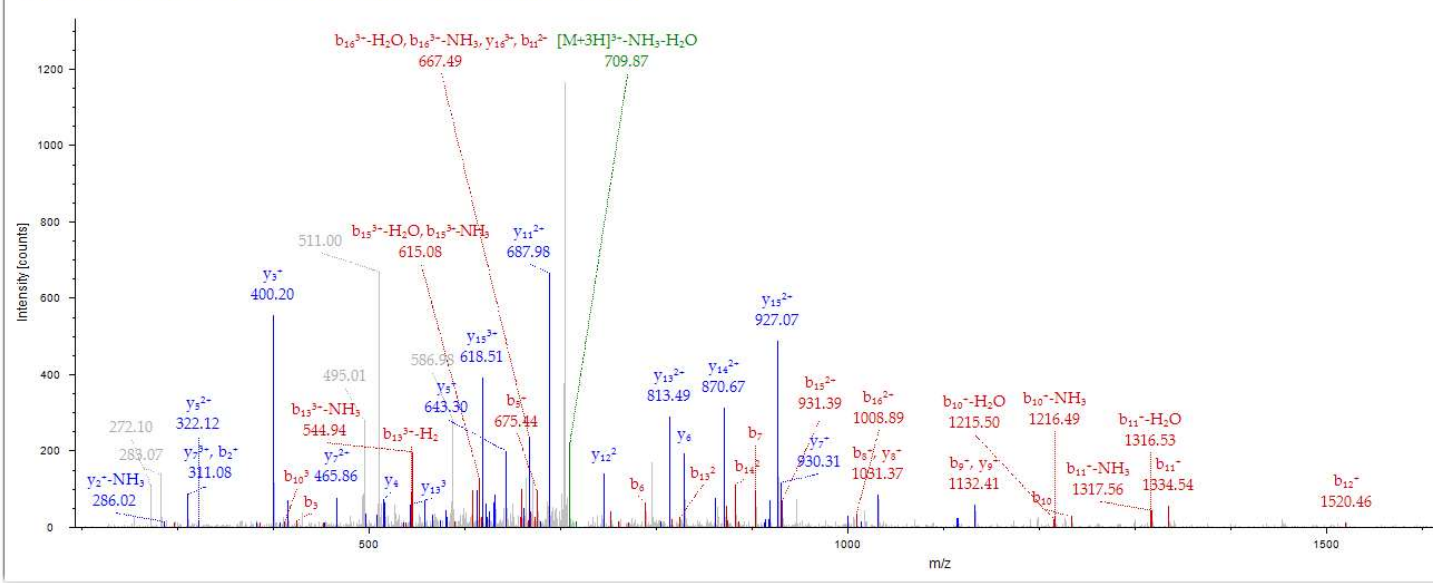
Sequence: YFLNHIDQTTTWQDPRK, Charge: +3, Monoisotopic m/z: 721.69275 Da (+0.64 mmu/+0.89 ppm), MH+: 2163.06369 Da, RT: 13.4807 min,
Identified with: Sequest HT (v1.17); XCorr:4.23, Percolator q-Value:0, Percolator PEP:0.0693, Ions matched by search engine: 0/0
Fragment match tolerance used for search: 0.6 Da
Fragments used for search: b; b-H_2O; b-NH_3; y; y-H_2O; y-NH_3

#1	b^+	b^{2+}	b^{3+}	Seq.	y^+	y^{2+}	y^{3+}	#2
1	164.07061	82.53894	55.36172	Y				17
2	311.13902	156.07315	104.38452	F	1999.99845	1000.50286	667.33767	16
3	424.22308	212.61518	142.07921	L	1852.93003	926.96866	618.31486	15
4	538.26601	269.63664	180.09352	N	1739.84597	870.42662	580.62017	14
5	675.32492	338.16610	225.77983	H	1625.80304	813.40516	542.60587	13
6	788.40899	394.70813	263.47451	I	1488.74413	744.87570	496.91956	12
7	903.43593	452.22160	301.81683	D	1375.66007	688.33367	459.22487	11
8	1031.49451	516.25089	344.50302	Q	1260.63312	630.82020	420.88256	10
9	1132.54218	566.77473	378.18558	T	1132.57455	566.79091	378.19637	9
10	1233.58986	617.29857	411.86814	T	1031.52687	516.26707	344.51381	8
11	1334.63754	667.82241	445.55070	T	930.47919	465.74323	310.83125	7
12	1520.71685	760.86207	507.57714	W	829.43151	415.21939	277.14869	6
13	1648.77543	824.89135	550.26333	Q	643.35220	322.17974	215.12225	5
14	1763.80237	882.40483	588.60564	D	515.29362	258.15045	172.43606	4
15	1860.85514	930.93121	620.95656	P	400.26668	200.63698	134.09374	3
16	2016.95625	1008.98176	672.99027	R	303.21392	152.11060	101.74282	2
17				K	147.11280	74.06004	49.70912	1

#1	b-H_2O^+	b-H_2O^{2+}	b-H_2O^{3+}	b-NH_3^+	b-NH_3^{2+}	b-NH_3^{3+}	Seq.	y-H_2O^+	y-H_2O^{2+}	y-H_2O^{3+}	y-NH_3^+	y-NH_3^{2+}	y-NH_3^{3+}	#2
1							Y							17
2							F	1981.98788	991.49758	661.33415	1982.97190	991.98959	661.66215	16
3							L	1834.91947	917.96337	612.31134	1835.90349	918.45538	612.63935	15
4				521.23946	261.12337	174.41800	N	1721.83541	861.42134	574.61665	1722.81942	861.91335	574.94466	14
5				658.29837	329.65282	220.10431	H	1607.79248	804.39988	536.60234	1608.77649	804.89189	536.93035	13
6				771.38244	386.19486	257.79900	I	1470.73357	735.87042	490.91604	1471.71758	736.36243	491.24405	12
7	885.42536	443.21632	295.81331	886.40938	443.70833	296.14131	D	1357.64950	679.32839	453.22135	1358.63352	679.82040	453.54936	11
8	1013.48394	507.24561	338.49950	1014.46796	507.73762	338.82750	Q	1242.62256	621.81492	414.87904	1243.60658	622.30693	415.20704	10
9	1114.53162	557.76945	372.18206	1115.51564	558.26146	372.51006	T	1114.56398	557.78563	372.19285	1115.54800	558.27764	372.52085	9
10	1215.57930	608.29329	405.86462	1216.56331	608.78530	406.19262	T	1013.51630	507.26179	338.51029	1014.50032	507.75380	338.83829	8
11	1316.62698	658.81713	439.54718	1317.61099	659.30913	439.87518	T	912.46863	456.73795	304.82773	913.45264	457.22996	305.15573	7
12	1502.70629	751.85678	501.57361	1503.69031	752.34879	501.90162	W	811.42095	406.21411	271.14517	812.40496	406.70612	271.47317	6
13	1630.76487	815.88607	544.25981	1631.74888	816.37808	544.58781	Q	625.34163	313.17446	209.11873	626.32565	313.66646	209.44673	5
14	1745.79181	873.39954	582.60212	1746.77583	873.89155	582.93013	D	497.28306	249.14517	166.43254	498.26707	249.63717	166.76054	4
15	1842.84457	921.92593	614.95304	1843.82859	922.41793	615.28105	P				383.24013	192.12370	128.41823	3
16	1998.94569	999.97648	666.98675	1999.92970	1000.46849	667.31475	R				286.18737	143.59732	96.06731	2
17							K				130.08626	65.54677	44.03360	1

Fragment Spectrum

11292018_Amanda_1.raw #2673 RT: 13.4807 min
ITMS, 722.0270@cid35.00, z=+3, Mono m/z=721.69275 Da, MH+=2163.06369 Da, Match Tol.=0.6 Da



D. Painted YAP2 sample 11292018_Amanda_5 does not contain peptides KLPDSFFKPPEPK, QSSFEIPDDVPLPAGWEMAK, and YFLNHIDQTTTWQDPRK of YAP2 within the YAP2-derived peptide set as identified by Sequest

Confidence	Sequence	Modifications	Quality PEP	Theo. MH+ [Da]	XCorr A Sequest HT
High	SQLPTLEQDGGTQNPVSSPGMSQELR	1xOxidation [M21]	0.00607993	2772.31	6.52
High	SQLPTLEQDGGTQNPVSSPGMSQELR		0.0113767	2756.316	5.98
High	DESTDSGLSMSSYSVPR		0.0969116	1817.786	5.05
High	TMTTNSSDPFLNSGTYHSR	1xOxidation [M2]	0.0668259	2131.935	4.83
High	TPDDFLNSVDEMDTGDTINQSTLPSQQNR	1xOxidation [M12]	0.030534	3254.439	4.67
High	DESTDSGLSMSSYSVPR	1xOxidation [M10]	0.104162	1833.781	4.55
High	QASTDAGTAGALTPQHVR		0.030148	1780.894	4.04
High	NKTTSWLDPR		0.214779	1217.627	3.1
High	LQQLQMEK		0.338173	1017.54	3.07
High	LKQQELLR		0.295662	1027.626	2.9
High	TTSWLDPR		0.236017	975.4894	2.83
High	LQQLQMEKER		0.171489	1302.683	2.83
High	TANVPQTVPMR		0.114955	1213.636	2.64
High	TANVPQTVPMR	1xOxidation [M10]	0.446823	1229.631	2.61
High	CQELALR	1xCarbamidomethyl [C1]	0.414696	889.456	2.43
High	NINPSTANSPK		0.144957	1142.58	2.43
High	ISQSAPVK		0.445128	829.4778	2.29
High	FAMNQR		0.522696	766.3665	1.93
High	LQQLQMEKER	1xOxidation [M6]	0.313021	1318.678	1.62

E. R89 of painted YAP2/ZO-1 complex sample 11292018_Amanda_10 is identified by the presence of peptide KLPDSFFKPPEPK tandem mass spectrum matched via Sequest

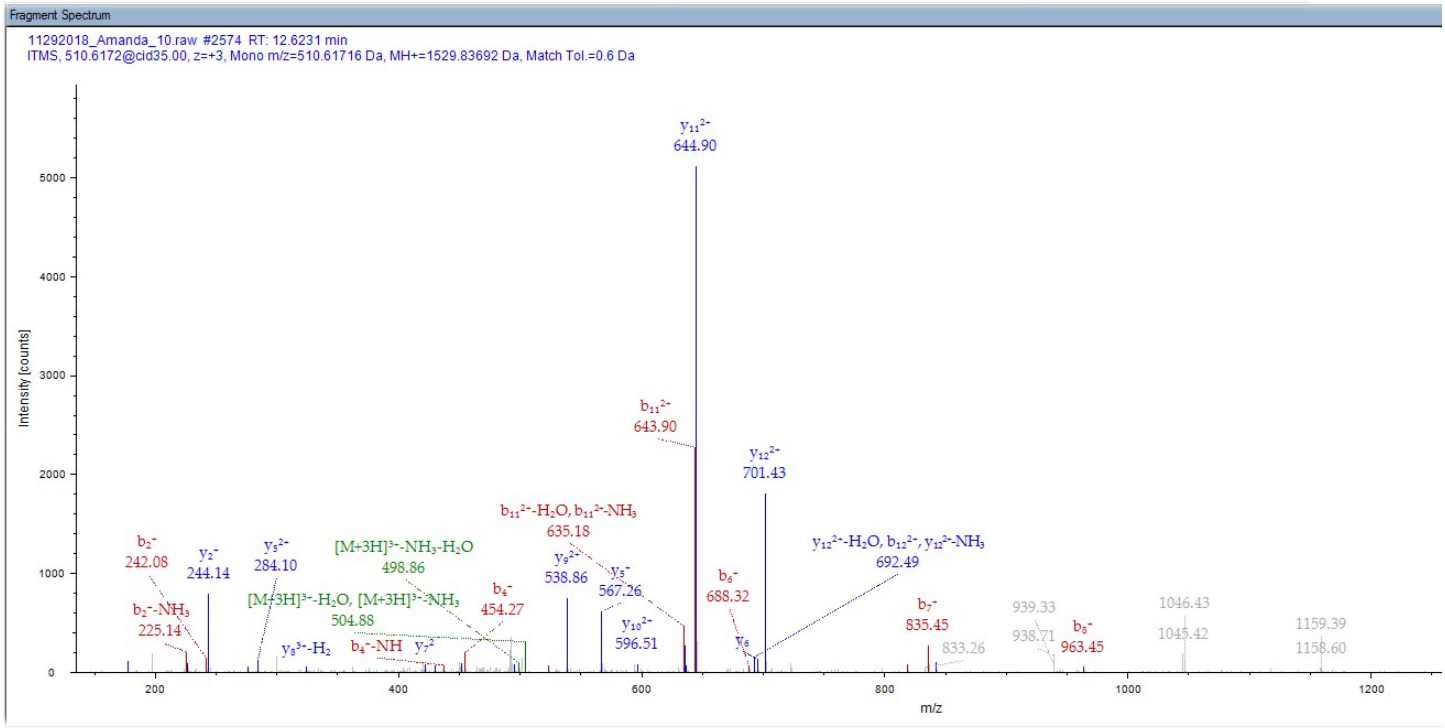
Peptide Summary

Sequence: KLPDSFFKPPEPK, Charge: +3, Monoisotopic m/z: 510.61716 Da (+0.23 mmu/+0.44 ppm), MH+: 1529.83692 Da, RT: 12.6231 min,
Identified with: Sequest HT (v1.17); XCorr:3.43, Percolator q-Value:0, Percolator PEP:0.0107, Ions matched by search engine: 0/0
Fragment match tolerance used for search: 0.6 Da
Fragments used for search: b; b-H_2O; b-NH_3; y; y-H_2O; y-NH_3

#1	b^+	b^{2+}	b^{3+}	Seq.	y^+	y^{2+}	y^{3+}	#2
1	129.10224	65.05476	43.70560	K				13
2	242.18630	121.59679	81.40029	L	1401.74128	701.37428	467.91861	12
3	339.23907	170.12317	113.75121	P	1288.65721	644.83225	430.22392	11
4	454.26601	227.63664	152.09352	D	1191.60445	596.30586	397.87300	10
5	541.29804	271.15266	181.10420	S	1076.57751	538.79239	359.53069	9
6	688.36645	344.68686	230.12700	F	989.54548	495.27638	330.52001	8
7	835.43487	418.22107	279.14981	F	842.47707	421.74217	281.49721	7
8	963.52983	482.26855	321.84813	K	695.40865	348.20796	232.47440	6
9	1060.58259	530.79493	354.19905	P	567.31369	284.16048	189.77608	5
10	1157.63536	579.32132	386.54997	P	470.26092	235.63410	157.42516	4
11	1286.67795	643.84261	429.56417	E	373.20816	187.10772	125.07424	3
12	1383.73071	692.36900	461.91509	P	244.16557	122.58642	82.06004	2
13				K	147.11280	74.06004	49.70912	1

#1	b-H_2O^+	b-H_2O^{2+}	b-H_2O^{3+}	b-NH_3^+	b-NH_3^{2+}	b-NH_3^{3+}	Seq.	y-H_2O^+	y-H_2O^{2+}	y-H_2O^{3+}	y-NH_3^+	y-NH_3^{2+}	y-NH_3^{3+}	#2
1				112.07569	56.54148	38.03008	K							13
2				225.15975	113.08352	75.72477	L	1383.73071	692.36900	461.91509	1384.71473	692.86100	462.24309	12
3				322.21252	161.60990	108.07569	P	1270.64665	635.82696	424.22040	1271.63067	636.31897	424.54841	11
4	436.25545	218.63136	146.09000	437.23946	219.12337	146.41800	D	1173.59389	587.30058	391.86948	1174.57790	587.79259	392.19748	10
5	523.28747	262.14738	175.10068	524.27149	262.63938	175.42868	S	1058.56694	529.78711	353.52717	1059.55096	530.27912	353.85517	9
6	670.35589	335.68158	224.12348	671.33990	336.17359	224.45149	F	971.53491	486.27110	324.51649	972.51893	486.76310	324.84449	8
7	817.42430	409.21579	273.14628	818.40832	409.70780	273.47429	F	824.46650	412.73689	275.49368	825.45052	413.22890	275.82169	7
8	945.51926	473.26327	315.84461	946.50328	473.75528	316.17261	K	677.39809	339.20268	226.47088	678.38210	339.69469	226.79889	6
9	1042.57203	521.78965	348.19553	1043.55604	522.28166	348.52353	P	549.30312	275.15520	183.77256	550.28714	275.64721	184.10056	5
10	1139.62479	570.31603	380.54645	1140.60881	570.80804	380.87445	P	452.25036	226.62882	151.42164	453.23438	227.12083	151.74964	4
11	1268.66739	634.83733	423.56065	1269.65140	635.32934	423.88865	E	355.19760	178.10244	119.07072	356.18161	178.59444	119.39872	3
12	1365.72015	683.36371	455.91157	1366.70416	683.85572	456.23957	P				227.13902	114.07315	76.38452	2
13							K				130.08626	65.54677	44.03360	1

Fragment Spectrum



F. R161 of painted YAP2/ZO-1 complex sample 11292018_Amanda_10 is identified by the presence of peptide QSSFEIPDDVPLPAGWEMAK tandem mass spectrum matched via Sequest

Peptide Summary

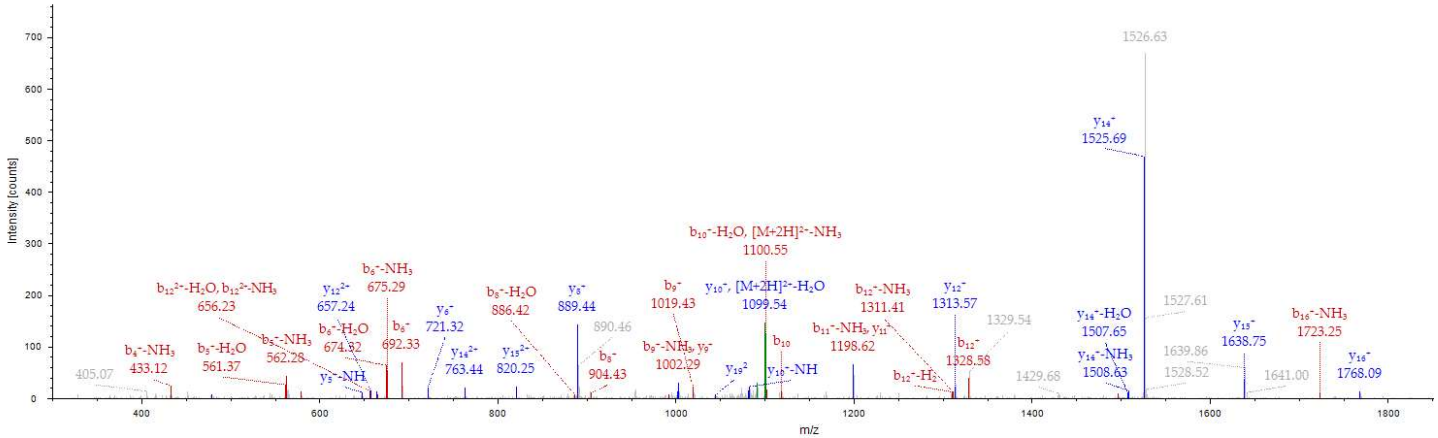
Sequence: QSSFEIPDDVPLPAGWEMAK, Charge: +2, Monoisotopic m/z: 1109.03113 Da (+0.87 mmu/+0.78 ppm), MH+: 2217.05498 Da, RT: 20.1155 min,
Identified with: Sequest HT (v1.17); XCorr:4.70, Percolator q-Value:0, Percolator PEP:0.00268, Ions matched by search engine: 0/0
Fragment match tolerance used for search: 0.6 Da
Fragments used for search: b; b-H_2O; b-NH_3; y; y-H_2O; y-NH_3

#1	b^+	b^{2+}	Seq.	y^+	y^{2+}	#2
1	129.06585	65.03657	Q			20
2	216.09788	108.55258	S	2088.99467	1045.00097	19
3	303.12991	152.06859	S	2001.96264	1001.48496	18
4	450.19832	225.60280	F	1914.93061	957.96894	17
5	579.24092	290.12410	E	1767.86220	884.43474	16
6	692.32498	346.66613	I	1638.81960	819.91344	15
7	789.37775	395.19251	P	1525.73554	763.37141	14
8	904.40469	452.70598	D	1428.68278	714.84503	13
9	1019.43163	510.21945	D	1313.65583	657.33156	12
10	1118.50005	559.75366	V	1198.62889	599.81808	11
11	1215.55281	608.28004	P	1099.56048	550.28388	10
12	1328.63687	664.82207	L	1002.50771	501.75750	9
13	1425.68964	713.34846	P	889.42365	445.21546	8
14	1496.72675	748.86701	A	792.37089	396.68908	7
15	1553.74821	777.37775	G	721.33377	361.17052	6
16	1739.82753	870.41740	W	664.31231	332.65979	5
17	1868.87012	934.93870	E	478.23300	239.62014	4
18	1999.91060	1000.45894	M	349.19040	175.09884	3
19	2070.94772	1035.97750	A	218.14992	109.57860	2
20			K	147.11280	74.06004	1

#1	b-H_2O^+	b-H_2O^{2+}	b-NH_3^+	b-NH_3^{2+}	Seq.	y-H_2O^+	y-H_2O^{2+}	y-NH_3^+	y-NH_3^{2+}	#2
1			112.03930	56.52329	Q					20
2	198.08732	99.54730	199.07133	100.03930	S	2070.98410	1035.99569	2071.96812	1036.48770	19
3	285.11935	143.06331	286.10336	143.55532	S	1983.95208	992.47968	1984.93609	992.97168	18
4	432.18776	216.59752	433.17178	217.08953	F	1896.92005	948.96366	1897.90406	949.45567	17
5	561.23035	281.11881	562.21437	281.61082	E	1749.85163	875.42945	1750.83565	875.92146	16
6	674.31442	337.66085	675.29843	338.15285	I	1620.80904	810.90816	1621.79306	811.40017	15
7	771.36718	386.18723	772.35120	386.67924	P	1507.72498	754.36613	1508.70899	754.85813	14
8	886.39412	443.70070	887.37814	444.19271	D	1410.67221	705.83974	1411.65623	706.33175	13
9	1001.42107	501.21417	1002.40508	501.70618	D	1295.64527	648.32627	1296.62929	648.81828	12
10	1100.48948	550.74838	1101.47350	551.24039	V	1180.61833	590.81280	1181.60234	591.30481	11
11	1197.54224	599.27476	1198.52626	599.76677	P	1081.54991	541.27859	1082.53393	541.77060	10
12	1310.62631	655.81679	1311.61032	656.30880	L	984.49715	492.75221	985.48116	493.24422	9
13	1407.67907	704.34317	1408.66309	704.83518	P	871.41309	436.21018	872.39710	436.70219	8
14	1478.71619	739.86173	1479.70020	740.35374	A	774.36032	387.68380	775.34434	388.17581	7
15	1535.73765	768.37246	1536.72167	768.86447	G	703.32321	352.16524	704.30722	352.65725	6
16	1721.81696	861.41212	1722.80098	861.90413	W	646.30174	323.65451	647.28576	324.14652	5
17	1850.85956	925.93342	1851.84357	926.42542	E	460.22243	230.61485	461.20645	231.10686	4
18	1981.90004	991.45366	1982.88406	991.94567	M			332.16385	166.58557	3
19	2052.93715	1026.97222	2053.92117	1027.46422	A			201.12337	101.06532	2
20					K			130.08626	65.54677	1

Fragment Spectrum

11292018_Amanda_10.raw #3913 RT: 20.1155 min
ITMS, 1109.5333@cid35.00, z=+2, Mono m/z=1109.03113 Da, MH+=2217.05498 Da, Match Tol.=0.6 Da



G. R187 of painted YAP2/ZO-1 complex sample 11292018_Amanda_10 is identified by the presence of peptide YFLNHIDQTTTWQDPRK tandem mass spectrum matched via Sequest

Peptide Summary

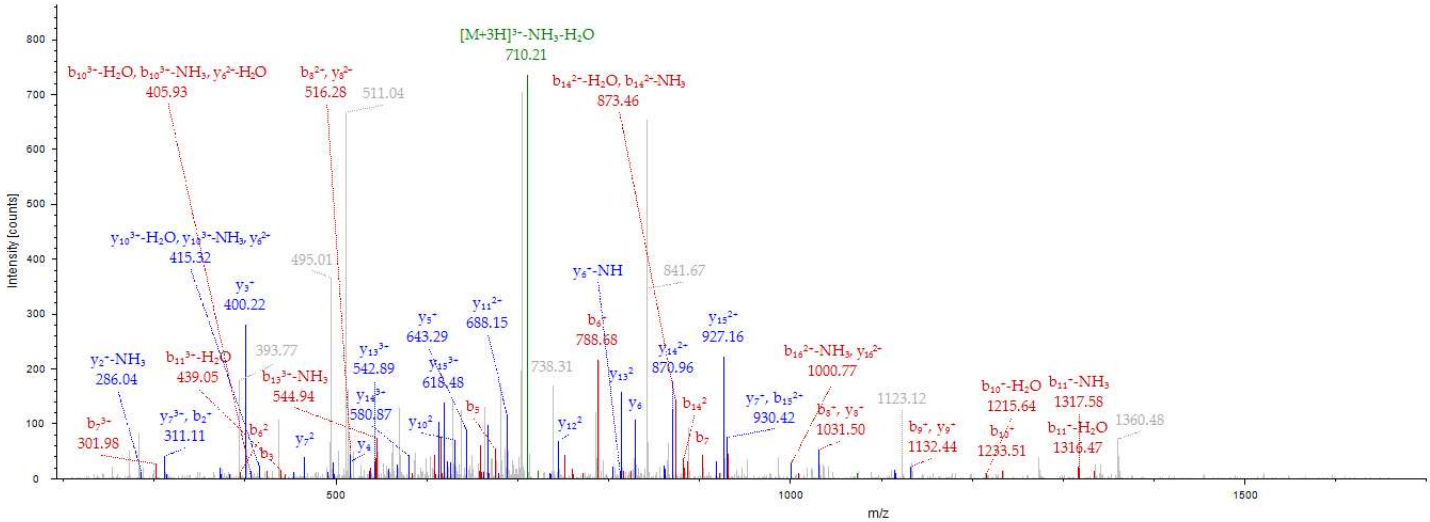
Sequence: YFLNHIDQTTTWQDPRK, Charge: +3, Monoisotopic m/z: 721.69263 Da (+0.52 mmu/+0.72 ppm), MH+: 2163.06333 Da, RT: 13.3667 min,
Identified with: Sequest HT (v1.17); XCorr:3.38, Percolator q-Value:0, Percolator PEP:2.98e-05, Ions matched by search engine: 0/0
Fragment match tolerance used for search: 0.6 Da
Fragments used for search: b; b-H_2O; b-NH_3; y; y-H_2O; y-NH_3

#1	b^+	b^{2+}	b^{3+}	Seq.	y^+	y^{2+}	y^{3+}	#2
1	164.07061	82.53894	55.36172	Y				17
2	311.13902	156.07315	104.38452	F	1999.99845	1000.50286	667.33767	16
3	424.22308	212.61518	142.07921	L	1852.93003	926.96866	618.31486	15
4	538.26601	269.63664	180.09352	N	1739.84597	870.42662	580.62017	14
5	675.32492	338.16610	225.77983	H	1625.80304	813.40516	542.60587	13
6	788.40899	394.70813	263.47451	I	1488.74413	744.87570	496.91956	12
7	903.43593	452.22160	301.81683	D	1375.66007	688.33367	459.22487	11
8	1031.49451	516.25089	344.50302	Q	1260.63312	630.82020	420.88256	10
9	1132.54218	566.77473	378.18558	T	1132.57455	566.79091	378.19637	9
10	1233.58986	617.29857	411.86814	T	1031.52687	516.26707	344.51381	8
11	1334.63754	667.82241	445.55070	T	930.47919	465.74323	310.83125	7
12	1520.71685	760.86207	507.57714	W	829.43151	415.21939	277.14869	6
13	1648.77543	824.89135	550.26333	Q	643.35220	322.17974	215.12225	5
14	1763.80237	882.40483	588.60564	D	515.29362	258.15045	172.43606	4
15	1860.85514	930.93121	620.95656	P	400.26668	200.63698	134.09374	3
16	2016.95625	1008.98176	672.99027	R	303.21392	152.11060	101.74282	2
17				K	147.11280	74.06004	49.70912	1

#1	b-H_2O^+	b-H_2O^{2+}	b-H_2O^{3+}	b-NH_3^+	b-NH_3^{2+}	b-NH_3^{3+}	Seq.	y-H_2O^+	y-H_2O^{2+}	y-H_2O^{3+}	y-NH_3^+	y-NH_3^{2+}	y-NH_3^{3+}	#2
1							Y							17
2							F	1981.98788	991.49758	661.33415	1982.97190	991.98959	661.66215	16
3							L	1834.91947	917.96337	612.31134	1835.90349	918.45538	612.63935	15
4				521.23946	261.12337	174.41800	N	1721.83541	861.42134	574.61665	1722.81942	861.91335	574.94466	14
5				658.29837	329.65282	220.10431	H	1607.79248	804.39988	536.60234	1608.77649	804.89189	536.93035	13
6				771.38244	386.19486	257.79900	I	1470.73357	735.87042	490.91604	1471.71758	736.36243	491.24405	12
7	885.42536	443.21632	295.81331	886.40938	443.70833	296.14131	D	1357.64950	679.32839	453.22135	1358.63352	679.82040	453.54936	11
8	1013.48394	507.24561	338.49950	1014.46796	507.73762	338.82750	Q	1242.62256	621.81492	414.87904	1243.60658	622.30693	415.20704	10
9	1114.53162	557.76945	372.18206	1115.51564	558.26146	372.51006	T	1114.56398	557.78563	372.19285	1115.54800	558.27764	372.52085	9
10	1215.57930	608.29329	405.86462	1216.56331	608.78530	406.19262	T	1013.51630	507.26179	338.51029	1014.50032	507.75380	338.83829	8
11	1316.62698	658.81713	439.54718	1317.61099	659.30913	439.87518	T	912.46863	456.73795	304.82773	913.45264	457.22996	305.15573	7
12	1502.70629	751.85678	501.57361	1503.69031	752.34879	501.90162	W	811.42095	406.21411	271.14517	812.40496	406.70612	271.47317	6
13	1630.76487	815.88607	544.25981	1631.74888	816.37808	544.58781	Q	625.34163	313.17446	209.11873	626.32565	313.66646	209.44673	5
14	1745.79181	873.39954	582.60212	1746.77583	873.89155	582.93013	D	497.28306	249.14517	166.43254	498.26707	249.63717	166.76054	4
15	1842.84457	921.92593	614.95304	1843.82859	922.41793	615.28105	P				383.24013	192.12370	128.41823	3
16	1998.94569	999.97648	666.98675	1999.92970	1000.46849	667.31475	R				286.18737	143.59732	96.06731	2
17							K				130.08626	65.54677	44.03360	1

Fragment Spectrum

11292018_Amanda_10.raw #2667 RT: 13.3667 min
ITMS, 722.0267@cid35.00, z=+3, Mono m/z=721.69263 Da, MH+=2163.06333 Da, Match Tol.=0.6 Da



Supplementary Figure 11: Control peptides in YAP2 do not change in the presence of dye coverage or complexation with ZO-1. A) Control peptide QASTDAGTAGALTPQHVR is present in the unpainted YAP2 sample 11292018_Amanda_1. B) Control peptide TANVPQTVPMR is present in the unpainted YAP2 sample 11292018_Amanda_1. C) Control peptide QASTDAGTAGALTPQHVR is present in the painted YAP2 sample 11292018_Amanda_5. D) Control peptide TANVPQTVPMR is present in the painted YAP2 sample 11292018_Amanda_5. E) Control peptide QASTDAGTAGALTPQHVR is present in the painted YAP2/ZO-1 complex sample 11292018_Amanda_10. F) Control peptide TANVPQTVPMR is present in the painted YAP2/ZO-1 complex sample 11292018_Amanda_10.

A. Control peptide QASTDAGTAGALTPQHVR is present in the unpainted YAP2 sample 11292018_Amanda_1.

Peptide Summary

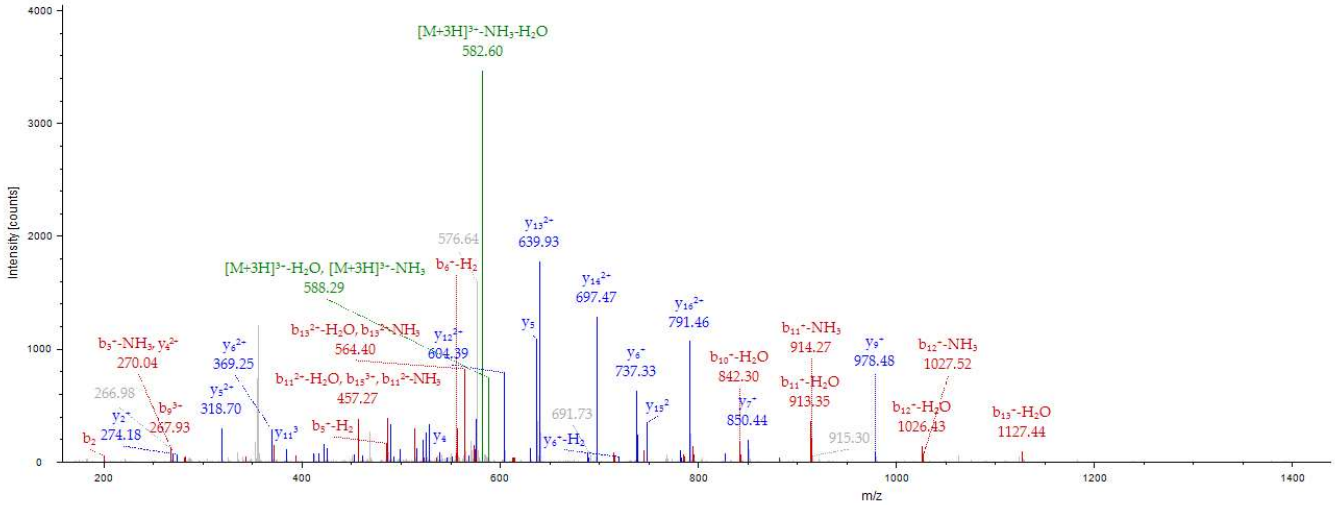
Sequence: QASTDAGTAGALTPQHVR, Charge: +3, Monoisotopic m/z: 594.30280 Da (+0.06 mmu/+0.1 ppm), MH+: 1780.89383 Da, RT: 9.3495 min,
Identified with: Sequest HT (v1.17); XCorr:3.77, Percolator q-Value:0, Percolator PEP:0.000937, Ions matched by search engine: 0/0
Fragment match tolerance used for search: 0.6 Da
Fragments used for search: b; b-H_2O; b-NH_3; y; y-H_2O; y-NH_3

#1	b^+	b^{2+}	b^{3+}	Seq.	y^+	y^{2+}	y^{3+}	#2
1	129.06585	65.03657	43.69347	Q				18
2	200.10297	100.55512	67.37251	A	1652.83507	826.92117	551.61654	17
3	287.13500	144.07114	96.38318	S	1581.79796	791.40262	527.93750	16
4	388.18267	194.59498	130.06574	T	1494.76593	747.88660	498.92683	15
5	503.20962	252.10845	168.40806	D	1393.71825	697.36276	465.24427	14
6	574.24673	287.62700	192.08709	A	1278.69131	639.84929	426.90195	13
7	631.26819	316.13774	211.09425	G	1207.65419	604.33074	403.22292	12
8	732.31587	366.66158	244.77681	T	1150.63273	575.82000	384.21576	11
9	803.35299	402.18013	268.45585	A	1049.58505	525.29616	350.53320	10
10	860.37445	430.69086	287.46300	G	978.54794	489.77761	326.85416	9
11	931.41156	466.20942	311.14204	A	921.52648	461.26688	307.84701	8
12	1044.49563	522.75145	348.83673	L	850.48936	425.74832	284.16797	7
13	1145.54331	573.27529	382.51929	T	737.40530	369.20629	246.47328	6
14	1242.59607	621.80167	414.87021	P	636.35762	318.68245	212.79072	5
15	1370.65465	685.83096	457.55640	Q	539.30486	270.15607	180.43980	4
16	1507.71356	754.36042	503.24270	H	411.24628	206.12678	137.75361	3
17	1606.78197	803.89463	536.26551	V	274.18737	137.59732	92.06731	2
18				R	175.11895	88.06311	59.04450	1

#1	b-H_2O^+	b-H_2O^{2+}	b-H_2O^{3+}	b-NH_3^+	b-NH_3^{2+}	b-NH_3^{3+}	Seq.	y-H_2O^+	y-H_2O^{2+}	y-H_2O^{3+}	y-NH_3^+	y-NH_3^{2+}	y-NH_3^{3+}	#2
1				112.03930	56.52329	38.01795	Q							18
2				183.07642	92.04185	61.69699	A	1634.82451	817.91589	545.61302	1635.80852	818.40790	545.94103	17
3	269.12443	135.06585	90.37966	270.10845	135.55786	90.70767	S	1563.78739	782.39734	521.93398	1564.77141	782.88934	522.26199	16
4	370.17211	185.58969	124.06222	371.15613	186.08170	124.39023	T	1476.75537	738.88132	492.92331	1477.73938	739.37333	493.25131	15
5	485.19905	243.10316	162.40454	486.18307	243.59517	162.73254	D	1375.70769	688.35748	459.24075	1376.69170	688.84949	459.56875	14
6	556.23617	278.62172	186.08357	557.22018	279.11373	186.41158	A	1260.68074	630.84401	420.89843	1261.66476	631.33602	421.22644	13
7	613.25763	307.13245	205.09073	614.24165	307.62446	205.41873	G	1189.64363	595.32545	397.21939	1190.62765	595.81746	397.54740	12
8	714.30531	357.65629	238.77329	715.28932	358.14830	239.10129	T	1132.62217	566.81472	378.21224	1133.60618	567.30673	378.54025	11
9	785.34242	393.17485	262.45233	786.32644	393.66686	262.78033	A	1031.57449	516.29088	344.52968	1032.55850	516.78289	344.85769	10
10	842.36389	421.68558	281.45948	843.34790	422.17759	281.78748	G	960.53737	480.77233	320.85064	961.52139	481.26433	321.17865	9
11	913.40100	457.20414	305.13852	914.38502	457.69615	305.46652	A	903.51591	452.26159	301.84349	904.49993	452.75360	302.17149	8
12	1026.48506	513.74617	342.83321	1027.46908	514.23818	343.16121	L	832.47880	416.74304	278.16445	833.46281	417.23504	278.49246	7
13	1127.53274	564.27001	376.51577	1128.51676	564.76202	376.84377	T	719.39473	360.20100	240.46976	720.37875	360.69301	240.79777	6
14	1224.58551	612.79639	408.86669	1225.56952	613.28840	409.19469	P				619.33107	310.16917	207.11521	5
15	1352.64408	676.82568	451.55288	1353.62810	677.31769	451.88088	Q				522.27831	261.64279	174.76429	4
16	1489.70300	745.35514	497.23918	1490.68701	745.84714	497.56719	H				394.21973	197.61350	132.07809	3
17	1588.77141	794.88934	530.26199	1589.75542	795.38135	530.58999	V				257.16082	129.08405	86.39179	2
18							R				158.09240	79.54984	53.36899	1

Fragment Spectrum

11292018_Amanda_1.raw #2067 RT: 9.3495 min
ITMS, 594.3028@cid35.00, z=+3, Mono m/z=594.30280 Da, MH+=1780.89383 Da, Match Tol.=0.6 Da



B. Control peptide TANVPQTVPMR is present in the unpainted YAP2 sample 11292018_Amanda_1.

Peptide Summary

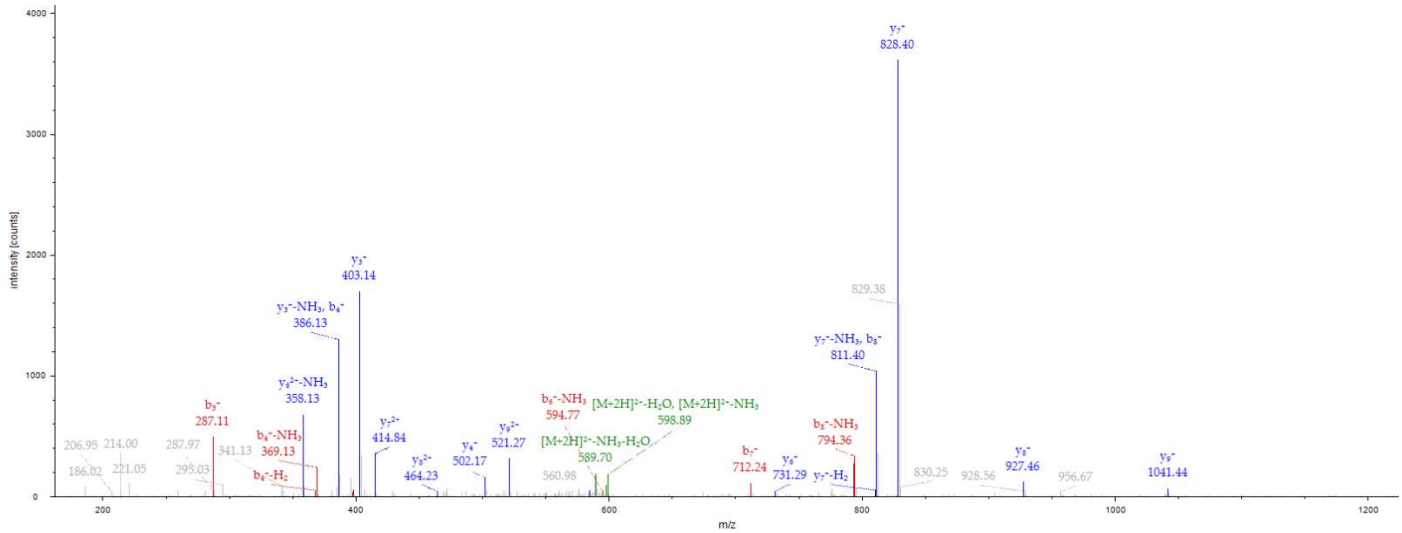
Sequence: TANVPQTVPMR, Charge: +2, Monoisotopic m/z: 607.32172 Da (+0.19 mmu/+0.32 ppm), MH+: 1213.63616 Da, RT: 10.8735 min,
Identified with: Sequest HT (v1.17); XCorr:2.43, Percolator q-Value:0, Percolator PEP:0.32, Ions matched by search engine: 0/0
Fragment match tolerance used for search: 0.6 Da
Fragments used for search: b; b-H_2O; b-NH_3; y; y-H_2O; y-NH_3

#1	b^+	b^{2+}	Seq.	y^+	y^{2+}	#2
1	102.05496	51.53112	T			11
2	173.09207	87.04967	A	1112.58809	556.79768	10
3	287.13500	144.07114	N	1041.55098	521.27913	9
4	386.20341	193.60534	V	927.50805	464.25766	8
5	483.25617	242.13173	P	828.43963	414.72346	7
6	611.31475	306.16101	Q	731.38687	366.19707	6
7	712.36243	356.68485	T	603.32829	302.16778	5
8	811.43084	406.21906	V	502.28061	251.64395	4
9	908.48361	454.74544	P	403.21220	202.10974	3
10	1039.52409	520.26568	M	306.15944	153.58336	2
11			R	175.11895	88.06311	1

#1	b-H_2O^+	b-H_2O^{2+}	b-NH_3^+	b-NH_3^{2+}	Seq.	y-H_2O^+	y-H_2O^{2+}	y-NH_3^+	y-NH_3^{2+}	#2
1	84.04439	42.52583			T					11
2	155.08150	78.04439			A	1094.57752	547.79240	1095.56154	548.28441	10
3	269.12443	135.06585	270.10845	135.55786	N	1023.54041	512.27384	1024.52443	512.76585	9
4	368.19285	184.60006	369.17686	185.09207	V	909.49748	455.25238	910.48150	455.74439	8
5	465.24561	233.12644	466.22962	233.61845	P	810.42907	405.71817	811.41309	406.21018	7
6	593.30419	297.15573	594.28820	297.64774	Q	713.37631	357.19179	714.36032	357.68380	6
7	694.35187	347.67957	695.33588	348.17158	T	585.31773	293.16250	586.30174	293.65451	5
8	793.42028	397.21378	794.40429	397.70579	V			485.25407	243.13067	4
9	890.47304	445.74016	891.45706	446.23217	P			386.18565	193.59646	3
10	1021.51353	511.26040	1022.49754	511.75241	M			289.13289	145.07008	2
11					R			158.09240	79.54984	1

Fragment Spectrum

11292018_Amanda_1.raw #2360 RT: 10.8735 min
ITMS, 607.3217@cid35.00, z=+2, Mono m/z=607.32172 Da, MH+=1213.63616 Da, Match Tol.=0.6 Da



C. Control peptide QASTDAGTAGALTPQHVR is present in the painted YAP2 sample 11292018_Amanda_5.

Peptide Summary

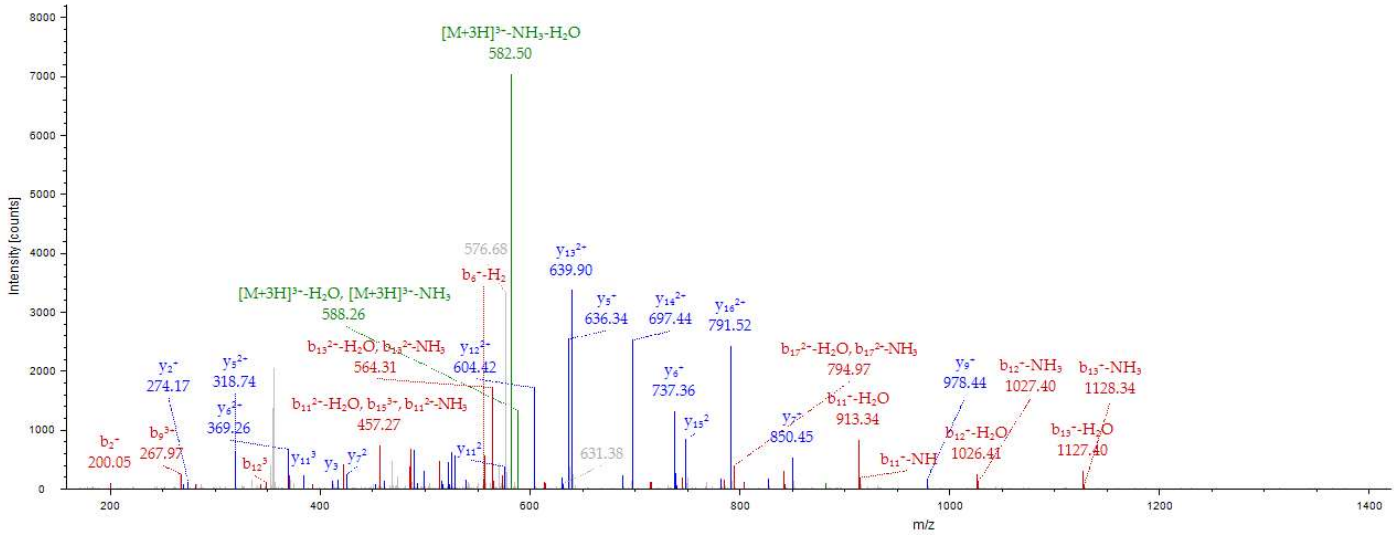
Sequence: QASTDAGTAGALTPQHVR, Charge: +3, Monoisotopic m/z: 594.30402 Da (+1.28 mmu/+2.16 ppm), MH+: 1780.89750 Da, RT: 9.3039 min,
Identified with: Sequest HT (v1.17); XCorr:3.29, Percolator q-Value:0, Percolator PEP:0.015, Ions matched by search engine: 0/0
Fragment match tolerance used for search: 0.6 Da
Fragments used for search: b; b-H_2O; b-NH_3; y; y-H_2O; y-NH_3

#1	b^+	b^{2+}	b^{3+}	Seq.	y^+	y^{2+}	y^{3+}	#2
1	129.06585	65.03657	43.69347	Q				18
2	200.10297	100.55512	67.37251	A	1652.83507	826.92117	551.61654	17
3	287.13500	144.07114	96.38318	S	1581.79796	791.40262	527.93750	16
4	388.18267	194.59498	130.06574	T	1494.76593	747.88660	498.92683	15
5	503.20962	252.10845	168.40806	D	1393.71825	697.36276	465.24427	14
6	574.24673	287.62700	192.08709	A	1278.69131	639.84929	426.90195	13
7	631.26819	316.13774	211.09425	G	1207.65419	604.33074	403.22292	12
8	732.31587	366.66158	244.77681	T	1150.63273	575.82000	384.21576	11
9	803.35299	402.18013	268.45585	A	1049.58505	525.29616	350.53320	10
10	860.37445	430.69086	287.46300	G	978.54794	489.77761	326.85416	9
11	931.41156	466.20942	311.14204	A	921.52648	461.26688	307.84701	8
12	1044.49563	522.75145	348.83673	L	850.48936	425.74832	284.16797	7
13	1145.54331	573.27529	382.51929	T	737.40530	369.20629	246.47328	6
14	1242.59607	621.80167	414.87021	P	636.35762	318.68245	212.79072	5
15	1370.65465	685.83096	457.55640	Q	539.30486	270.15607	180.43980	4
16	1507.71356	754.36042	503.24270	H	411.24628	206.12678	137.75361	3
17	1606.78197	803.89463	536.26551	V	274.18737	137.59732	92.06731	2
18				R	175.11895	88.06311	59.04450	1

#1	b-H_2O^+	b-H_2O^{2+}	b-H_2O^{3+}	b-NH_3^+	b-NH_3^{2+}	b-NH_3^{3+}	Seq.	y-H_2O^+	y-H_2O^{2+}	y-H_2O^{3+}	y-NH_3^+	y-NH_3^{2+}	y-NH_3^{3+}	#2
1				112.03930	56.52329	38.01795	Q							18
2				183.07642	92.04185	61.69699	A	1634.82451	817.91589	545.61302	1635.80852	818.40790	545.94103	17
3	269.12443	135.06585	90.37966	270.10845	135.55786	90.70767	S	1563.78739	782.39734	521.93398	1564.77141	782.88934	522.26199	16
4	370.17211	185.58969	124.06222	371.15613	186.08170	124.39023	T	1476.75537	738.88132	492.92331	1477.73938	739.37333	493.25131	15
5	485.19905	243.10316	162.40454	486.18307	243.59517	162.73254	D	1375.70769	688.35748	459.24075	1376.69170	688.84949	459.56875	14
6	556.23617	278.62172	186.08357	557.22018	279.11373	186.41158	A	1260.68074	630.84401	420.89843	1261.66476	631.33602	421.22644	13
7	613.25763	307.13245	205.09073	614.24165	307.62446	205.41873	G	1189.64363	595.32545	397.21939	1190.62765	595.81746	397.54740	12
8	714.30531	357.65629	238.77329	715.28932	358.14830	239.10129	T	1132.62217	566.81472	378.21224	1133.60618	567.30673	378.54025	11
9	785.34242	393.17485	262.45233	786.32644	393.66686	262.78033	A	1031.57449	516.29088	344.52968	1032.55850	516.78289	344.85769	10
10	842.36389	421.68558	281.45948	843.34790	422.17759	281.78748	G	960.53737	480.77233	320.85064	961.52139	481.26433	321.17865	9
11	913.40100	457.20414	305.13852	914.38502	457.69615	305.46652	A	903.51591	452.26159	301.84349	904.49993	452.75360	302.17149	8
12	1026.48506	513.74617	342.83321	1027.46908	514.23818	343.16121	L	832.47880	416.74304	278.16445	833.46281	417.23504	278.49246	7
13	1127.53274	564.27001	376.51577	1128.51676	564.76202	376.84377	T	719.39473	360.20100	240.46976	720.37875	360.69301	240.79777	6
14	1224.58551	612.79639	408.86669	1225.56952	613.28840	409.19469	P				619.33107	310.16917	207.11521	5
15	1352.64408	676.82568	451.55288	1353.62810	677.31769	451.88088	Q				522.27831	261.64279	174.76429	4
16	1489.70300	745.35514	497.23918	1490.68701	745.84714	497.56719	H				394.21973	197.61350	132.07809	3
17	1588.77141	794.88934	530.26199	1589.75542	795.38135	530.58999	V				257.16082	129.08405	86.39179	2
18							R				158.09240	79.54984	53.36899	1

Fragment Spectrum

11292018_Amanda_5.raw #1814 RT: 9.3039 min
ITMS, 594.3040@cid35.00, z=+3, Mono m/z=594.30402 Da, MH+=1780.89750 Da, Match Tol.=0.6 Da



D. Control peptide TANVPQTVPMR is present in the painted YAP2 sample 11292018_Amanda_5.

Peptide Summary

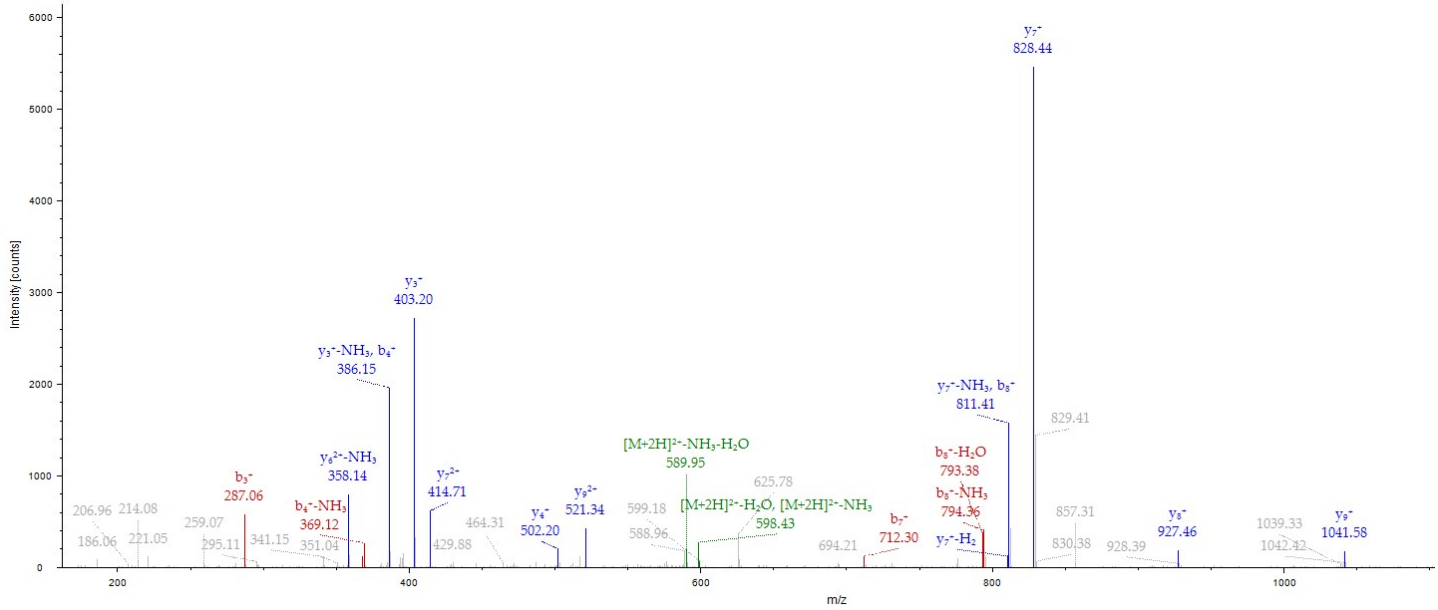
Sequence: TANVPQTVPMR, Charge: +2, Monoisotopic m/z: 607.32208 Da (+0.56 mmu/+0.92 ppm), MH+: 1213.63689 Da, RT: 10.6620 min,
Identified with: Sequest HT (v1.17); XCorr:2.64, Percolator q-Value:0, Percolator PEP:0.149, Ions matched by search engine: 0/0
Fragment match tolerance used for search: 0.6 Da
Fragments used for search: b; b-H_2O; b-NH_3; y; y-H_2O; y-NH_3

#1	b^+	b^{2+}	Seq.	y^+	y^{2+}	#2
1	102.05496	51.53112	T			11
2	173.09207	87.04967	A	1112.58809	556.79768	10
3	287.13500	144.07114	N	1041.55098	521.27913	9
4	386.20341	193.60534	V	927.50805	464.25766	8
5	483.25617	242.13173	P	828.43963	414.72346	7
6	611.31475	306.16101	Q	731.38687	366.19707	6
7	712.36243	356.68485	T	603.32829	302.16778	5
8	811.43084	406.21906	V	502.28061	251.64395	4
9	908.48361	454.74544	P	403.21220	202.10974	3
10	1039.52409	520.26568	M	306.15944	153.58336	2
11			R	175.11895	88.06311	1

#1	b-H_2O^+	b-H_2O^{2+}	b-NH_3^+	b-NH_3^{2+}	Seq.	y-H_2O^+	y-H_2O^{2+}	y-NH_3^+	y-NH_3^{2+}	#2
1	84.04439	42.52583			T					11
2	155.08150	78.04439			A	1094.57752	547.79240	1095.56154	548.28441	10
3	269.12443	135.06585	270.10845	135.55786	N	1023.54041	512.27384	1024.52443	512.76585	9
4	368.19285	184.60006	369.17686	185.09207	V	909.49748	455.25238	910.48150	455.74439	8
5	465.24561	233.12644	466.22962	233.61845	P	810.42907	405.71817	811.41309	406.21018	7
6	593.30419	297.15573	594.28820	297.64774	Q	713.37631	357.19179	714.36032	357.68380	6
7	694.35187	347.67957	695.33588	348.17158	T	585.31773	293.16250	586.30174	293.65451	5
8	793.42028	397.21378	794.40429	397.70579	V			485.25407	243.13067	4
9	890.47304	445.74016	891.45706	446.23217	P			386.18565	193.59646	3
10	1021.51353	511.26040	1022.49754	511.75241	M			289.13289	145.07008	2
11					R			158.09240	79.54984	1

Fragment Spectrum

11292018_Amanda_5.raw #2052 RT: 10.6620 min
ITMS, 607.3221@cid35.00, z=+2, Mono m/z=607.32208 Da, MH+=1213.63689 Da, Match Tol.=0.6 Da



E. Control peptide QASTDAGTAGALTPQHVR is present in the painted YAP2/ZO-1 complex sample 11292018_Amanda_10.

Peptide Summary

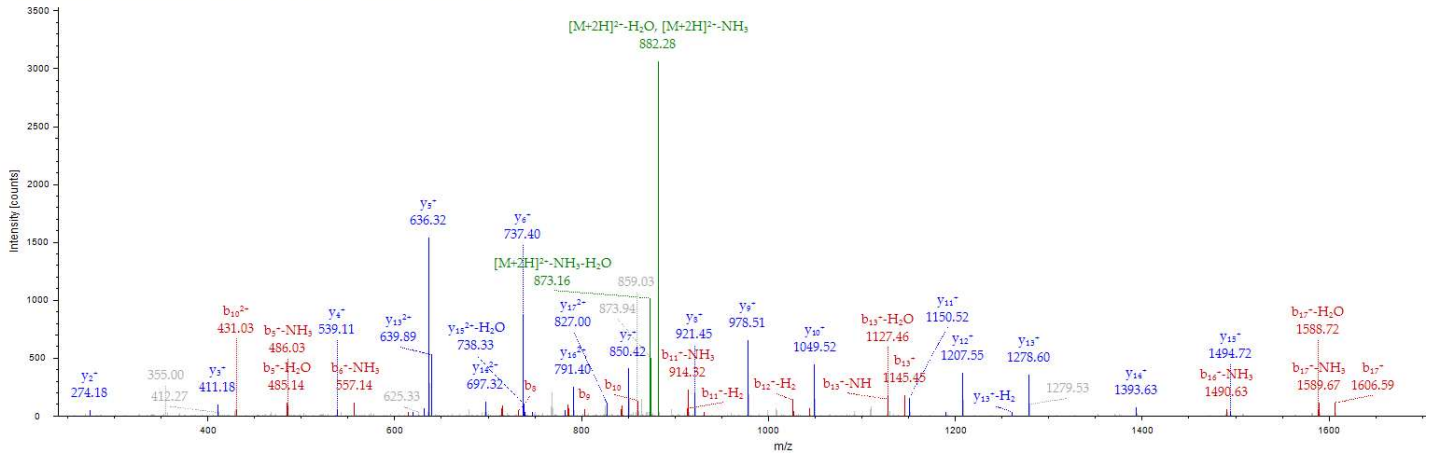
Sequence: QASTDAGTAGALTPQHVR, Charge: +2, Monoisotopic m/z: 890.95129 Da (+0.83 mmu/+0.93 ppm), MH+: 1780.89531 Da, RT: 9.3282 min,
Identified with: Sequest HT (v1.17); XCorr:4.13, Percolator q-Value:0, Percolator PEP:6.43e-05, Ions matched by search engine: 0/0
Fragment match tolerance used for search: 0.6 Da
Fragments used for search: b; b-H_2O; b-NH_3; y; y-H_2O; y-NH_3

#1	b-H_2O^+	b-H_2O^{2+}	b-NH_3^+	b-NH_3^{2+}	Seq.	y-H_2O^+	y-H_2O^{2+}
1			112.03930	56.52329	Q		
2			183.07642	92.04185	A	1634.82451	817.91589
3	269.12443	135.06585	270.10845	135.55786	S	1563.78739	782.39734
4	370.17211	185.58969	371.15613	186.08170	T	1476.75537	738.88132
5	485.19905	243.10316	486.18307	243.59517	D	1375.70769	688.35748
6	556.23617	278.62172	557.22018	279.11373	A	1260.68074	630.84401
7	613.25763	307.13245	614.24165	307.62446	G	1189.64363	595.32545
8	714.30531	357.65629	715.28932	358.14830	T	1132.62217	566.81472
9	785.34242	393.17485	786.32644	393.66686	A	1031.57449	516.29088
10	842.36389	421.68558	843.34790	422.17759	G	960.53737	480.77233
11	913.40100	457.20414	914.38502	457.69615	A	903.51591	452.26159
12	1026.48506	513.74617	1027.46908	514.23818	L	832.47880	416.74304
13	1127.53274	564.27001	1128.51676	564.76202	T	719.39473	360.20100
14	1224.58551	612.79639	1225.56952	613.28840	P		
15	1352.64408	676.82568	1353.62810	677.31769	Q		
16	1489.70300	745.35514	1490.68701	745.84714	H		
17	1588.77141	794.88934	1589.75542	795.38135	V		
18					R		

#1	b-H_2O^+	b-H_2O^{2+}	b-NH_3^+	b-NH_3^{2+}	Seq.	y-H_2O^+	y-H_2O^{2+}	y-NH_3^+	y-NH_3^{2+}	#2
1			112.03930	56.52329	Q					18
2			183.07642	92.04185	A	1634.82451	817.91589	1635.80852	818.40790	17
3	269.12443	135.06585	270.10845	135.55786	S	1563.78739	782.39734	1564.77141	782.88934	16
4	370.17211	185.58969	371.15613	186.08170	T	1476.75537	738.88132	1477.73938	739.37333	15
5	485.19905	243.10316	486.18307	243.59517	D	1375.70769	688.35748	1376.69170	688.84949	14
6	556.23617	278.62172	557.22018	279.11373	A	1260.68074	630.84401	1261.66476	631.33602	13
7	613.25763	307.13245	614.24165	307.62446	G	1189.64363	595.32545	1190.62765	595.81746	12
8	714.30531	357.65629	715.28932	358.14830	T	1132.62217	566.81472	1133.60618	567.30673	11
9	785.34242	393.17485	786.32644	393.66686	A	1031.57449	516.29088	1032.55850	516.78289	10
10	842.36389	421.68558	843.34790	422.17759	G	960.53737	480.77233	961.52139	481.26433	9
11	913.40100	457.20414	914.38502	457.69615	A	903.51591	452.26159	904.49993	452.75360	8
12	1026.48506	513.74617	1027.46908	514.23818	L	832.47880	416.74304	833.46281	417.23504	7
13	1127.53274	564.27001	1128.51676	564.76202	T	719.39473	360.20100	720.37875	360.69301	6
14	1224.58551	612.79639	1225.56952	613.28840	P			619.33107	310.16917	5
15	1352.64408	676.82568	1353.62810	677.31769	Q			522.27831	261.64279	4
16	1489.70300	745.35514	1490.68701	745.84714	H			394.21973	197.61350	3
17	1588.77141	794.88934	1589.75542	795.38135	V			257.16082	129.08405	2
18					R			158.09240	79.54984	1

Fragment Spectrum

11292018_Amanda_10.raw #1862 RT: 9.3282 min
ITMS, 890.9513@cid35.00, z=+2, Mono m/z=890.95129 Da, MH+=1780.89531 Da, Match Tol.=0.6 Da



F. Control peptide TANVPQTVPMR is present in the painted /YAP2/ZO-1 complex sample 11292018_Amanda_10.

Peptide Summary

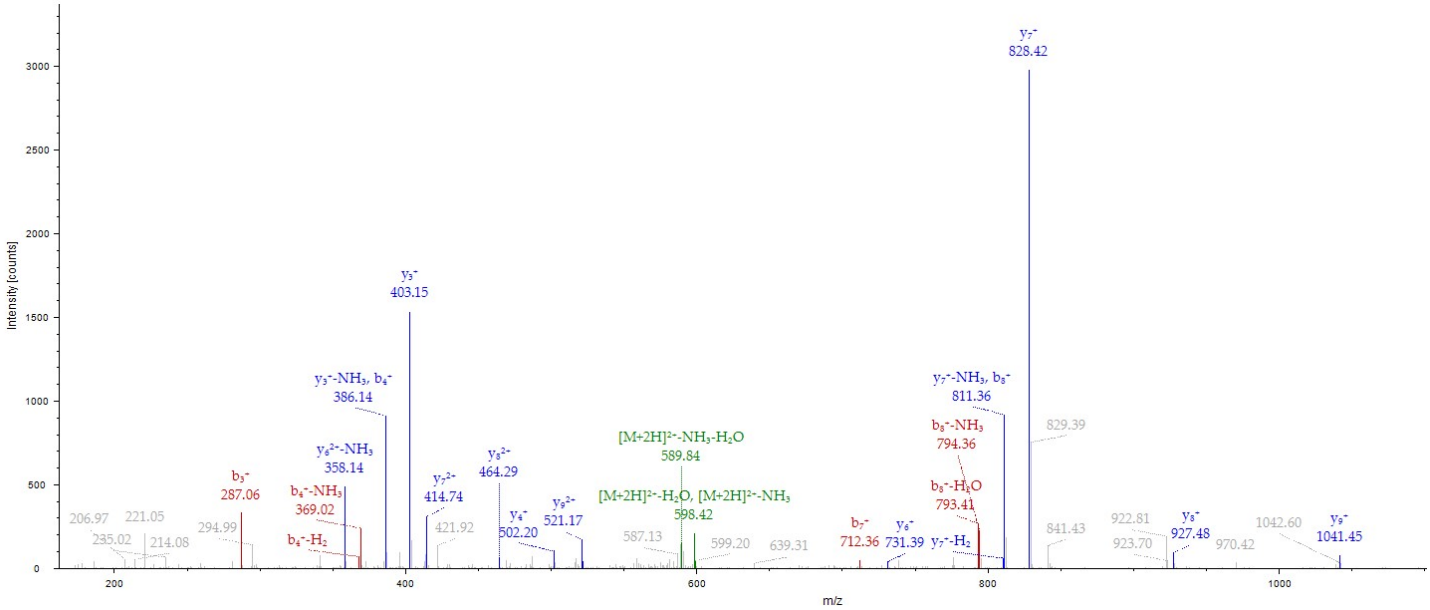
Sequence: TANVPQTVPMR, Charge: +2, Monoisotopic m/z: 607.32166 Da (+0.13 mmu/+0.22 ppm), MH+: 1213.63603 Da, RT: 10.8499 min,
Identified with: Sequest HT (v1.17); XCorr:2.71, Percolator q-Value:0, Percolator PEP:0.0885, Ions matched by search engine: 0/0
Fragment match tolerance used for search: 0.6 Da
Fragments used for search: b; b-H_2O; b-NH_3; y; y-H_2O; y-NH_3

#1	b^+	b^{2+}	Seq.	y^+	y^{2+}	#2
1	102.05496	51.53112	T			11
2	173.09207	87.04967	A	1112.58809	556.79768	10
3	287.13500	144.07114	N	1041.55098	521.27913	9
4	386.20341	193.60534	V	927.50805	464.25766	8
5	483.25617	242.13173	P	828.43963	414.72346	7
6	611.31475	306.16101	Q	731.38687	366.19707	6
7	712.36243	356.68485	T	603.32829	302.16778	5
8	811.43084	406.21906	V	502.28061	251.64395	4
9	908.48361	454.74544	P	403.21220	202.10974	3
10	1039.52409	520.26568	M	306.15944	153.58336	2
11			R	175.11895	88.06311	1

#1	b-H_2O^+	b-H_2O^{2+}	b-NH_3^+	b-NH_3^{2+}	Seq.	y-H_2O^+	y-H_2O^{2+}	y-NH_3^+	y-NH_3^{2+}	#2
1	84.04439	42.52583			T					11
2	155.08150	78.04439			A	1094.57752	547.79240	1095.56154	548.28441	10
3	269.12443	135.06585	270.10845	135.55786	N	1023.54041	512.27384	1024.52443	512.76585	9
4	368.19285	184.60006	369.17686	185.09207	V	909.49748	455.25238	910.48150	455.74439	8
5	465.24561	233.12644	466.22962	233.61845	P	810.42907	405.71817	811.41309	406.21018	7
6	593.30419	297.15573	594.28820	297.64774	Q	713.37631	357.19179	714.36032	357.68380	6
7	694.35187	347.67957	695.33588	348.17158	T	585.31773	293.16250	586.30174	293.65451	5
8	793.42028	397.21378	794.40429	397.70579	V			485.25407	243.13067	4
9	890.47304	445.74016	891.45706	446.23217	P			386.18565	193.59646	3
10	1021.51353	511.26040	1022.49754	511.75241	M			289.13289	145.07008	2
11					R			158.09240	79.54984	1

Fragment Spectrum

11292018_Amanda_10.raw #2167 RT: 10.8499 min
ITMS, 607.3217@cid35.00, z=+2, Mono m/z=607.32166 Da, MH+=1213.63603 Da, Match Tol.=0.6 Da



A

Control Peptide TANVPQTVPMR found in all samples

Hotspot Peptide KLPDSFFKPPEPK disappears when painted, reappears when complexed; cleavage blocked at R89

Control Peptide QASTDAGTAGALTPQHVR found in all samples

```
Unpainted Amanda_1:    71 AVMNPKTANV PQTVPMRLRK LPDSFFKPPE PKSHSRQAST DAGTAGALTP QHVRAHSSPA 130
Painted Amanda_5:      71 AVMNPKTANV PQTVPMRLRK LPDSFFKPPE PKSHSRQAST DAGTAGALTP QHVRAHSSPA 130
Complexed Sample 10:   71 AVMNPKTANV PQTVPMRLRK LPDSFFKPPE PKSHSRQAST DAGTAGALTP QHVRAHSSPA 130

Unpainted Amanda_1:   141 TLTPTGVVSG PAATPTAQHL RQSSFEIPDD VPLPAGWEMA KTSSGGRYFL NHIDQTTTWQ DPRK 203
Painted Amanda_5:     141 TLTPTGVVSG PAATPTAQHL RQSSFEIPDD VPLPAGWEMA KTSSGGRYFL NHIDQTTTWQ DPRK 203
Complexed Amanda_10:  141 TLTPTGVVSG PAATPTAQHL RQSSFEIPDD VPLPAGWEMA KTSSGGRYFL NHIDQTTTWQ DPRK 203
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Hotspot Peptide QSSFEIPDDVPLPAGWEMAK disappears when painted, reappears when complexed; cleavage blocked at R161

Hotspot Peptide YFLNHIDQTTTWQDPRK disappears when painted, reappears when complexed; cleavage blocked at R187

B

R89 Interaction Site (Peptide KLPDSFFKPPEPK)

Fisher's Exact Test, Freeman-Halton Extension: p = 0.002

	Unpainted Samples	Painted Samples	Complexed Samples
Peptide Present	6	1	6
Peptide Absent	0	5	0

R161 Interaction Site (Peptide QSSFEIPDDVPLPAGWEMAK)

Fisher's Exact Test, Freeman-Halton Extension: p = 0.004

	Unpainted Samples	Painted Samples	Complexed Samples
Peptide Present	5	0	5
Peptide Absent	1	6	1

R187 Interaction Site (Peptide YFLNHIDQTTTWQDPR)

Fisher's Exact Test, Freeman-Halton Extension: p = 0.012

	Unpainted Samples	Painted Samples	Complexed Samples
Peptide Present	6	1	5
Peptide Absent	0	5	1

Supplementary Figure 12: YAP2 hotspots for its interaction with ZO-1 are determined by differential analysis of unpainted YAP2, painted YAP2, and painted YAP2/ZO-1 complex. A) To determine the presence of YAP2 hotspots for an independent mass spectrometry experiment, the sequence of YAP2 for an unpainted sample, a painted sample, and a complexed sample are aligned, and peptides identified in each sample are highlighted in green, using the trials depicted in Supplementary Figures 7 and 8. Peptides which are present in the unpainted sample, absent in the painted sample, and return in the complexed sample are considered indicative of regions that are solvent-accessible in the painted sample, but not solvent-accessible in the complexed sample, such as KLPDSFFKPPEPK. The missed tryptic cleavage site, such as R89 for the peptide KLPDSFFKPPEPK, is the identified hotspot. Experiments were completed with 2 technical replicates per trial for three independent trials, resulting in 6 samples per condition. Significant difference between subgroups was evaluated by Fisher's exact test with the Freeman-Halton expansion for *m x n* tables. B) Hotspots were considered statistically significant when a peptide was present in 5 out of 6 unpainted samples, absent in 5 out of 6 painted samples, and present in 5 out of 6 painted complexed samples (p = 0.03 via Fisher's exact test with the Freeman-Halton extension; greater than 95% confidence to reject the null hypothesis that presence or absence of a peptide is group-independent). Hotspots R89 (p = 0.0012), R161 (p = 0.004), and R187 (p = 0.012) were statistically significant.

Supplementary Figure 13. Sequence Alignment of YAP2 from 11 diverse species shows that hotspots identified via protein painting are found in the N-terminal proline-rich region that is not as heavily conserved in evolution. *Homo sapiens YAP2* is shown in red, and hotspots identified in complex with ZO-1 are highlighted in yellow.

```
CLUSTAL O(1.2.4) multiple sequence alignment

Species represented:
Homo sapiens (Human; NP_003248.3)
Aliuropoda melanoleuca (Giant Panda; XP_011226750.1)
Odobenus rosmarus divergens (Walrus, XP_012423426.1)
Balaenoptera acutorostrata scammoni (Minke whale, XP_007191278.1)
Melopsittacus undulates (Parakeet, XP_005149868.2)
Struthio camelus australis (Ostrich, XP_009685064.1)
Pogona vitticeps (Central bearded dragon lizard, XP_020651649.1)
Python bivittatus (Burmese python, XP_007434822.1)
Callorhinchus milii (Elephant shark, XP_007890013.1)
Salmo salar (Atlantic salmon, XP_014024139.1)
Hippocampus comes (Tiger tail seahorse, XP_019742899.1)


S.salar        ----------------------------------------msnplqpmpgqqvihvaqd 19
H.comes        ---------------------------------------mdpsqhnppaghqivhvrgd 20
C.milli        ------------------------------------------------------------ 0
P.bivittatus   ------------------------------------------------------------ 0
A.melanoleuca  ------------------------------------------------------------ 0
P.vitticeps    ------------------------------------------------------------ 0
M.undulatus    ------------------------------------------------------------ 0
S.australis    ------------------------------------------------------------ 0
O.divergens    ------------------------------------------------------------ 0
H.sapiens      mdpgqqpppqpapqgqgqppsqppqgqgppsgpgqpapaatqaapqappaghqivhvrgd 60
B.scammoni     ------------------------------------------------------------ 0

S.salar        sdtdlealfnsvmnpkpsswrk-------kmlpesffkepdsgshsrqsstdsgshpprl 72
H.comes        setdlealfnavmnpkntivppsvpmrmr-klpdsffkppepkshsrqastdagtg-gal 78
C.milli        ------------------------------------------------------------ 0
P.bivittatus   ------------------------------------------------------------ 0
A.melanoleuca  -------mt---------narlalalefapgtgarlwrprrp--lpwpastdagta-gal 41
P.vitticeps    ---------------------------mk-hfterhle--ratfygiwastdagta-gal 29
M.undulatus    ------------------------------------------------wqastdagta-gal 13
S.australis    ------------------------------------------------------------ 0
O.divergens    ------------mnpktanvpqtvpmrlr-klpdsffkppepkshsrqastdagta-gal 46
H.sapiens      setdlealfnavmnpktanvpqtvpmrlr-KLPDSFFKPPEPKshsrqastdagta-gal 118
B.scammoni     ------------------------------------------------------------ 0


S.salar        tvqhvrshsspaslqlglganptps----------pvhhshirhqsfdvaeelplppgwe 122
H.comes        tphhvrahsspaslqmgtvsggslsg-----mpaaaaspqhlrqssyeipddvplppgwe 133
C.milli        ---------------------------------------hlrqpsyeypddvplppgwe 20
P.bivittatus   ------------------------------------------------------------ 0
A.melanoleuca  tpqhvrahsspaslqlgavspgnltatgvvsgpaaaptaqhlrqssfeipddvplpagwe 101
P.vitticeps    tpqhvrahsspaslqlgtispgtltpssvvpgaa---psqhlrqssfeipddvplppgwe 86
M.undulatus    tpqhvrahsspaslqlgavspgtltpsgvvtgpga-pasqhlrqssfeipddvplppgwe 72
S.australis    ------------------------------------------------------------ 0
O.divergens    pqhvrahsspaslqlgavspgsltptgvvsgpaaaptaqhlrqssfeipddvplpagwe  106
H.sapiens      tpqhvrahsspaslqlgavspgtltptgvvsgpaatptaqhlrQSSFEIPDDVPLPAGWE 178
B.scammoni     ------------------------------------------------------------ 0


S.salar        maytstgqkyflnhvekittwhdprktmtaamnqmslhapppsatpqqrnmalsqpnlge 182
H.comes        maktasgqryflnhidqtttwqdprkallqmnqapp----pssvpvqqqn--lmnpasgp 187
C.milli        maktpsgqryylnhvdqtttwqdprktmpvsvsvpa----sp-vsvqqnl--mnttpsgp 73
P.bivittatus   -------------------------------mnvta----pt-sppvqqn--imnsaa-- 20
A.melanoleuca  maktssgqryflnhidqtttwqdprktmlsqmsvta----pt-sppvqqs--lmtsasgp 154
P.vitticeps    maktpsgqryflnhidqtttwqdprkallsqmnvta----pt-sppvqqn--imnsatgp 139
M.undulatus    maktpsgqryflnhidqtttwqdprkamlsqmnvta----pt-spsvqqn--imnsasgp 125
S.australis    ------------------------------------------------------------ 0
O.divergens    maktssgqryflnhidqtttwqdprkamlsqmsvta----pt-sppvqqs--lmtsasgp 159
```

```
H.sapiens       MAKtssgqRYFLNHIDQTTTWQDPRkamlsqmnvta----pt-sppvqqn--mmnsasgp 231
B.scammoni      maktssgqryflnhidqtttwqdprkamlsqmnvts----pt-tppvqqn--mmnsasgp 53


S.salar         lpdgweqaenpsgetyfidhktqrtswidprvdarlmmsqhhhqqqlqhtqqqqpqqaqa 242
H.comes         lpegweqaitsegeiyyinhknkttswldprldprfaln-qqrisqs-----------ap 235
C.milli         fpdgweqgmtpdgeiyyinhknkttswldprldprfamd-qqrltqs-----------ap 121
P.bivittatus    ---------------------------------------amsqrisqs-----------ap 31
A.melanoleuca   lpdgweqgvtqdgeiyyinhknkttswldprldprfa--mnqrisqs-----------ap 201
P.vitticeps     lpdgweqamtqdgeiyyinhknkttswldprldprfa--msqrisqs-----------vp 186
M.undulatus     lpdgweqamtqdgeiyyinhknkttswldprldprfa--mnqrisqs-----------ap 172
S.australis     ---------------------------------------mnqrisqs-----------ap 10
O.divergens     lpdgweqavtqdgeiyyinhknkttswldprldprfa--mnqrvsqs-----------ap 206
H.sapiens       lpdgweqamtqdgeiyyinhknkttswldprldprfa--mnqrisqs-----------ap 278
B.scammoni      lpdgweqamtqdgeiyyinhknkttswldprldprfgkamnqrisqs-----------ap 102
                                                          ::  *

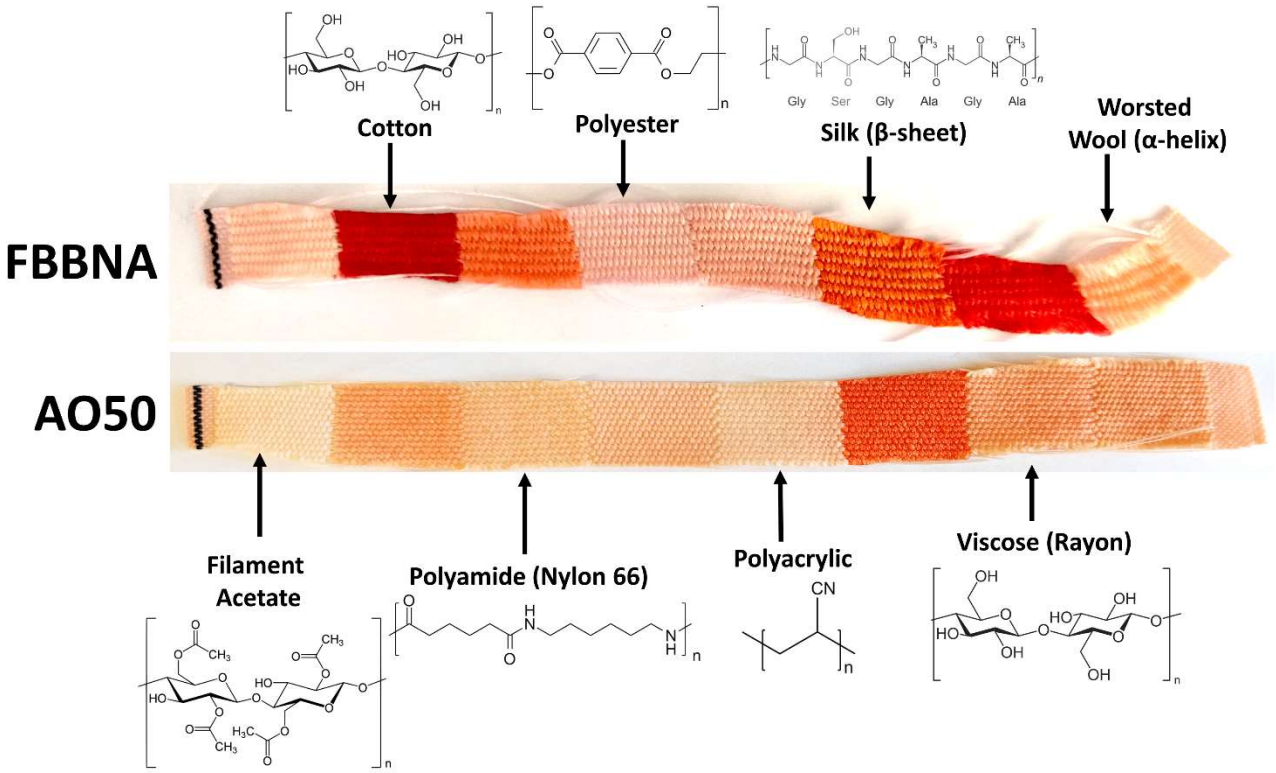
S.salar         lgspgslpqq--qssqagmmslssplgvgvssqhqqkmrlqriqlereriqrrqeelmrq 300
H.comes         vkqggqlp---------------------sgtlsgvnqlrlq--kmekerlrlkqel-lrq 272
C.milli         vkptssmgphsphspqggvi-------sgsn--kqqqmrlqqlqfekerlrlkhqellrq 172
P.bivittatus    vkqp--ppplapqsppggvl-------gggnsnqqqqirlqqlqmekerlrlkhqellrq 82
A.melanoleuca   vkqp---pplapqsppggvl-------gsggssqqqqmrlqqlqmekerlrlkqqellrq 251
P.vitticeps     vk-p---pplapqsppggvl-------gsnnsnqqqqmrlqqlqmekerlrlkhqellrq 235
M.undulatus     vkqp---pplapqspqggvm-------ggsssnqqqqmrlqqlqmekerlrlkhqellrq 222
S.australis     vkqp---pplapqspqggvm-------ggsssnqqqqmrlqqlqmekerlrlkhqellrq 60
O.divergens     vkqp---pplapqsppggvl-------gsggssqqqqmrlqqlqmekerlrlkqqellrq 256
H.sapiens       vkqp---pplapqspqggvm-------ggsnsnqqqqmrlqqlqmekerlrlkqqellrq 328
B.scammoni      vkqp---pplapqspqggvm-------gggnsnqqqqmrlqqlqmekerlrlkqqellrq 152
                                     :         :::***  ::*:**:: :::  :**

S.salar         --------------------evalcrqlpmdsesmapvptpggnpaqpmtqgnmptngad 340
H.comes         -----------------rpqelalrnqlptsmeqdgggtnpvssp-maqdartmtanssd 314
C.milli         --------------------eialrnqlpt-meqdggsqnvvsspamgqdvrtmttnssd 211
P.bivittatus    vrpqalrninpstanspkrqelalrsqlps-meqdg-sqnrvsspgmsqelrtmtttssd 140
A.melanoleuca   ----amrninpstanspkcqelalrsqlpt-leqdagtpnpvpspgmsqelrtmttsgsd 306
P.vitticeps     ----alrninpstanspkrqelalrsqlps-meqdv-sqnpvsspgmsqelrtmttnssd 289
M.undulatus     ----alrninpstanspkhqelalrsqlpt-meqdggsqnpvsspgmsqelrtmttnssd 277
S.australis     ----alrninpstanspkhqelalrsqlpt-meqdggsqnpvsspgmsqelrtmttnssd 115
O.divergens     --------------------elalrsqlpt-leqdggtpnpvpspgmsqelrtmttsgsd 295
H.sapiens       ----amrninpstanspkcqelalrsqlpt-leqdggtqnpvsspgmsqelrtmttnssd 383
B.scammoni      vrpqamrninpstanspkcqelalrsqlpt-leqdggtqnpvsspgmsqelrtmttnssd 211
                                    *:**  ***   *.     .   .*       .* :..:*

S.salar         pflnsghfqhsreqstdsglglgcysipttpedflnnmeemdtgesmaqagnmnvpqhsr 400
H.comes         pflnsgty-hsrdestdsglsmssysvprtpddflnsvdemdtgdplp-ss--mgtqpsr 370
C.milli         pflnsgty-hsrdestdsglgmssysvprtpddflnsveemdtgeslgpnn--masqqnr 268
P.bivittatus    pflnsgty-hsrdestdsglsmssysvprtpddflnsvdemdtgdtinqst--ipshqnr 197
A.melanoleuca   pflnsgty-hsrdestdsglsmssysvprtpddflnsvdemdtgdtinqst--lpsqqnr 363
P.vitticeps     pflnsgty-hsrdestdsglsmssysvprtpddflnsvdemdtgdsinqsn--mpshqnr 346
M.undulatus     pflnsgty-hsrdestdsglsmssysvprtpddflnsvdemdtgdsisqsn--ipshqnr 334
S.australis     pflnsgty-hsrdestdsglsmssysvprtpddflnsvdemdtgdsisqsn--ipshqnr 172
O.divergens     pflnsgty-hsrdestdsglsmssysvprtpddflnsvdemdtgdtinqst--lpsqqnr 352
H.sapiens       pflnsgty-hsrdestdsglsmssysvprtpddflnsvdemdtgdtinqst--lpsqqnr 440
B.scammoni      pflnsgty-hsrdestdsglsmssysvprtpddflnsvdemdtgdtinqst--lpsqqnr 268
                ****** : ***::******.:..**:* **:****.::*****: :         : .*

S.salar         fpdfldslpgtnvdlgtlegadl-------i---------pilndvesvln----ksepf 440
H.comes         fpdyldaipgtdvdlgtlegesmavegeelmpslqealsseilndmesvlaatkidkesf 430
C.milli         fpdyletipgtnvdlgtlegesmnvegeelmpslqealssdilndmesvlaatkldkesf 328
P.bivittatus    fpdyleaipgtnvdlgtlegdamniegeelmpslqealssdilndmesvlaatkldkesf 257
A.melanoleuca   fpdyleaipgtnvdlgtlegdgmniegeelmpslqealssdilndmesvlaatkldkesf 423
P.vitticeps     fpdyleaipgtnvdlgtlegdamniegeelmpslqealssdilndmesvlaatkldkesf 406
M.undulatus     fpdyleaipgtnvdlgtlegdgmniegeelmpslqealssdilndmesvlaatkldkesf 394
S.australis     fpdyleaipgtnvdlgtlegdgmniegeelmpslqealcsdilndmesvlaatkldkesf 232
O.divergens     fpdyleaipgtnvdlgtlegdgmniegeelmpslqealssdilndmesvlaatkldkesf 412
H.sapiens       fpdyleaipgtnvdlgtlegdgmniegeelmpslqealssdilndmesvlaatkldkesf 500
B.scammoni      fpdyleaipgtnvdlgtlegdgmniegeelmpslqealssdilndmesvlaatkldkesf 328
                ***:*:::***:********  :       :          ****:****     ..* *
```

```
S.salar        ltwl  444
H.comes        ltwl  434
C.milli        ltwl  332
P.bivittatus   ltwl  261
A.melanoleuca  ltwl  427
P.vitticeps    ltwl  410
M.undulatus    ltwl  398
S.australis    ltwl  236
O.divergens    ltwl  416
H.sapiens      ltwl  504
B.scammoni     ltwl  332
               ****
```



Supplementary Figure 14. Fabric of various types dyed with FBBNA or AO50 reveals significant binding of FBBNA to carbohydrate-based fabrics cotton and viscose. A fabric strip composed of sections of filament acetate, cotton, polyamide, polyester, polyacrylic, silk, viscose, and worsted wool was incubated with FBBNA or AO50 dye for 2 hours with shaking, washed until rinse was clear, then dried. FBBNA showed a strong affinity for cotton and viscose (structures shown), but had poor affinity for other carbohydrate-based fibers such as filament acetate. FBBNA also showed strong affinity for silk and polyamide, which are more representative of proteins. FBBNA bound poorly to polyester, polyacrylic, and worsted wool (protein covered with a cuticle layer). AO50 on the other hand bound poorly to all fabrics but silk.

Supplementary Figure 15: One individual hotspot on PD-1 was identified when complexed with PD-L1. Hotspots are identified as the tryptic cleavage sites blocked by dye treatment but protected during complexation with a protein binding partner. This leads to an identification pattern for hotspots where hotspot peptides are present in unpainted samples, absent in painted samples due to blockage of the tryptic cleavage site by dye coverage, and present in the dyed complexed samples where complexation protects the tryptic cleavage site from dye coverage. A) Peptide MSPSNQTDKLAAFPEDR from PD-1, following tryptic cleavage at R69 (missed cleavage at K78), is identified by MS/MS in the unpainted sample 11222015_Angela_PD1.3. B) All PD-1 peptides identified within 1% FDR in the PD-1 painted sample 11222015_Angela_PD1_AO50; neither the MSPSNQTDKLAAFPEDR nor the shorter KLAAFPEDR peptides were identified in the sample, indicating blockage of R69 and K78 by the dye treatment. C) Peptide KLAAFPEDR from PD-1, following tryptic cleavage at K78, is identified by MS/MS in the painted complexed sample 11222015_Angela_Com_AO50.

A. R69 and K78 of unpainted PD-1 sample 11222015_Angela_PD1 is identified by the presence of peptide MSPSNQTDKLAAFPEDR tandem mass spectrum matched via Sequest

Peptide Summary

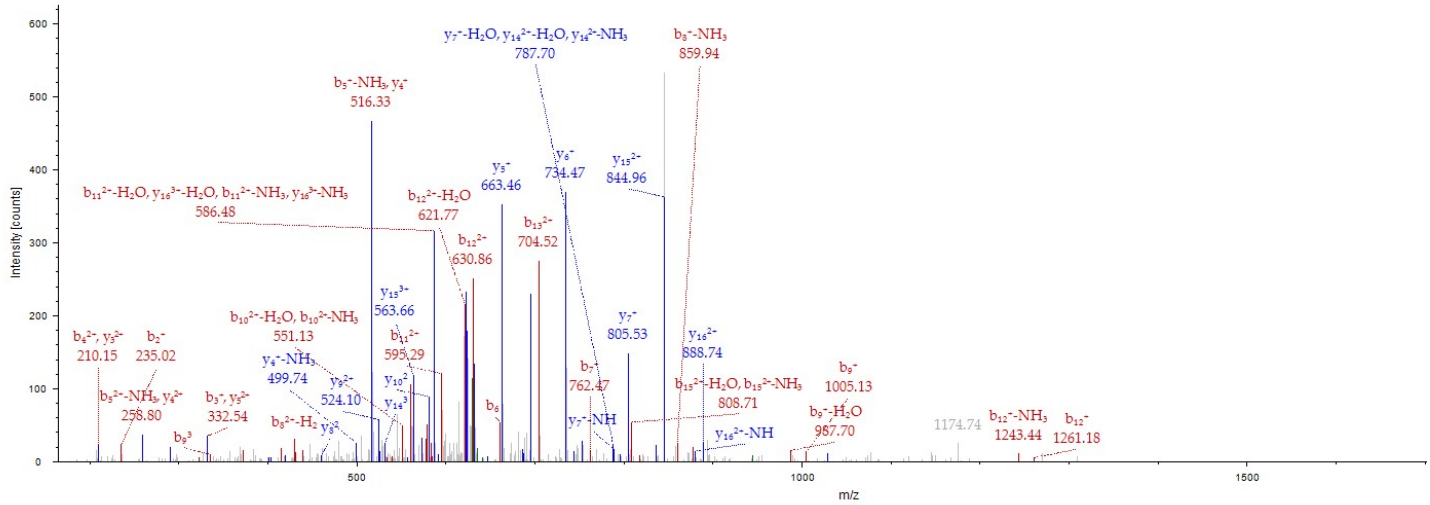
Sequence: MSPSNQTDKLAAFPEDR, M1-Oxidation (15.99492 Da)
Charge: +3, Monoisotopic m/z: 641.63409 Da (-1.18 mmu/-1.84 ppm), MH+: 1922.88773 Da, RT: 26.4456 min,
Identified with: Sequest HT (v1.17); XCorr:3.04, Percolator q-Value:0, Percolator PEP:0.0222, Ions matched by search engine: 0/0
Fragment match tolerance used for search: 0.6 Da

#1	b^+	b^{2+}	b^{3+}	Seq.	y^+	y^{2+}	y^{3+}	#2
1	148.04268	74.52498	50.01908	M-Oxidation				17
2	235.07470	118.04099	79.02975	S	1775.85587	888.43157	592.62347	16
3	332.12747	166.56737	111.38067	P	1688.82384	844.91556	563.61280	15
4	419.15950	210.08339	140.39135	S	1591.77107	796.38918	531.26188	14
5	533.20242	267.10485	178.40566	N	1504.73905	752.87316	502.25120	13
6	661.26100	331.13414	221.09185	Q	1390.69612	695.85170	464.23689	12
7	762.30868	381.65798	254.77441	T	1262.63754	631.82241	421.55070	11
8	877.33562	439.17145	293.11673	D	1161.58986	581.29857	387.86814	10
9	1005.43059	503.21893	335.81505	K	1046.56292	523.78510	349.52582	9
10	1118.51465	559.76096	373.50973	L	918.46796	459.73762	306.82750	8
11	1189.55176	595.27952	397.18877	A	805.38389	403.19559	269.13282	7
12	1260.58888	630.79808	420.86781	A	734.34678	367.67703	245.45378	6
13	1407.65729	704.33228	469.89061	F	663.30967	332.15847	221.77474	5
14	1504.71005	752.85867	502.24154	P	516.24125	258.62426	172.75194	4
15	1633.75265	817.37996	545.25573	E	419.18849	210.09788	140.40101	3
16	1748.77959	874.89343	583.59805	D	290.14590	145.57659	97.38682	2
17				R	175.11895	88.06311	59.04450	1

#1	$b\text{-}H_2O^+$	$b\text{-}H_2O^{2+}$	$b\text{-}H_2O^{3+}$	$b\text{-}NH_3^+$	$b\text{-}NH_3^{2+}$	$b\text{-}NH_3^{3+}$	Seq.	$y\text{-}H_2O^+$	$y\text{-}H_2O^{2+}$	$y\text{-}H_2O^{3+}$	$y\text{-}NH_3^+$	$y\text{-}NH_3^{2+}$	$y\text{-}NH_3^{3+}$	#2
1							M-Oxidation							17
2	217.06414	109.03571	73.02623				S	1757.84530	879.42629	586.61995	1758.82932	879.91830	586.94796	16
3	314.11690	157.56209	105.37715				P	1670.81327	835.91028	557.60928	1671.79729	836.40228	557.93728	15
4	401.14893	201.07810	134.38783				S	1573.76051	787.38389	525.25835	1574.74453	787.87590	525.58636	14
5	515.19186	258.09957	172.40214	516.17588	258.59158	172.73014	N	1486.72848	743.86788	496.24768	1487.71250	744.35989	496.57568	13
6	643.25044	322.12886	215.08833	644.23445	322.62086	215.41634	Q	1372.68555	686.84642	458.23337	1373.66957	687.33842	458.56137	12
7	744.29812	372.65270	248.77089	745.28213	373.14470	249.09889	T	1244.62698	622.81713	415.54718	1245.61099	623.30913	415.87518	11
8	859.32506	430.16617	287.11320	860.30907	430.65818	287.44121	D	1143.57930	572.29329	381.86462	1144.56331	572.78530	382.19262	10
9	987.42002	494.21365	329.81152	988.40404	494.70566	330.13953	K	1028.55236	514.77982	343.52230	1029.53637	515.27182	343.85031	9
10	1100.50409	550.75568	367.50621	1101.48810	551.24769	367.83422	L	900.45739	450.73233	300.82398	901.44141	451.22434	301.15199	8
11	1171.54120	586.27424	391.18525	1172.52521	586.76625	391.51326	A	787.37333	394.19030	263.12929	788.35734	394.68231	263.45730	7
12	1242.57831	621.79279	414.86429	1243.56233	622.28480	415.19229	A	716.33621	358.67175	239.45026	717.32023	359.16375	239.77826	6
13	1389.64673	695.32700	463.88709	1390.63074	695.81901	464.21510	F	645.29910	323.15319	215.77122	646.28312	323.64520	216.09922	5
14	1486.69949	743.85338	496.23801	1487.68351	744.34539	496.56602	P	498.23069	249.61898	166.74841	499.21470	250.11099	167.07642	4
15	1615.74208	808.37468	539.25221	1616.72610	808.86669	539.58022	E	401.17792	201.09260	134.39749	402.16194	201.58461	134.72550	3
16	1730.76903	865.88815	577.59453	1731.75304	866.38016	577.92253	D	272.13533	136.57130	91.38329	273.11935	137.06331	91.71130	2
17							R				158.09240	79.54984	53.36899	1

Fragment Spectrum

10222015_Angela_PD1.RAW #2446 RT: 26.4456 min
ITMS, 641.9688@cid30.00, z=+3, Mono m/z=641.63409 Da, MH+=1922.88773 Da, Match Tol.=0.6 Da



B. Painted PD-1 sample 11222015_Angela_PD1_AO50 does not contain peptides MSPSNQTDKLAAFPEDR or KLAAFPEDR of PD-1 within the PD-1-derived peptide set as identified by Sequest

Confidence	Sequence	Modifications	Quality PEP	Master Protein Accessions	Theo. MH+ [Da]	XCorr A Sequest HT
High	VTQLPNGR		0.180939	167857792	884.4948	1.61
High	NDSGTYLCGAISLAPK	1xCarbamidomethyl [C8]	0.023518	167857792	1666.811	4.14
High	VTQLPNGRDFHMSVVR	1xOxidation [M12]	0.588718	167857792	1871.954	1.2
High	DFHMSVVR		0.181918	167857792	990.4826	1.74
High	DFHMSVVR	1xOxidation [M4]	0.517142	167857792	1006.477	1.56
High	FRVTQLPNGR		0.156064	167857792	1187.664	2.1

C. K78 of painted PD-1/PD-L1 complex sample 11222015_Angela_Com_AO50 is identified by the presence of peptide LAAFPEDR tandem mass spectrum matched via Sequest

Peptide Summary

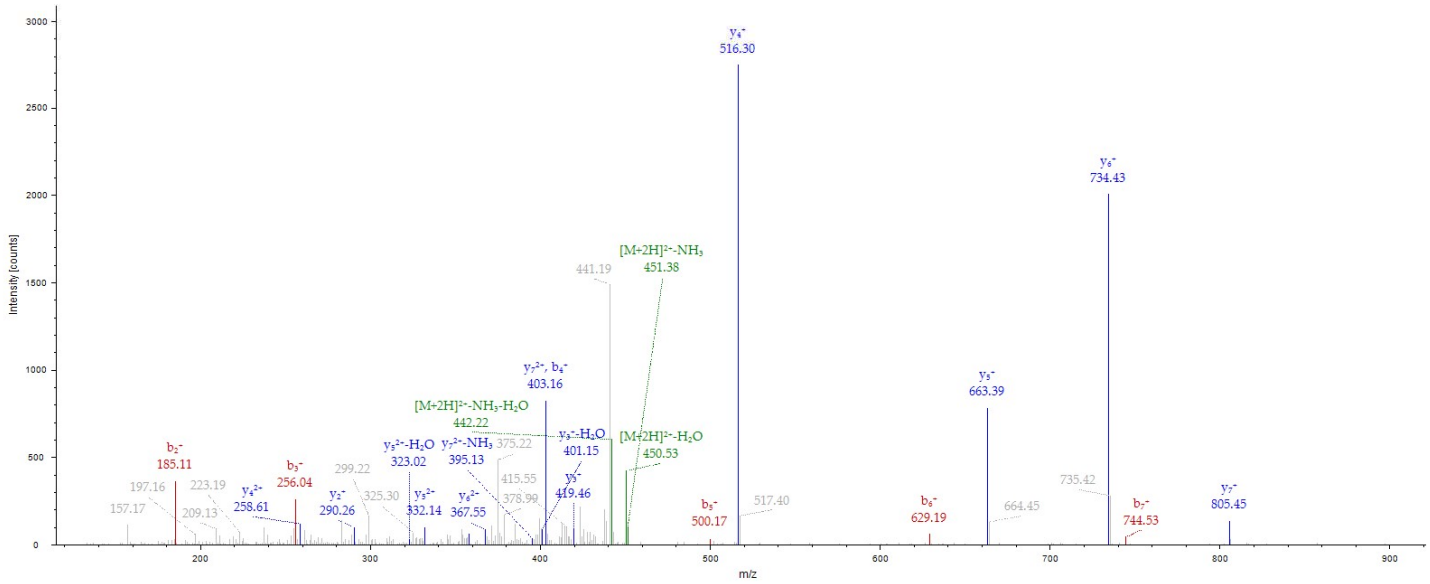
Sequence: LAAFPEDR, Charge: +2, Monoisotopic m/z: 459.73505 Da (-2.57 mmu/-5.6 ppm), MH+: 918.46282 Da, RT: 23.8086 min,
Identified with: Sequest HT (v1.17); XCorr:2.05, Percolator q-Value:0, Percolator PEP:0.0858, Ions matched by search engine: 0/0
Fragment match tolerance used for search: 0.6 Da
Fragments used for search: b; b-H_2O; y; y-H_2O; y-NH_3

#1	b^+	b^{2+}	Seq.	y^+	y^{2+}	#2
1	114.09134	57.54931	L			8
2	185.12845	93.06787	A	805.38389	403.19559	7
3	256.16557	128.58642	A	734.34678	367.67703	6
4	403.23398	202.12063	F	663.30967	332.15847	5
5	500.28675	250.64701	P	516.24125	258.62426	4
6	629.32934	315.16831	E	419.18849	210.09788	3
7	744.35628	372.68178	D	290.14590	145.57659	2
8			R	175.11895	88.06311	1

#1	b-H_2O^+	b-H_2O^{2+}	Seq.	y-H_2O^+	y-H_2O^{2+}	y-NH_3^+	y-NH_3^{2+}	#2
1			L					8
2			A	787.37333	394.19030	788.35734	394.68231	7
3			A	716.33621	358.67175	717.32023	359.16375	6
4			F	645.29910	323.15319	646.28312	323.64520	5
5			P	498.23069	249.61898	499.21470	250.11099	4
6	611.31877	306.16303	E	401.17792	201.09260	402.16194	201.58461	3
7	726.34572	363.67650	D	272.13533	136.57130	273.11935	137.06331	2
8			R			158.09240	79.54984	1

Fragment Spectrum

10222015_Angela_Com_A050.RAW #1821 RT: 23.8086 min
ITMS, 459.7354@cid30.00, z=+2, Mono m/z=459.73505 Da, MH+=918.46282 Da, Match Tol.=0.6 Da



Supplementary Figure 16: Control peptides in PD-1

A. Control peptide FRVTQLPNGR is present in the unpainted PD-1 sample 11222015_Angela_PD1.

Peptide Summary

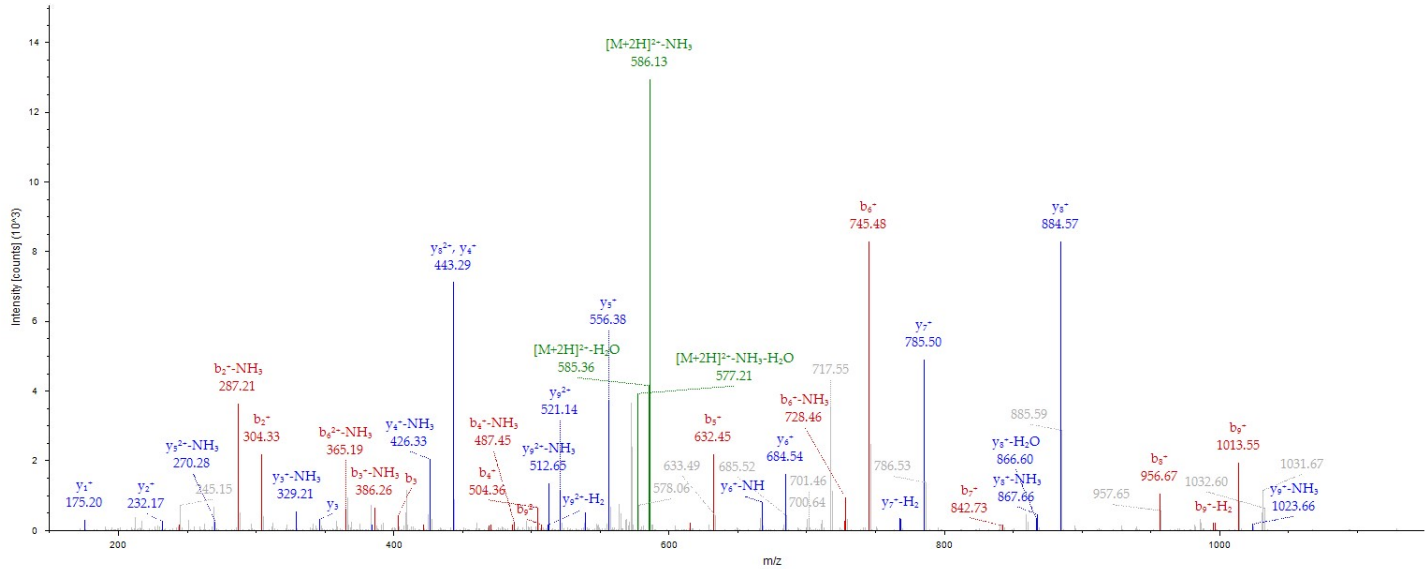
Sequence: FRVTQLPNGR, Charge: +2, Monoisotopic m/z: 594.33337 Da (-2.45 mmu/-4.12 ppm), MH+: 1187.65947 Da, RT: 24.7657 min,
Identified with: Sequest HT (v1.17); XCorr:2.37, Percolator q-Value:0, Percolator PEP:0.26, Ions matched by search engine: 0/0
Fragment match tolerance used for search: 0.6 Da
Fragments used for search: b; b-H_2O; b-NH_3; y; y-H_2O; y-NH_3

#1	b^+	b^{2+}	Seq.	y^+	y^{2+}	#2
1	148.07569	74.54148	F			10
2	304.17680	152.59204	R	1040.59595	520.80161	9
3	403.24522	202.12625	V	884.49484	442.75106	8
4	504.29289	252.65009	T	785.42643	393.21685	7
5	632.35147	316.67937	Q	684.37875	342.69301	6
6	745.43554	373.22141	L	556.32017	278.66372	5
7	842.48830	421.74779	P	443.23611	222.12169	4
8	956.53123	478.76925	N	346.18334	173.59531	3
9	1013.55269	507.27998	G	232.14042	116.57385	2
10			R	175.11895	88.06311	1

#1	b-H_2O^+	b-H_2O^{2+}	b-NH_3^+	b-NH_3^{2+}	Seq.	y-H_2O^+	y-H_2O^{2+}	y-NH_3^+	y-NH_3^{2+}	#2
1					F					10
2			287.15025	144.07876	R	1022.58539	511.79633	1023.56940	512.28834	9
3			386.21867	193.61297	V	866.48428	433.74578	867.46829	434.23778	8
4	486.28233	243.64480	487.26634	244.13681	T	767.41586	384.21157	768.39988	384.70358	7
5	614.34091	307.67409	615.32492	308.16610	Q			667.35220	334.17974	6
6	727.42497	364.21612	728.40899	364.70813	L			539.29362	270.15045	5
7	824.47773	412.74251	825.46175	413.23451	P			426.20956	213.60842	4
8	938.52066	469.76397	939.50468	470.25598	N			329.15679	165.08204	3
9	995.54213	498.27470	996.52614	498.76671	G			215.11387	108.06057	2
10					R			158.09240	79.54984	1

Fragment Spectrum

10222015_Angela_PD1.RAW #2188 RT: 24.7657 min
ITMS, 594.3341@cid30.00, z=+2, Mono m/z=594.33337 Da, MH+=1187.65947 Da, Match Tol.=0.6 Da



B. Control peptide NDSGTYLCGAISLAPK is present in the unpainted PD-1 sample 11222015_Angela_PD1.

Peptide Summary

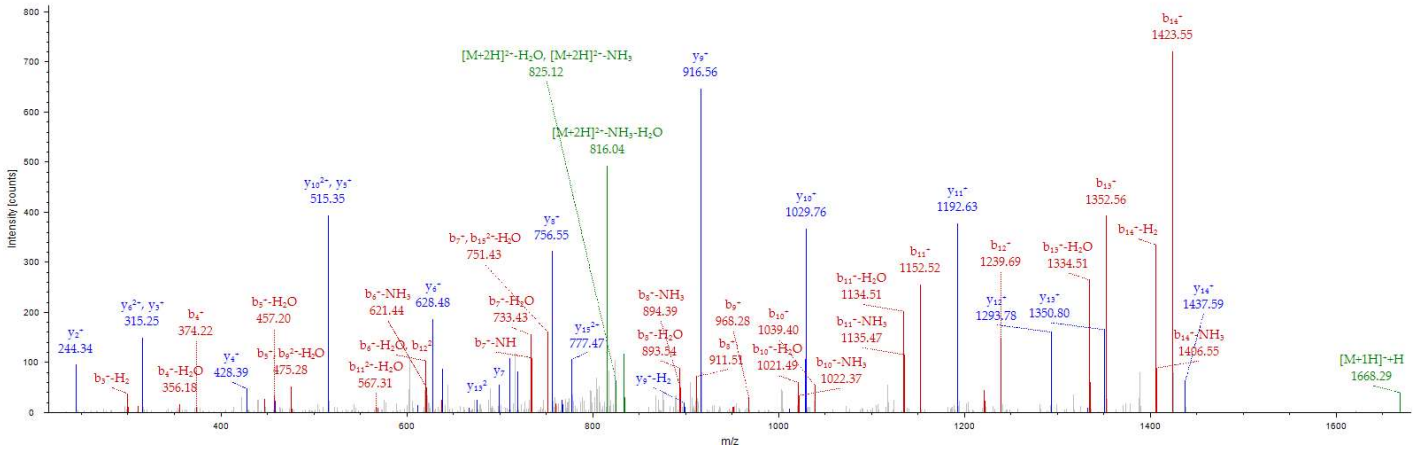
Sequence: NDSGTYLCGAISLAPK, C8-Carbamidomethyl (57.02146 Da)
Charge: +2, Monoisotopic m/z: 833.90509 Da (-3.8 mmu/-4.56 ppm), MH+: 1666.80290 Da, RT: 34.1300 min,
Identified with: Sequest HT (v1.17); XCorr:3.99, Percolator q-Value:0, Percolator PEP:0.0109, Ions matched by search engine: 0/0
Fragment match tolerance used for search: 0.6 Da

#1	b^+	b^{2+}	Seq.	y^+	y^{2+}	#2
1	115.05020	58.02874	N			16
2	230.07715	115.54221	D	1552.76757	776.88742	15
3	317.10918	159.05823	S	1437.74063	719.37395	14
4	374.13064	187.56896	G	1350.70860	675.85794	13
5	475.17832	238.09280	T	1293.68713	647.34721	12
6	638.24165	319.62446	Y	1192.63946	596.82337	11
7	751.32571	376.16649	L	1029.57613	515.29170	10
8	911.35636	456.18182	C-Carbami...	916.49206	458.74967	9
9	968.37782	484.69255	G	756.46142	378.73435	8
10	1039.41494	520.21111	A	699.43995	350.22361	7
11	1152.49900	576.75314	I	628.40284	314.70506	6
12	1239.53103	620.26915	S	515.31877	258.16303	5
13	1352.61509	676.81118	L	428.28675	214.64701	4
14	1423.65221	712.32974	A	315.20268	158.10498	3
15	1520.70497	760.85612	P	244.16557	122.58642	2
16			K	147.11280	74.06004	1

#1	$b-H_2O^+$	$b-H_2O^{2+}$	$b-NH_3^+$	$b-NH_3^{2+}$	Seq.	$y-H_2O^+$	$y-H_2O^{2+}$	$y-NH_3^+$	$y-NH_3^{2+}$	#2
1			98.02365	49.51547	N					16
2	212.06658	106.53693	213.05060	107.02894	D	1534.75701	767.88214	1535.74102	768.37415	15
3	299.09861	150.05294	300.08263	150.54495	S	1419.73006	710.36867	1420.71408	710.86068	14
4	356.12007	178.56368	357.10409	179.05568	G	1332.69803	666.85266	1333.68205	667.34466	13
5	457.16775	229.08751	458.15177	229.57952	T	1275.67657	638.34192	1276.66059	638.83393	12
6	620.23108	310.61918	621.21510	311.11119	Y	1174.62889	587.81808	1175.61291	588.31009	11
7	733.31515	367.16121	734.29916	367.65322	L	1011.56556	506.28642	1012.54958	506.77843	10
8	893.34579	447.17654	894.32981	447.66854	C-Carbami...	898.48150	449.74439	899.46551	450.23640	9
9	950.36726	475.68727	951.35127	476.17927	G	738.45085	369.72906	739.43487	370.22107	8
10	1021.40437	511.20582	1022.38839	511.69783	A	681.42939	341.21833	682.41340	341.71034	7
11	1134.48843	567.74786	1135.47245	568.23986	I	610.39227	305.69977	611.37629	306.19178	6
12	1221.52046	611.26387	1222.50448	611.75588	S	497.30821	249.15774	498.29223	249.64975	5
13	1334.60453	667.80590	1335.58854	668.29791	L			411.26020	206.13374	4
14	1405.64164	703.32446	1406.62566	703.81647	A			298.17613	149.59170	3
15	1502.69440	751.85084	1503.67842	752.34285	P			227.13902	114.07315	2
16					K			130.08626	65.54677	1

Fragment Spectrum

10222015_Angela_PD1.RAW #3527 RT: 34.1300 min
ITMS, 833.9061@cid30.00, z=+2, Mono m/z=833.90509 Da, MH+=1666.80290 Da, Match Tol.=0.6 Da



C. Control peptide FRVTQLPNGR is present in the painted PD-1 sample 11222015_Angela_PD1_AO50.

Peptide Summary

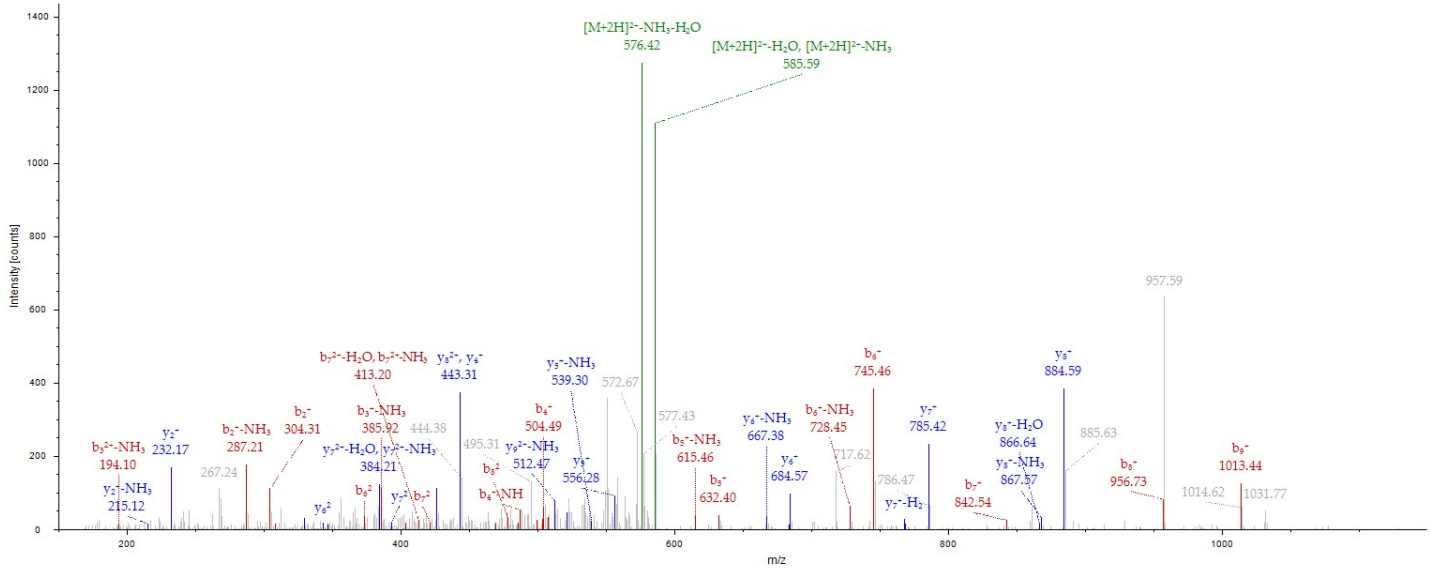
Sequence: FRVTQLPNGR, Charge: +2, Monoisotopic m/z: 594.33295 Da (-2.88 mmu/-4.84 ppm), MH+: 1187.65862 Da, RT: 31.3318 min,
Identified with: Sequest HT (v1.17); XCorr:2.10, Percolator q-Value:0, Percolator PEP:0.449, Ions matched by search engine: 0/0
Fragment match tolerance used for search: 0.6 Da
Fragments used for search: b; b-H_2O; b-NH_3; y; y-H_2O; y-NH_3

#1	b^+	b^{2+}	Seq.	y^+	y^{2+}	#2
1	148.07569	74.54148	F			10
2	304.17680	152.59204	R	1040.59595	520.80161	9
3	403.24522	202.12625	V	884.49484	442.75106	8
4	504.29289	252.65009	T	785.42643	393.21685	7
5	632.35147	316.67937	Q	684.37875	342.69301	6
6	745.43554	373.22141	L	556.32017	278.66372	5
7	842.48830	421.74779	P	443.23611	222.12169	4
8	956.53123	478.76925	N	346.18334	173.59531	3
9	1013.55269	507.27998	G	232.14042	116.57385	2
10			R	175.11895	88.06311	1

#1	b-H_2O^+	b-H_2O^{2+}	b-NH_3^+	b-NH_3^{2+}	Seq.	y-H_2O^+	y-H_2O^{2+}	y-NH_3^+	y-NH_3^{2+}	#2
1					F					10
2			287.15025	144.07876	R	1022.58539	511.79633	1023.56940	512.28834	9
3			386.21867	193.61297	V	866.48428	433.74578	867.46829	434.23778	8
4	486.28233	243.64480	487.26634	244.13681	T	767.41586	384.21157	768.39988	384.70358	7
5	614.34091	307.67409	615.32492	308.16610	Q			667.35220	334.17974	6
6	727.42497	364.21612	728.40899	364.70813	L			539.29362	270.15045	5
7	824.47773	412.74251	825.46175	413.23451	P			426.20956	213.60842	4
8	938.52066	469.76397	939.50468	470.25598	N			329.15679	165.08204	3
9	995.54213	498.27470	996.52614	498.76671	G			215.11387	108.06057	2
10					R			158.09240	79.54984	1

Fragment Spectrum

10222015_Angela_PD1_A050.RAW #2098 RT: 31.3318 min
ITMS, 594.3324@cid30.00, z=+2, Mono m/z=594.33295 Da, MH+=1187.65862 Da, Match Tol.=0.6 Da



D. Control peptide NDSGTYLCGAISLAPK is present in the painted PD-1 sample 11222015_Angela_PD1_AO50.

Peptide Summary

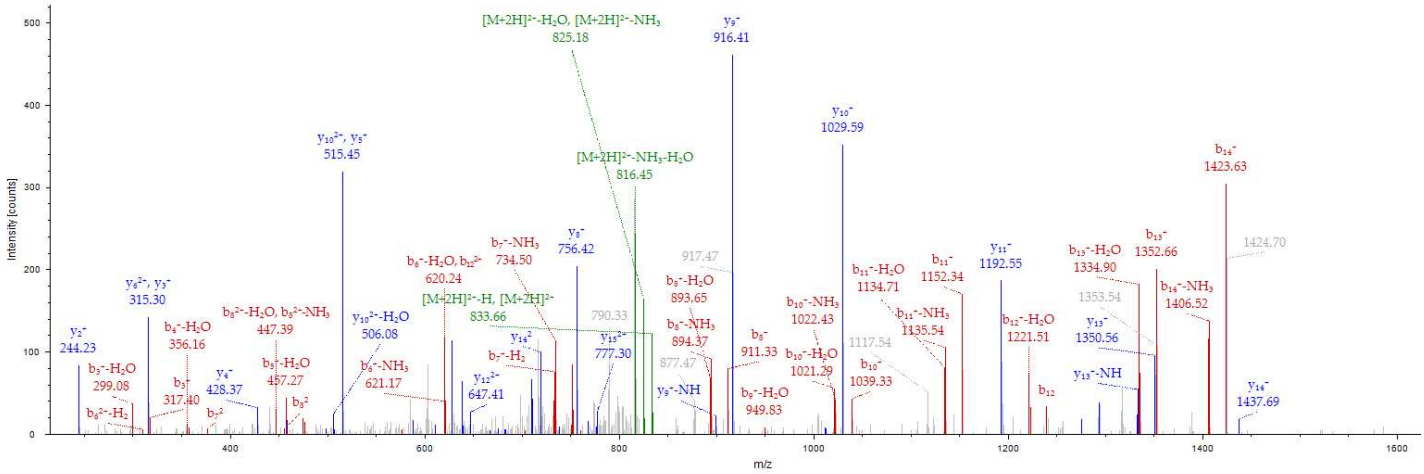
Sequence: NDSGTYLCGAISLAPK, C8-Carbamidomethyl (57.02146 Da)
Charge: +2, Monoisotopic m/z: 833.90796 Da (-0.93 mmu/-1.11 ppm), MH+: 1666.80864 Da, RT: 36.0712 min,
Identified with: Sequest HT (v1.17); XCorr:4.14, Percolator q-Value:0, Percolator PEP:0.169, Ions matched by search engine: 0/0
Fragment match tolerance used for search: 0.6 Da

#1	b^+	b^{2+}	Seq.	y^+	y^{2+}	#2
1	115.05020	58.02874	N			16
2	230.07715	115.54221	D	1552.76757	776.88742	15
3	317.10918	159.05823	S	1437.74063	719.37395	14
4	374.13064	187.56896	G	1350.70860	675.85794	13
5	475.17832	238.09280	T	1293.68713	647.34721	12
6	638.24165	319.62446	Y	1192.63946	596.82337	11
7	751.32571	376.16649	L	1029.57613	515.29170	10
8	911.35636	456.18182	C-Carbami...	916.49206	458.74967	9
9	968.37782	484.69255	G	756.46142	378.73435	8
10	1039.41494	520.21111	A	699.43995	350.22361	7
11	1152.49900	576.75314	I	628.40284	314.70506	6
12	1239.53103	620.26915	S	515.31877	258.16303	5
13	1352.61509	676.81118	L	428.28675	214.64701	4
14	1423.65221	712.32974	A	315.20268	158.10498	3
15	1520.70497	760.85612	P	244.16557	122.58642	2
16			K	147.11280	74.06004	1

#1	$b\text{-}H_2O^+$	$b\text{-}H_2O^{2+}$	$b\text{-}NH_3^+$	$b\text{-}NH_3^{2+}$	Seq.	$y\text{-}H_2O^+$	$y\text{-}H_2O^{2+}$	$y\text{-}NH_3^+$	$y\text{-}NH_3^{2+}$	#2
1			98.02365	49.51547	N					16
2	212.06658	106.53693	213.05060	107.02894	D	1534.75701	767.88214	1535.74102	768.37415	15
3	299.09861	150.05294	300.08263	150.54495	S	1419.73006	710.36867	1420.71408	710.86068	14
4	356.12007	178.56368	357.10409	179.05568	G	1332.69803	666.85266	1333.68205	667.34466	13
5	457.16775	229.08751	458.15177	229.57952	T	1275.67657	638.34192	1276.66059	638.83393	12
6	620.23108	310.61918	621.21510	311.11119	Y	1174.62889	587.81808	1175.61291	588.31009	11
7	733.31515	367.16121	734.29916	367.65322	L	1011.56556	506.28642	1012.54958	506.77843	10
8	893.34579	447.17654	894.32981	447.66854	C-Carbami...	898.48150	449.74439	899.46551	450.23640	9
9	950.36726	475.68727	951.35127	476.17927	G	738.45085	369.72906	739.43487	370.22107	8
10	1021.40437	511.20582	1022.38839	511.69783	A	681.42939	341.21833	682.41340	341.71034	7
11	1134.48843	567.74786	1135.47245	568.23986	I	610.39227	305.69977	611.37629	306.19178	6
12	1221.52046	611.26387	1222.50448	611.75588	S	497.30821	249.15774	498.29223	249.64975	5
13	1334.60453	667.80590	1335.58854	668.29791	L			411.26020	206.13374	4
14	1405.64164	703.32446	1406.62566	703.81647	A			298.17613	149.59170	3
15	1502.69440	751.85084	1503.67842	752.34285	P			227.13902	114.07315	2
16					K			130.08626	65.54677	1

Fragment Spectrum

10222015_Angela_PD1_A050.RAW #2731 RT: 36.0712 min
ITMS, 834.4083@cid30.00, z=+2, Mono m/z=833.90796 Da, MH+=1666.80864 Da, Match Tol.=0.6 Da



E. Control peptide FRVTQLPNGR is present in the painted PD-1/PD-L1 complexed sample 11222015_Angela_Com_AO50.

Peptide Summary

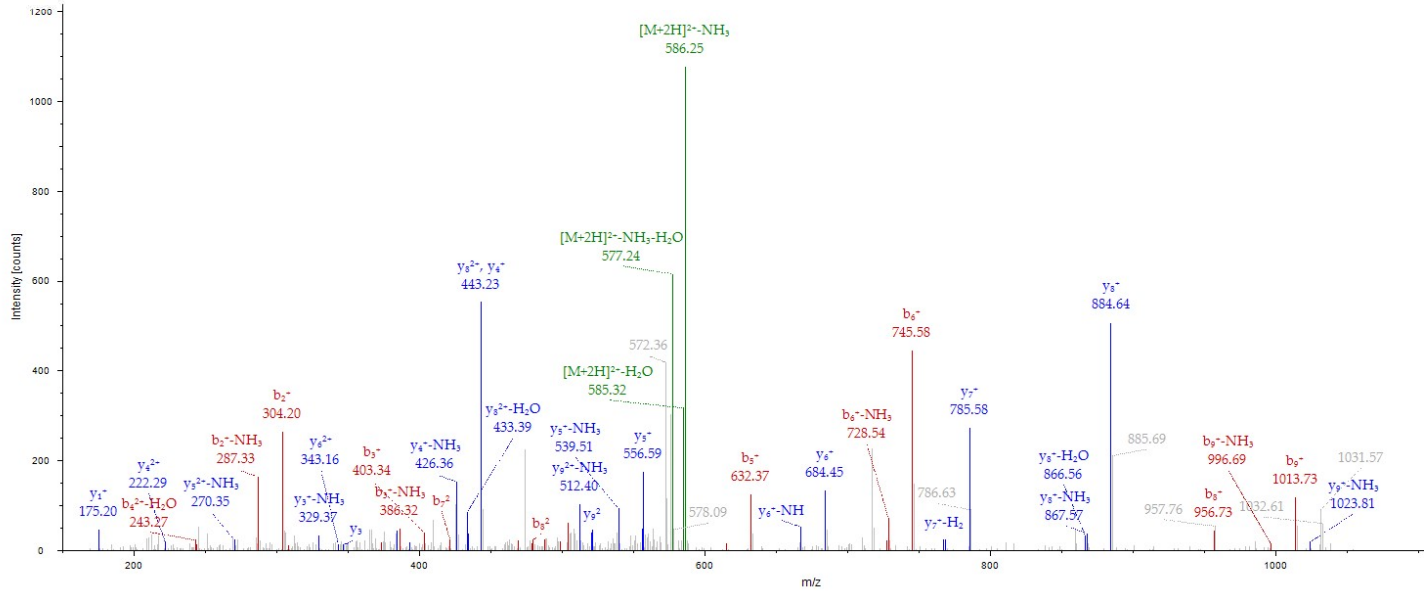
Sequence: FRVTQLPNGR, Charge: +2, Monoisotopic m/z: 594.33252 Da (-3.3 mmu/-5.56 ppm), MH+: 1187.65776 Da, RT: 36.5483 min,
Identified with: Sequest HT (v1.17); XCorr:1.78, Percolator q-Value:0, Percolator PEP:0.282, Ions matched by search engine: 0/0
Fragment match tolerance used for search: 0.6 Da
Fragments used for search: b; b-H_2O; b-NH_3; y; y-H_2O; y-NH_3

#1	b^+	b^{2+}	Seq.	y^+	y^{2+}	#2
1	148.07569	74.54148	F			10
2	304.17680	152.59204	R	1040.59595	520.80161	9
3	403.24522	202.12625	V	884.49484	442.75106	8
4	504.29289	252.65009	T	785.42643	393.21685	7
5	632.35147	316.67937	Q	684.37875	342.69301	6
6	745.43554	373.22141	L	556.32017	278.66372	5
7	842.48830	421.74779	P	443.23611	222.12169	4
8	956.53123	478.76925	N	346.18334	173.59531	3
9	1013.55269	507.27998	G	232.14042	116.57385	2
10			R	175.11895	88.06311	1

#1	b-H_2O^+	b-H_2O^{2+}	b-NH_3^+	b-NH_3^{2+}	Seq.	y-H_2O^+	y-H_2O^{2+}	y-NH_3^+	y-NH_3^{2+}	#2
1					F					10
2			287.15025	144.07876	R	1022.58539	511.79633	1023.56940	512.28834	9
3			386.21867	193.61297	V	866.48428	433.74578	867.46829	434.23778	8
4	486.28233	243.64480	487.26634	244.13681	T	767.41586	384.21157	768.39988	384.70358	7
5	614.34091	307.67409	615.32492	308.16610	Q			667.35220	334.17974	6
6	727.42497	364.21612	728.40899	364.70813	L			539.29362	270.15045	5
7	824.47773	412.74251	825.46175	413.23451	P			426.20956	213.60842	4
8	938.52066	469.76397	939.50468	470.25598	N			329.15679	165.08204	3
9	995.54213	498.27470	996.52614	498.76671	G			215.11387	108.06057	2
10					R			158.09240	79.54984	1

Fragment Spectrum

10222015_Angela_Com_A050.RAW #3480 RT: 36.5483 min
ITMS, 594.3341@cid30.00, z=+2, Mono m/z=594.33252 Da, MH+=1187.65776 Da, Match Tol.=0.6 Da



F. Control peptide NDSGTYLCGAISLAPK is present in the painted PD-1/PD-L1 complexed sample 11222015_Angela_Com_AO50.

Peptide Summary

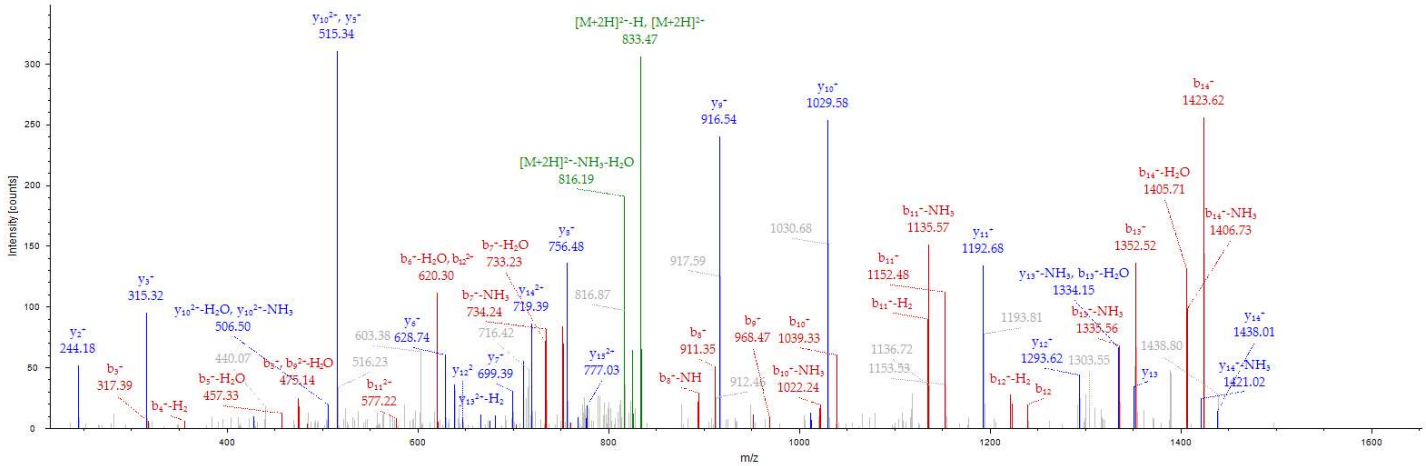
Sequence: NDSGTYLCGAISLAPK, C8-Carbamidomethyl (57.02146 Da)
Charge: +2, Monoisotopic m/z: 833.90869 Da (-0.2 mmu/-0.23 ppm), MH+: 1666.81011 Da, RT: 33.4988 min,
Identified with: Sequest HT (v1.17); XCorr:4.21, Percolator q-Value:0, Percolator PEP:0.00162, Ions matched by search engine: 0/0
Fragment match tolerance used for search: 0.6 Da

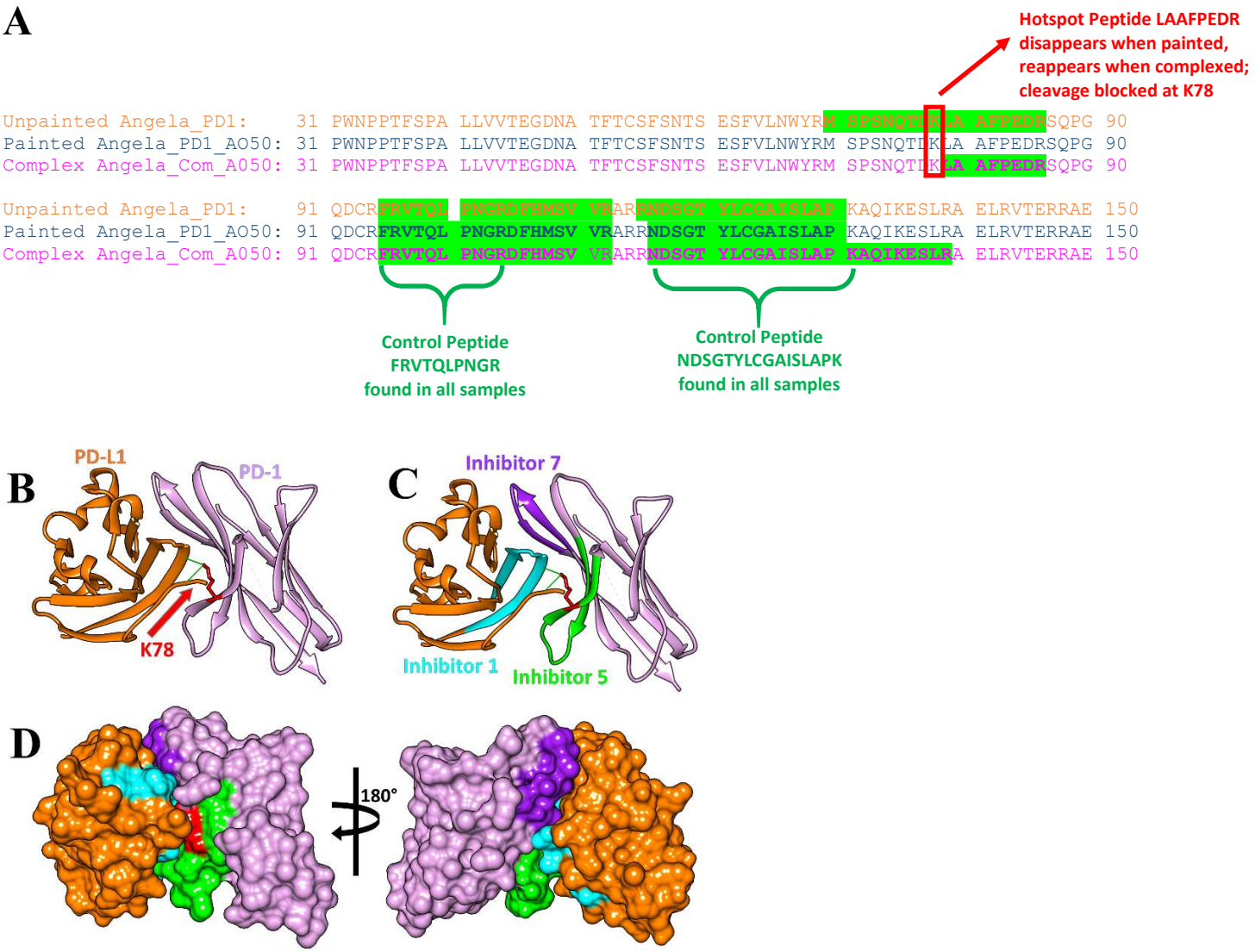
#1	b^+	b^{2+}	Seq.	y^+	y^{2+}	#2
1	115.05020	58.02874	N			16
2	230.07715	115.54221	D	1552.76757	776.88742	15
3	317.10918	159.05823	S	1437.74063	719.37395	14
4	374.13064	187.56896	G	1350.70860	675.85794	13
5	475.17832	238.09280	T	1293.68713	647.34721	12
6	638.24165	319.62446	Y	1192.63946	596.82337	11
7	751.32571	376.16649	L	1029.57613	515.29170	10
8	911.35636	456.18182	C-Carbami...	916.49206	458.74967	9
9	968.37782	484.69255	G	756.46142	378.73435	8
10	1039.41494	520.21111	A	699.43995	350.22361	7
11	1152.49900	576.75314	I	628.40284	314.70506	6
12	1239.53103	620.26915	S	515.31877	258.16303	5
13	1352.61509	676.81118	L	428.28675	214.64701	4
14	1423.65221	712.32974	A	315.20268	158.10498	3
15	1520.70497	760.85612	P	244.16557	122.58642	2
16			K	147.11280	74.06004	1

#1	$b\text{-}H_2O^+$	$b\text{-}H_2O^{2+}$	$b\text{-}NH_3^+$	$b\text{-}NH_3^{2+}$	Seq.	$y\text{-}H_2O^+$	$y\text{-}H_2O^{2+}$	$y\text{-}NH_3^+$	$y\text{-}NH_3^{2+}$	#2
1			98.02365	49.51547	N					16
2	212.06658	106.53693	213.05060	107.02894	D	1534.75701	767.88214	1535.74102	768.37415	15
3	299.09861	150.05294	300.08263	150.54495	S	1419.73006	710.36867	1420.71408	710.86068	14
4	356.12007	178.56368	357.10409	179.05568	G	1332.69803	666.85266	1333.68205	667.34466	13
5	457.16775	229.08751	458.15177	229.57952	T	1275.67657	638.34192	1276.66059	638.83393	12
6	620.23108	310.61918	621.21510	311.11119	Y	1174.62889	587.81808	1175.61291	588.31009	11
7	733.31515	367.16121	734.29916	367.65322	L	1011.56556	506.28642	1012.54958	506.77843	10
8	893.34579	447.17654	894.32981	447.66854	C-Carbami...	898.48150	449.74439	899.46551	450.23640	9
9	950.36726	475.68727	951.35127	476.17927	G	738.45085	369.72906	739.43487	370.22107	8
10	1021.40437	511.20582	1022.38839	511.69783	A	681.42939	341.21833	682.41340	341.71034	7
11	1134.48843	567.74786	1135.47245	568.23986	I	610.39227	305.69977	611.37629	306.19178	6
12	1221.52046	611.26387	1222.50448	611.75588	S	497.30821	249.15774	498.29223	249.64975	5
13	1334.60453	667.80590	1335.58854	668.29791	L			411.26020	206.13374	4
14	1405.64164	703.32446	1406.62566	703.81647	A			298.17613	149.59170	3
15	1502.69440	751.85084	1503.67842	752.34285	P			227.13902	114.07315	2
16					K			130.08626	65.54677	1

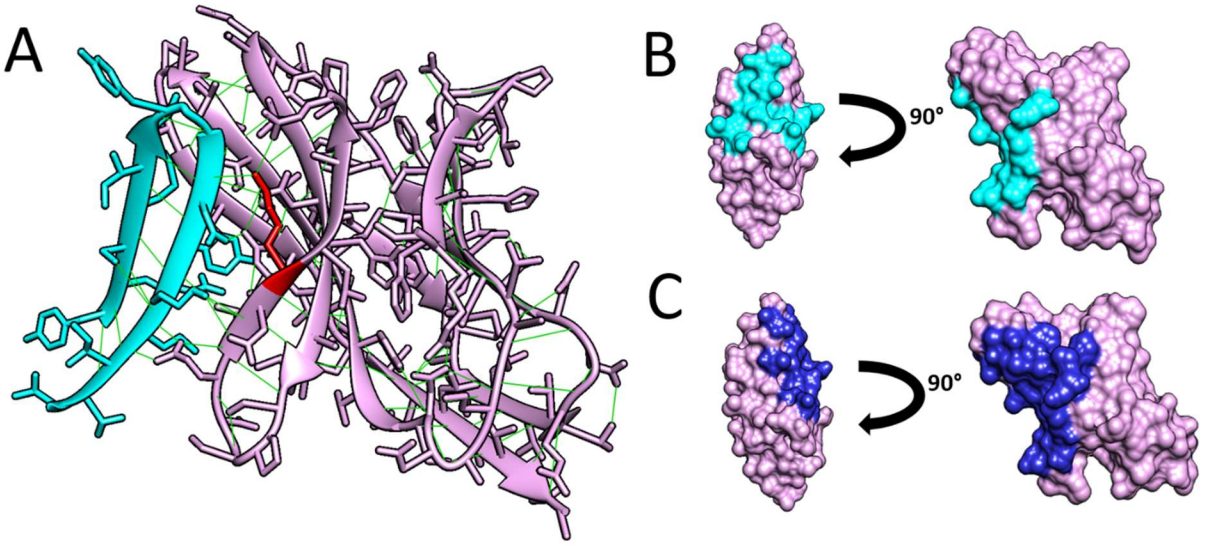
Fragment Spectrum

10222015_Angela_Com_A050.RAW #3020 RT: 33.4988 min
ITMS, 834.4067@cid30.00, z=+2, Mono m/z=833.90869 Da, MH+=1666.81011 Da, Match Tol.=0.6 Da





Supplementary Figure 17. PD-1 hotspot for its interaction with PD-L1 is determined by differential analysis of unpainted PD-1, painted PD-1, and painted PD-1/PD-L1 complex, and is validated with peptide inhibitors. A) To determine the presence of PD-1 hotspots for an independent mass spectrometry experiment, the sequence of PD-1 for an unpainted sample, a painted sample, and a complexed sample are aligned, and peptides identified in each sample are highlighted in green, using the trials depicted in Supplementary Figures 14 and 15. Peptides which are present in the unpainted sample, absent in the painted sample, and return in the complexed sample are considered indicative of regions that are solvent-accessible in the painted sample, but not solvent-accessible in the complexed sample, such as LAAFPEDR. The missed tryptic cleavage site, such as K78 for the peptide LAAFPEDR, is the identified hotspot. B) The K78 hotspot of PD-1 (red) is shown using the crystal structure (PDB 4ZQK) of the extracellular portions of PD-1 (light purple) and PD-L1 (orange) where K78 is found in the protein-protein interface and forms two hydrogen bonds to PD-L1, indicated with green lines. C) Parent sequences of Inhibitor 1 (Inhibitor 2 is a cyclized version of Inhibitor 1), Inhibitor 5 (Inhibitor 6 is a cyclized version of Inhibitor 5), and Inhibitor 7 (Inhibitor 8 is a cyclized version of inhibitor 7) are shown in cyan, light green, and dark purple respectively in the co-crystal structure of PD-1/PD-L1. All inhibitors are found in the protein-protein interface. Inhibitor 1 is designed to directly bind to the K78 hotspot, and was predicted to be the most potent if K78 represents a true positive. Scrambled Inhibitor 3 (Inhibitor 4 is a cyclized version of Inhibitor 3) was designed from non-adjacent interface sequences of PD-L1 and is not shown. D) Space-filling model showing parent sequences of Inhibitors 1, 5 and 7 relative to the protein-protein interface.



Supplementary Figure 18. Inhibitor 1 predicted binding to PD-1 overlaps pembrolizumab (Keytruda) binding. A) Inhibitor 1 (cyan) bound to PD-1 (purple), with protein painting hit Lys 78 shown in red and hydrogen bonds shown in green. B) Binding site of Inhibitor 1, cyan, on PD-1. C) Binding site of pembrolizumab, dark blue, on PD-1. Significant overlap is seen between the binding sites of Inhibitor 1 and pembrolizumab.