SUPPORTING INFORMATION

Design and characterization of mutant and wild-type huntingtin proteins produced from a toolkit of scalable eukaryotic expression systems

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Running title: A toolkit of HTT protein resources

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Table S1. Database search results for HTT$^{1-3144}$ Q23 expressed in EXPI293F cells. Proteins with five or more total no. spectra are listed. Of the peptides which do not correspond to HTT protein, most either have very high scores in the CRAPome (1), suggesting that these are non-specific contaminants of the co-immunoprecipitation step, not true HTT interactors, or they are very low abundance.

Protein Accession	Total no. spectra	No. unique peptides	Protein Accession	Total no. spectra	No. unique peptides	Protein Accession	Total no. spectra	No. unique peptides
P42858|HD	7681	1150	P0C0S5|H2AZ	14	4	O60391|NMD3B	8	2
Q13748|TBA3C	175	29	P0C0S8|H2A1	14	4	O75534|CSDE1	8	2
Q13885|TBB2A	111	26	P16104|H2AX	14	4	P49137|MAPK2	8	2
Q9BVA1|TBB2B	111	26	P20671|H2A1D	14	4	P62269|RS18	8	2
P07900|HS90A	110	24	P34931|HS71L	14	5	Q16695|H31T	8	2
Q6PEY2|TBA3E	64	13	Q16777|H2A2C	14	4	Q8NCG5|CHST4	8	2
Q9BYE2|TMPSD	56	7	Q6FI13|H2A2A	14	4	Q8WUA7|TB22A	8	2
Q9NY65|TBA8	54	9	Q71UI9|H2AV	14	4	Q9NRC6|SPTN5	8	2
Q71U36|TBA1A	51	12	Q7L7L0|H2A3	14	4	Q9NRD9|DUOX1	8	2
P04350|TBB4A	48	12	Q93077|H2A1C	14	4	O15381|NVL	7	2
P60709|ACTB	45	13	Q96KK5|H2A1H	14	4	Q15021|CND1	7	2
P11142|HSP7C	44	12	Q96QV6|H2A1A	14	4	Q5T9S5|CCD18	7	2
P63261|ACTG	44	13	Q99878|H2A1J	14	4	Q5VZP5|DUS27	7	2
P23396|RS3	43	11	Q9BTM1|H2AJ	14	4	Q6WRI0|IGS10	7	2
P54652|HSP72	43	12	Q9H2M9|RBGPR	14	2	Q709C8|VP13C	7	2
P15880|RS2	38	11	Q9NY93|DDX56	14	3	Q96JC1|VPS39	7	2
Q13509|TBB3	36	8	Q9P225|DYH2	14	4	Q9BSJ2|GCP2	7	2
tr|A0A0B4J269	32	7	Q9UKN7|MYO15	14	3	Q9P1Y6|PHRF1	7	2
P0DMV8|HS71A	31	9	Q58FF7|H90B3	13	5	Q9P1Z3|HCN3	7	2
P0DMV9|HS71B	31	9	Q8IVL1|NAV2	13	3	Q9P2E5|CHPF2	7	2
P07437|TBB5	30	7	Q9H853|TBA4B	13	3	Q9UL51|HCN2	7	2
Q9BWH6|RPAP1	27	4	P02549|SPTA1	12	2	Q9Y3Q4|HCN4	7	2
Q5VYK3|ECM29	26	6	P62805|H4	12	2	A6NE52|WDR97	6	1
Q8WXG9|GPR98	26	4	Q14568|HS902	12	5	O43314|VIP2	6	1
Q562R1|ACTBL	24	7	Q5CZC0|FSIP2	12	5	O43896|KIF1C	6	2
Q9UPN3|MACF1	23	4	Q5S007|LRRK2	12	2	P05164|PERM	6	2
A6NKT7|RGPD3	22	6	Q7Z5J8|ANKAR	12	3	P08670|VIME	6	1
P17066|HSP76	21	7	Q8TE73|DYH5	12	6	P19823|ITIH2	6	2
Q03001|DYST	21	3	Q96JB1|DYH8	12	4	Q13608|PEX6	6	1
P08238|HS90B	20	7	Q96PX9|PKH4B	12	2	Q13683|ITA7	6	1
Q9BQG0|MBB1A	20	5	Q9UDT6|CLIP2	12	3	Q2LD37|K1109	6	1
P11021|GRP78	19	6	O60890|OPHN1	11	3	Q5JV73|FRPD3	6	3
Q58FG1|HS904	19	4	Q02224|CENPE	11	3	Q5JWR5|DOP1	6	1
Q8NET8|TRPV3	19	3	Q6ZQQ6|WDR87	11	2	Q6N069|NAA16	6	2
O60814|H2B1K	18	3	Q7Z333|SETX	11	4	Q86XA9|HTR5A	6	1
P06899|H2B1J	18	3	Q86UQ4|ABCAD	11	4	Q8NCM8|DYHC2	6	1
P23527|H2B1O	18	3	Q9Y4F4|TGRM1	11	3	Q8TD57|DYH3	6	2
P33778|H2B1B	18	3	P21817|RYR1	10	2	Q92997|DVL3	6	1
P57053|H2BFS	18	3	Q12955|ANK3	10	5	Q96A08|H2B1A	6	1
P58876|H2B1D	18	3	Q1XH10|SKDA1	10	2	Q96QT4|TRPM7	6	1
P62807|H2B1C	18	3	Q5T1B0|AXDN1	10	2	Q99996|AKAP9	6	3
Q16778|H2B2E	18	3	Q86U86|PB1	10	3	Q9HCK8|CHD8	6	3
Q5QNW6|H2B2F	18	3	Q86VI3|IQGA3	10	3	Q9NYC9|DYH9	6	1
Q8N257|H2B3B	18	3	Q8IZD9|DOCK3	10	3	Q9P2D1|CHD7	6	3
Q93079|H2B1H	18	3	Q13136|LIPA1	9	4	P09131|P3	5	1
Q99877|H2B1N	18	3	Q15751|HERC1	9	3	Q13576|IQGA2	5	2
Q99879|H2B1M	18	3	Q6PI48|SYDM	9	3	Q15746|MYLK	5	1
Q99880|H2B1L	18	3	Q9HBJ7|UBP29	9	3	Q86VV8|RTTN	5	3
Q12931|TRAP1	16	5	Q9P0L2|MARK1	9	1	Q8NBX0|SCPDL	5	2
Q8IVF2|AHNK2	16	1	Q9P217|ZSWM5	9	3	Q8TD26|CHD6	5	2
O14647|CHD2	15	2	tr|Q5TEC6|Q5TEC6	9	3	Q9ULT8|HECD1	5	3
P04908|H2A1B	14	4	O15078|CE290	8	3			

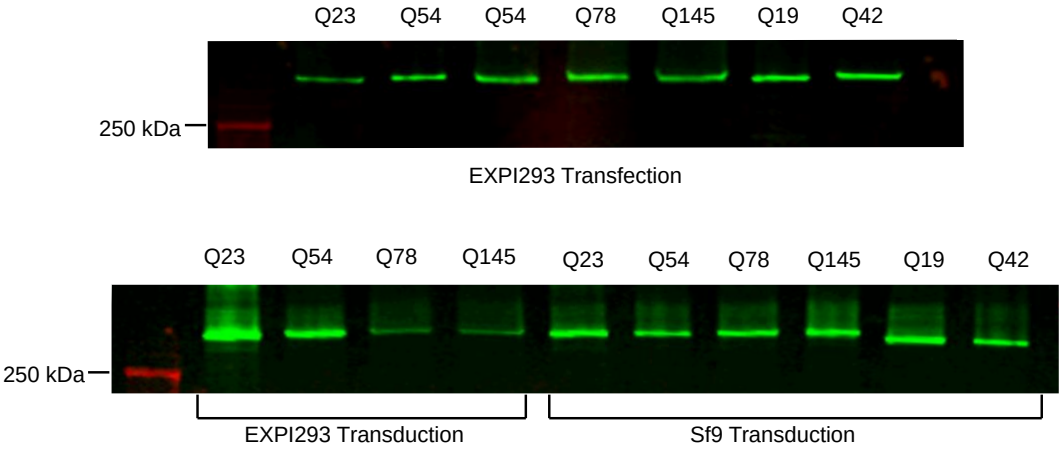
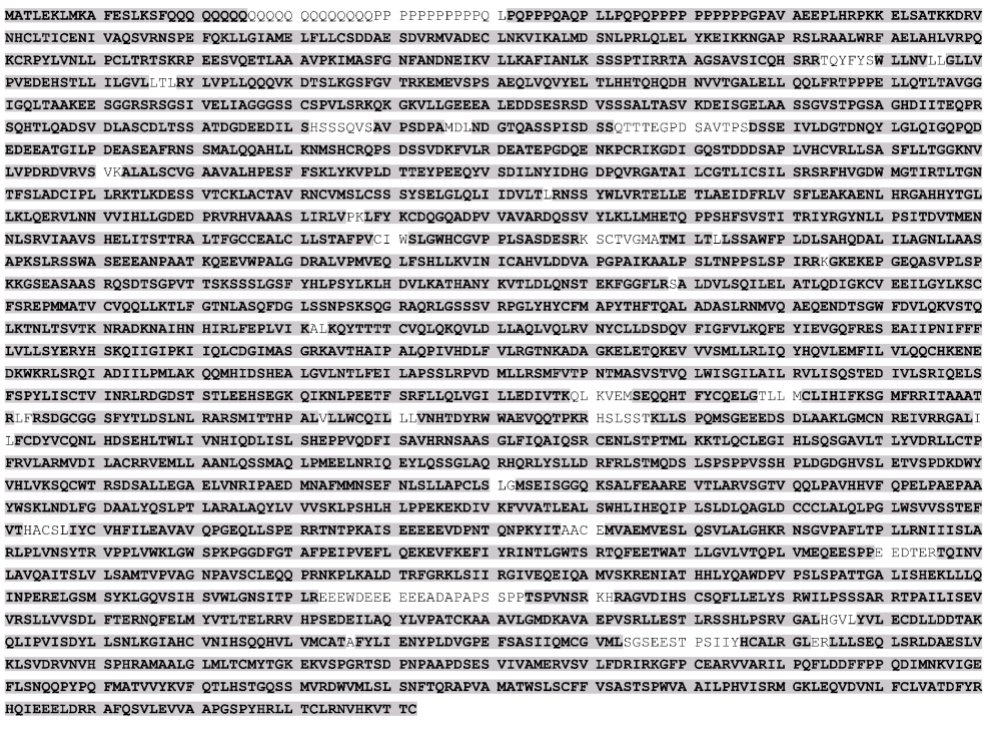


Figure S1. Western blot analysis of HTT samples from different expression systems. C-terminally FLAG-tagged HTT derived from EXPI293F expression, either by transient transfection or baculoviral transduction in EXPI293F cells or baculoviral transduction in Sf9 cells, were subject to Western Blot analysis with ab109115 which binds an epitope at amino acids 1-100.

A)



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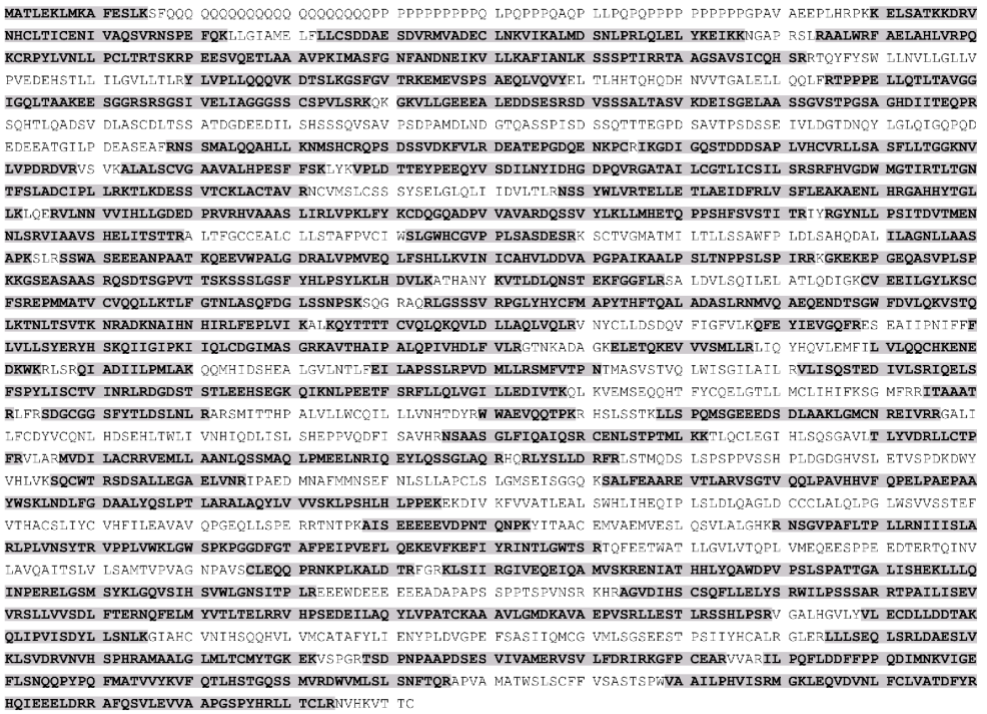
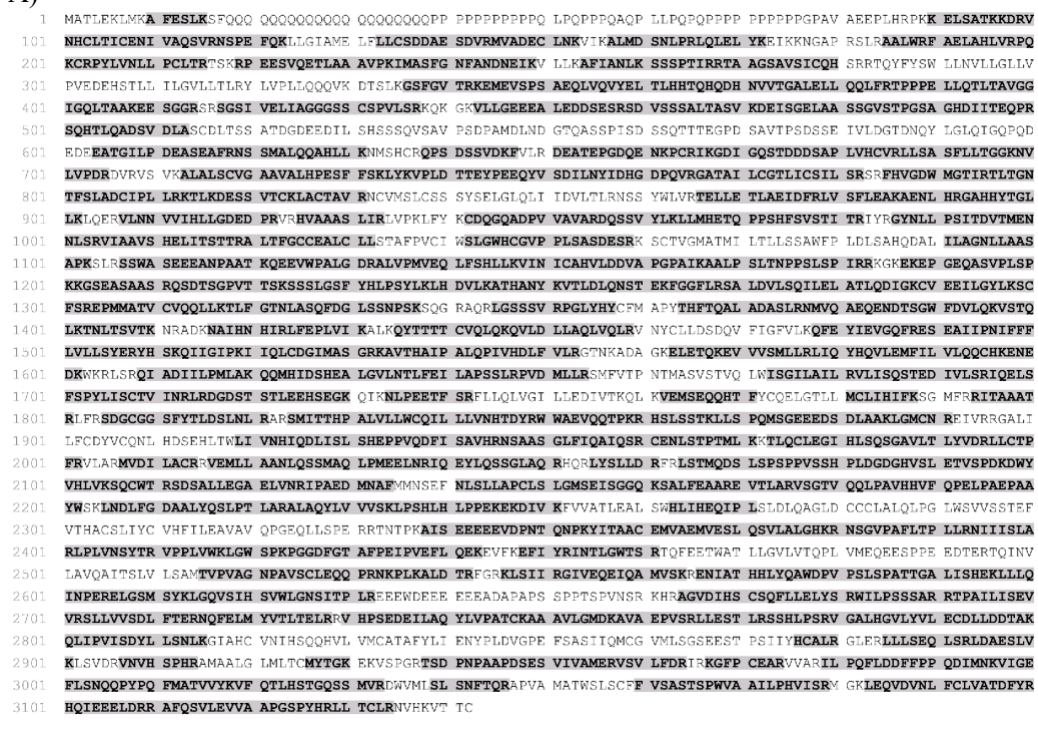


Figure S2. Primary sequence coverage maps of Sf9 HTT$^{1\text{-}3144}$ Q23. A) 97% coverage obtained by combining the database search results obtained from 5 enzymes (pepsin, WaLP, MaLP, lysargiNase, and trypsin), and B) 66% coverage obtained from 2 enzymes (lysargiNase and trypsin). NB: table displays HTT Q21 sequence as UniProt database used in sequence search.

A)



B)

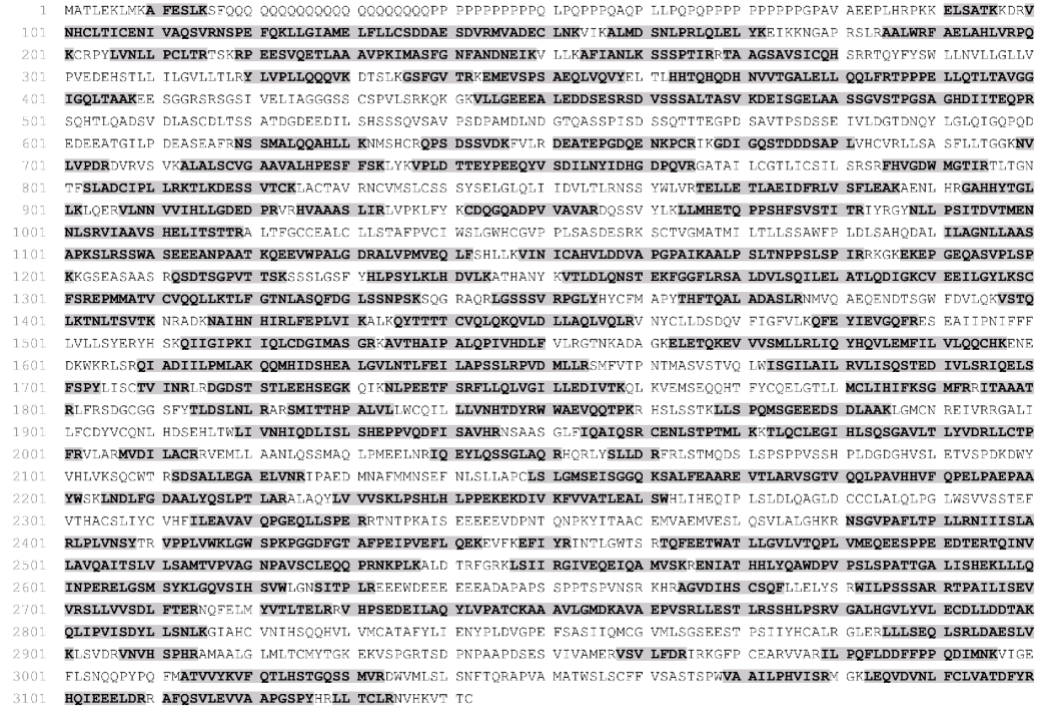


Figure S3. Primary sequence coverage maps of EXPI293F HTT$^{1\text{-}3144}$ Q23 digested with trypsin. A) 77% coverage obtained from solution, and B) 62% coverage from a gel band. NB: table displays HTT Q21 sequence as UniProt database used in sequence search.

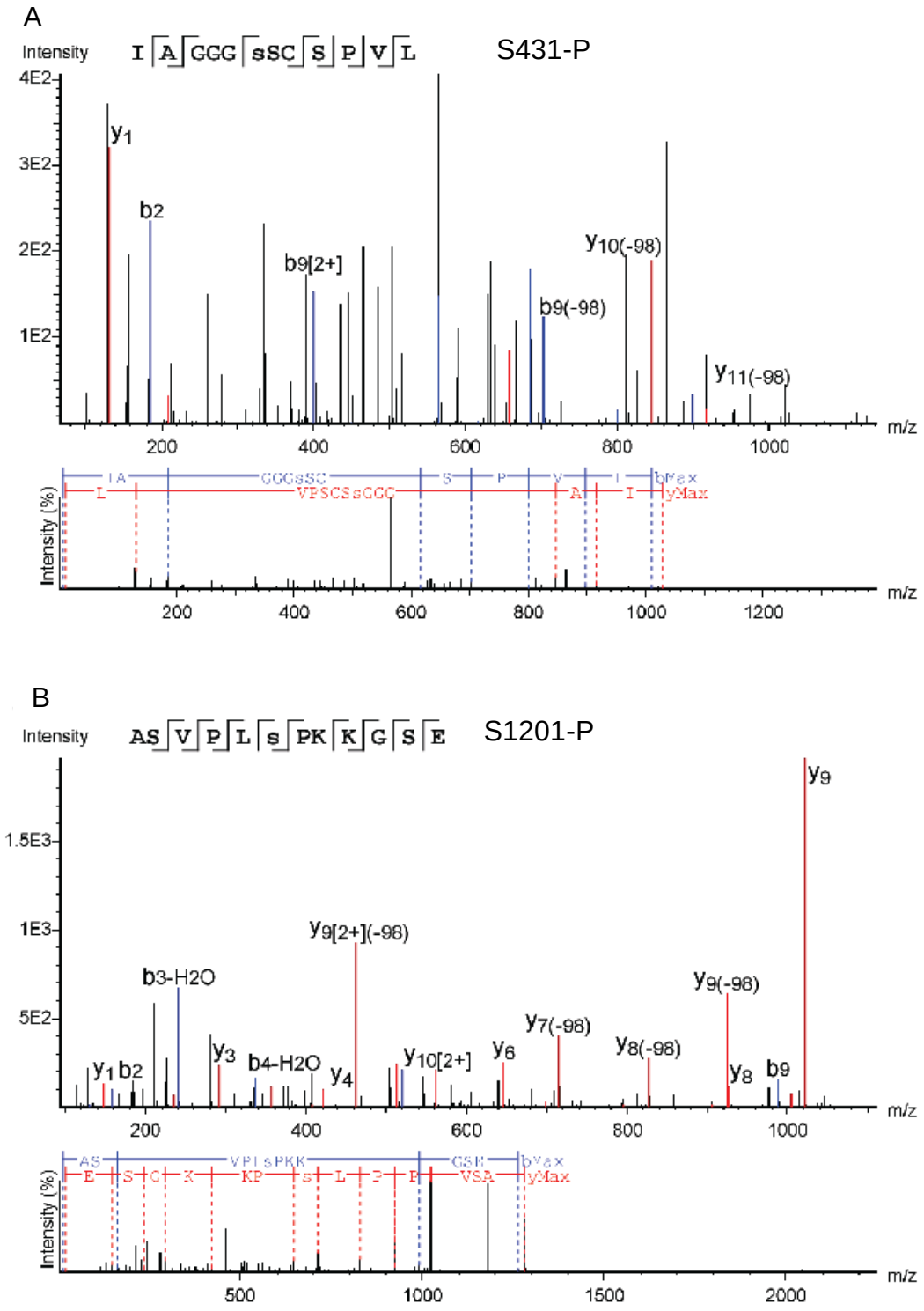
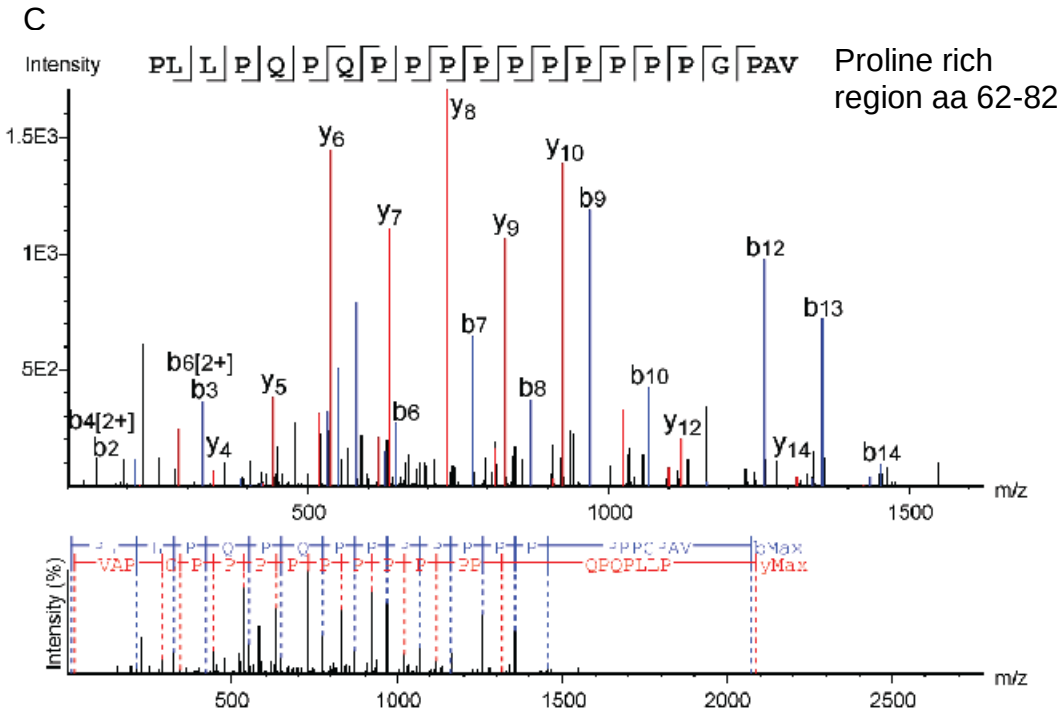


Figure S4. Exemplary spectra of pepsin digested HTT$^{1\text{-}3144}$ Q23 from Sf9. Full data can be found through PRIDE (2) with accession PXD010865.



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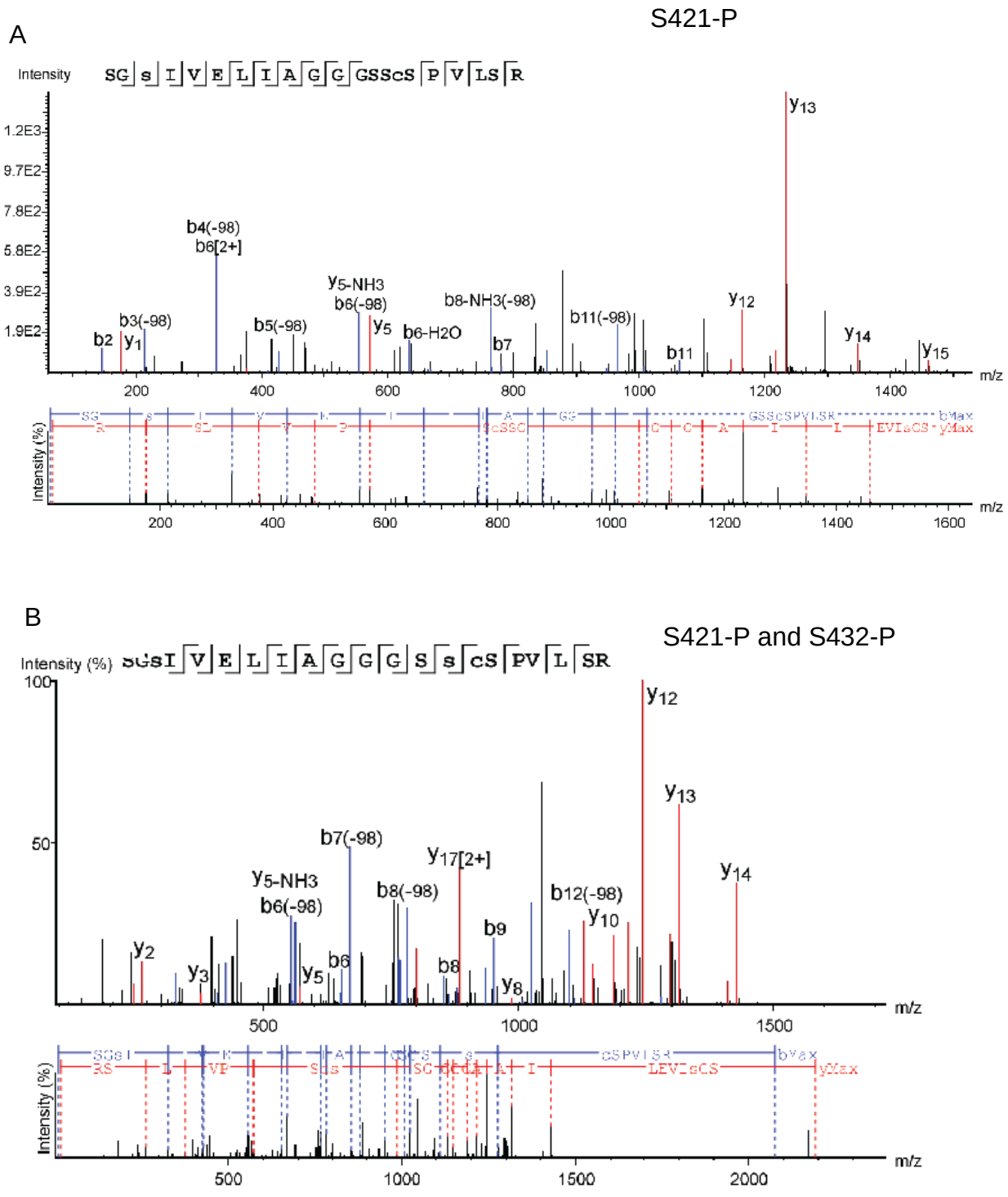
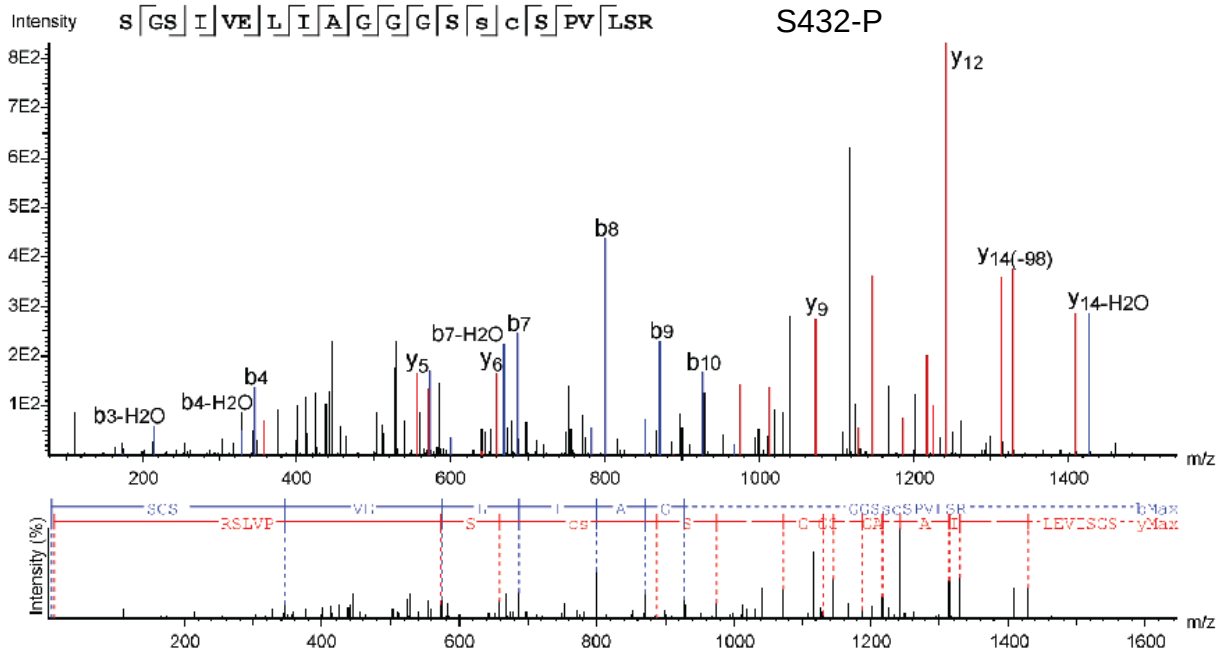


Figure S5. Exemplary spectra of trypsin or lysargiNase digested HTT$^{1\text{-}3144}$ Q23 from Sf9. Full data can be found through PRIDE (2) with accession PXD010865.

C



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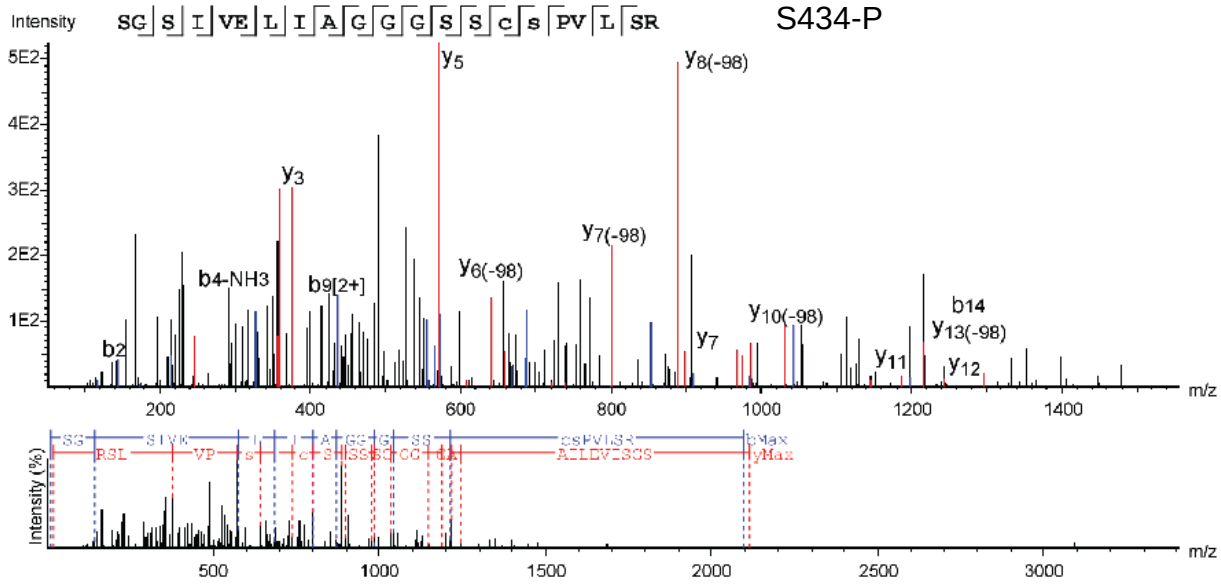

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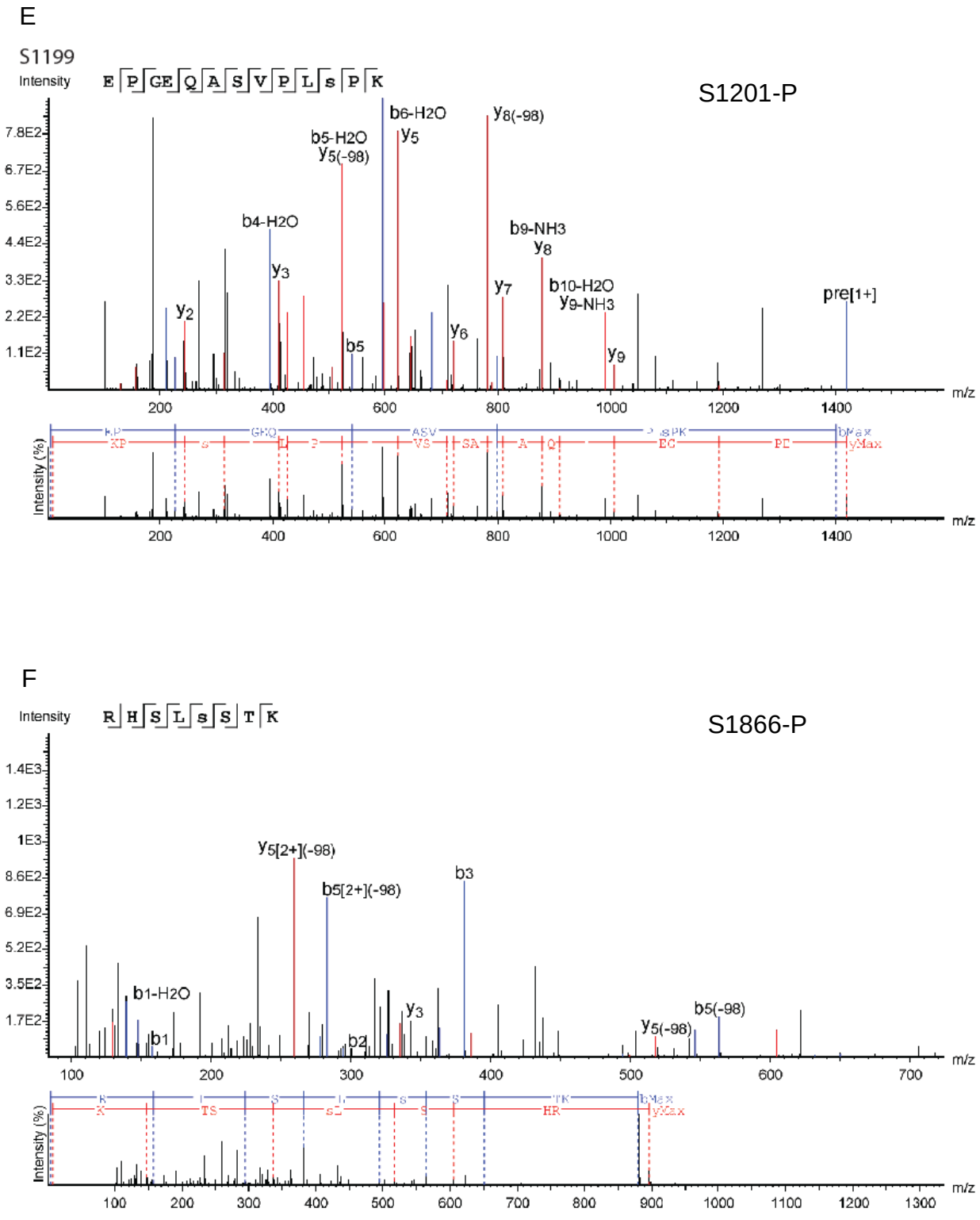

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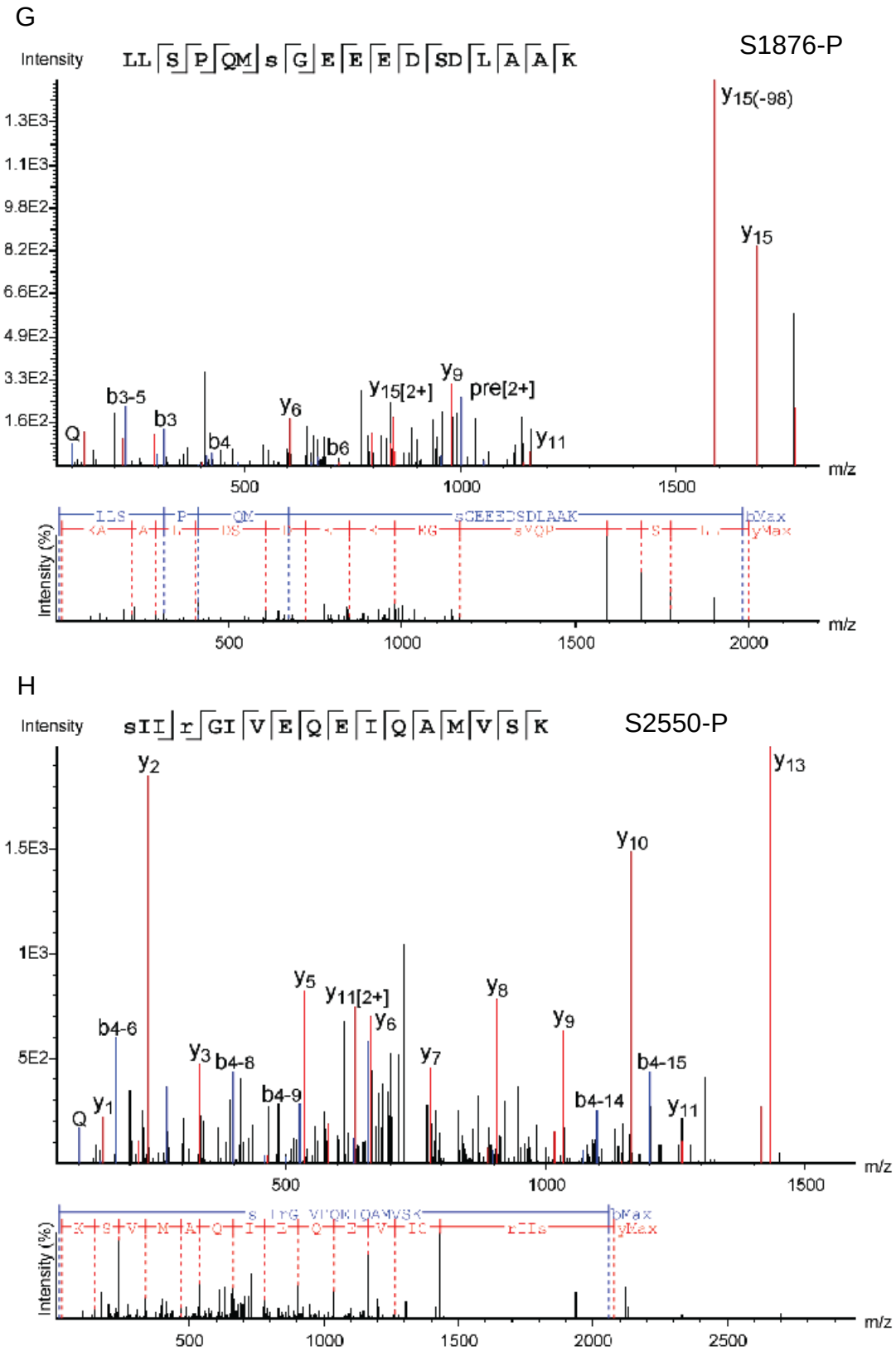

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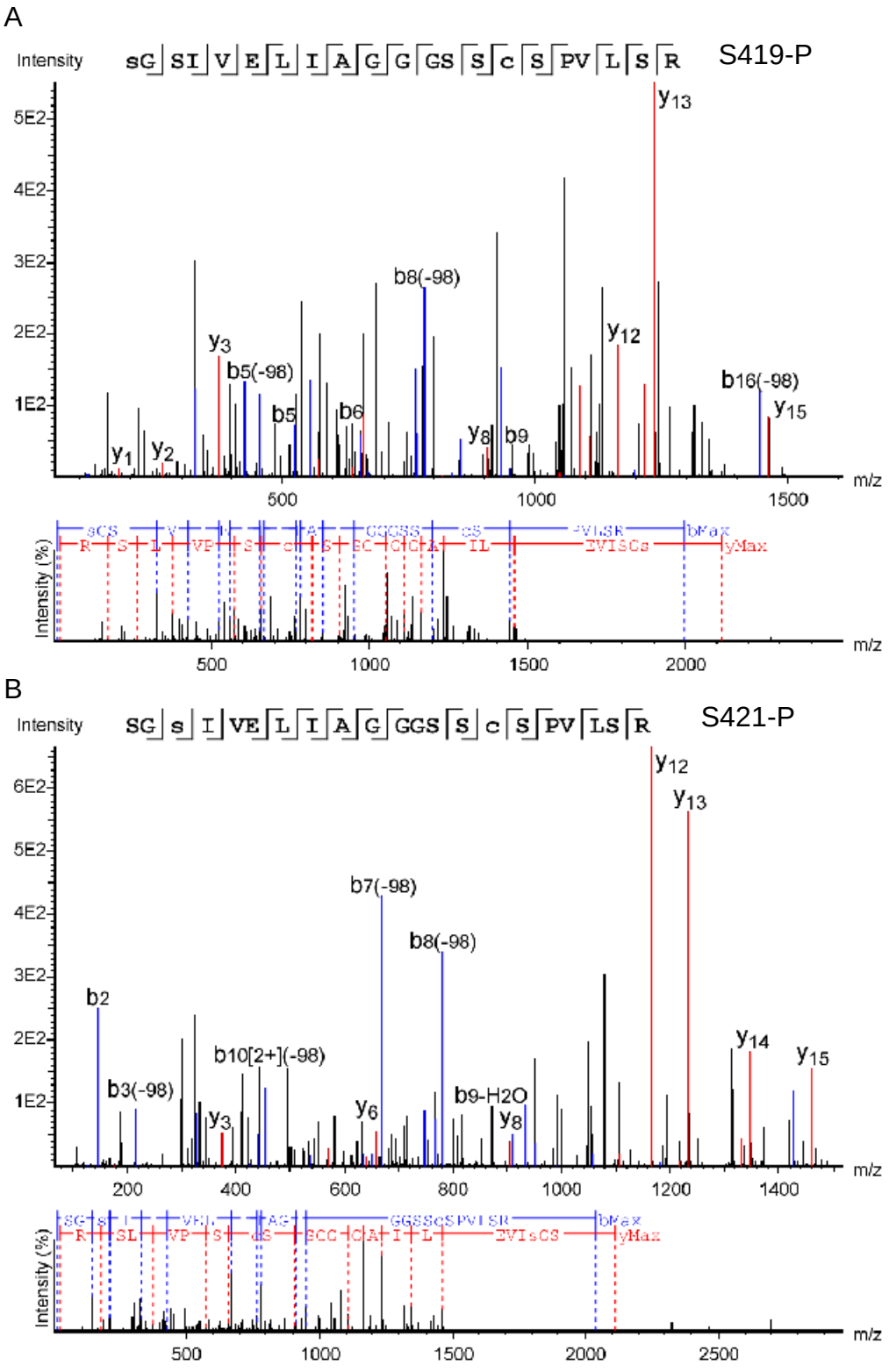
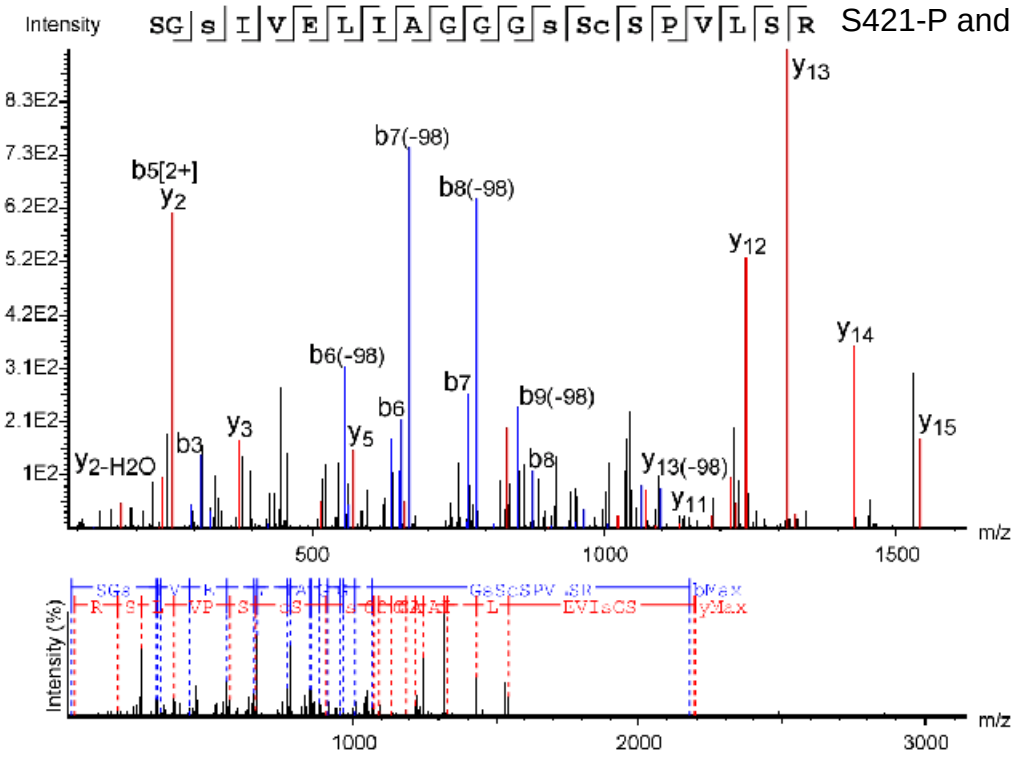


Figure S6. Exemplary spectra of trypsin digested HTT$^{1-3144}$ Q23 from EXPI293F.

Full data can be found through PRIDE (2) with accession PXD010865.

C



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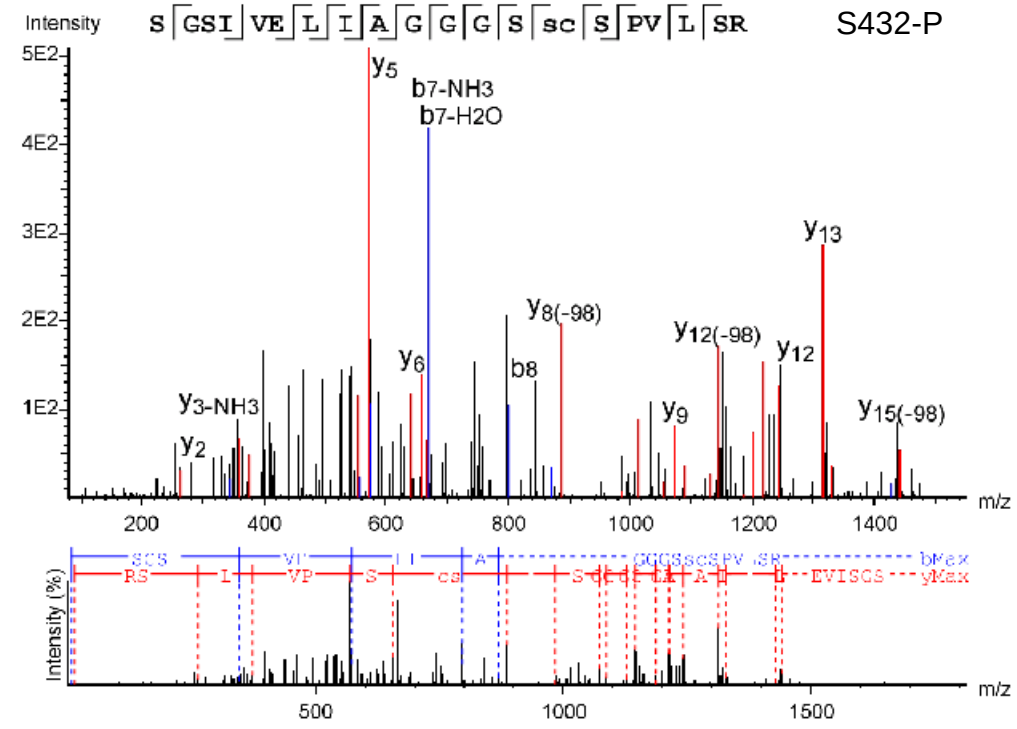

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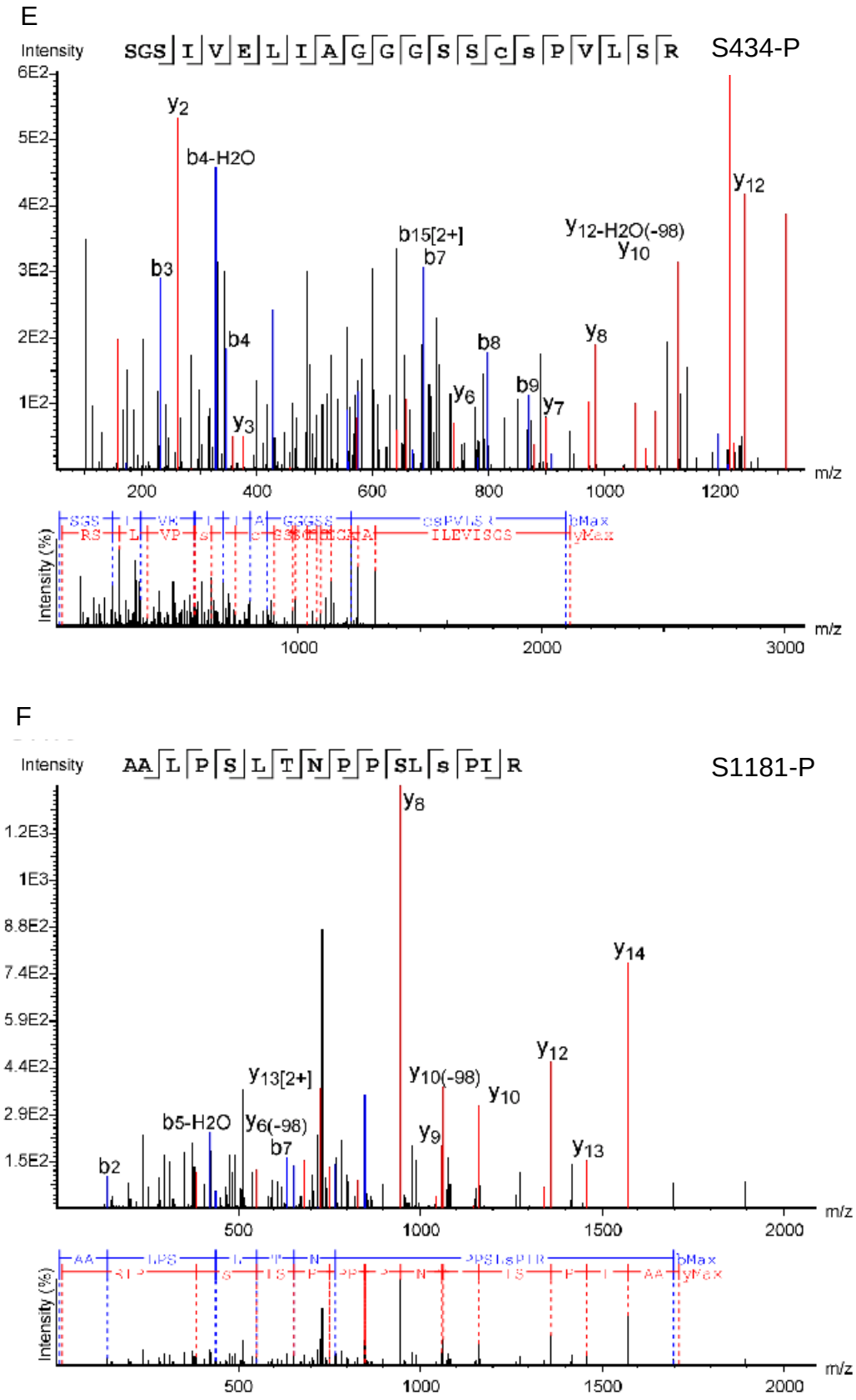

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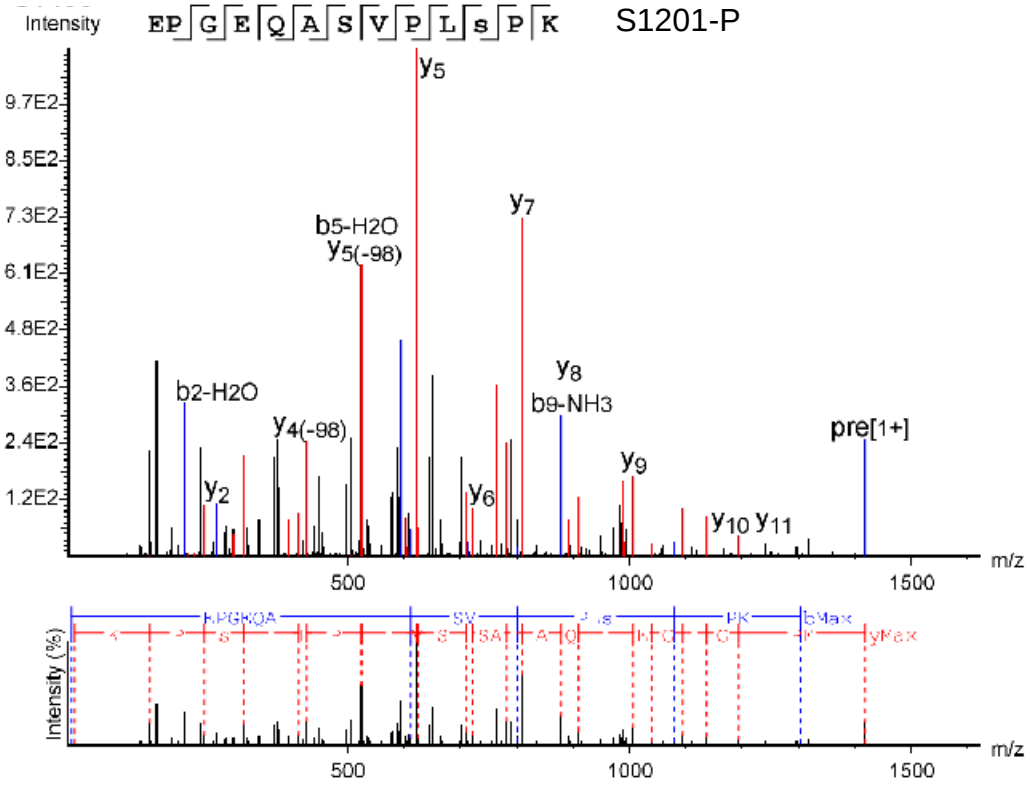

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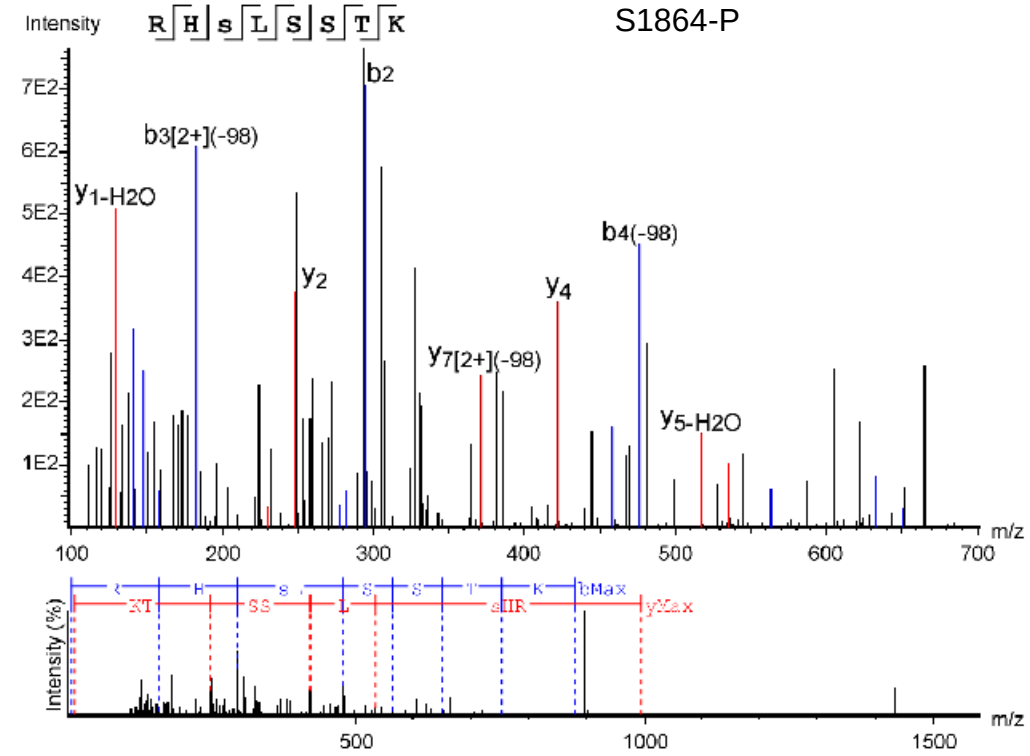

Figure S6 continued.

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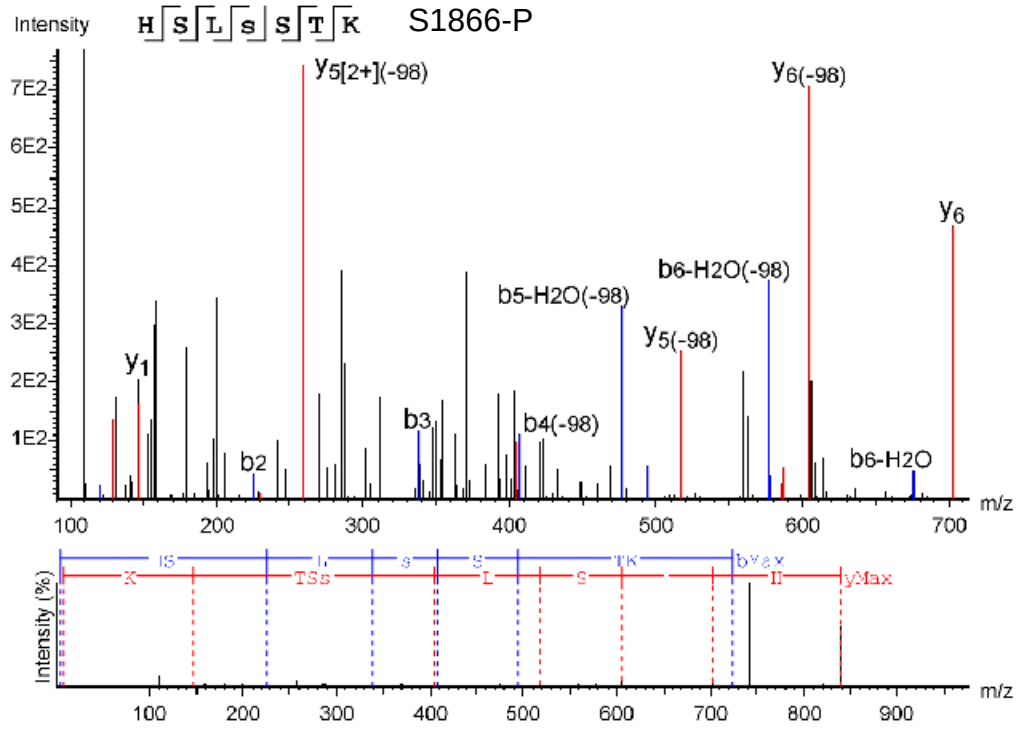

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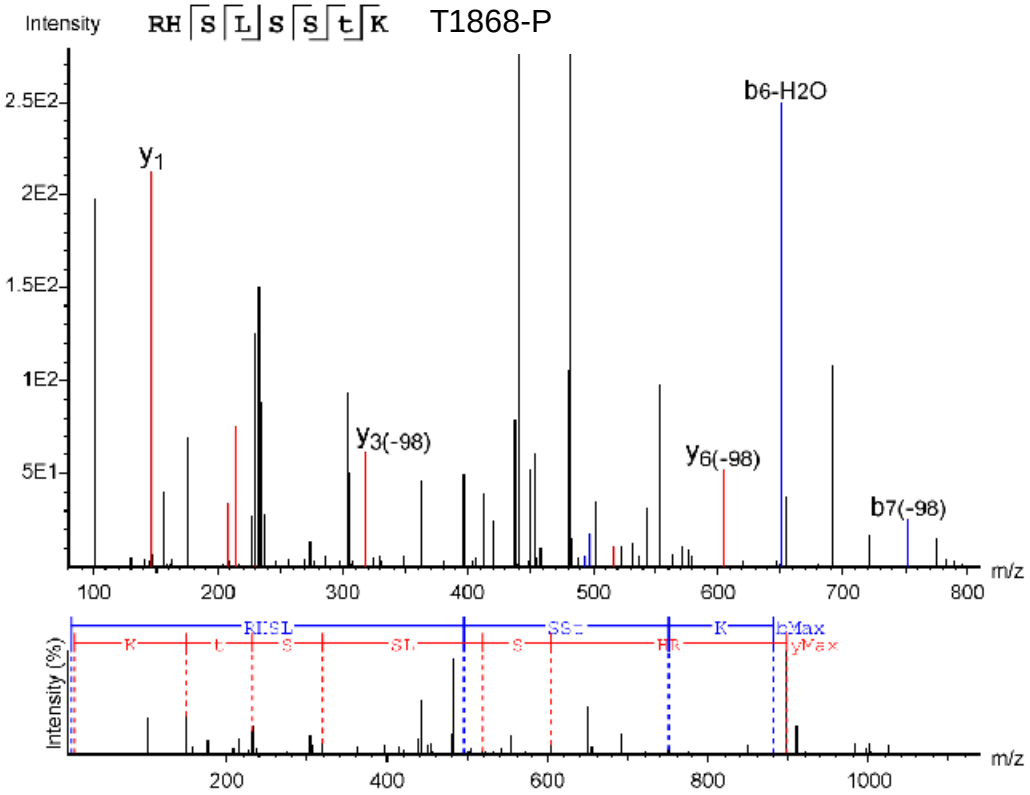

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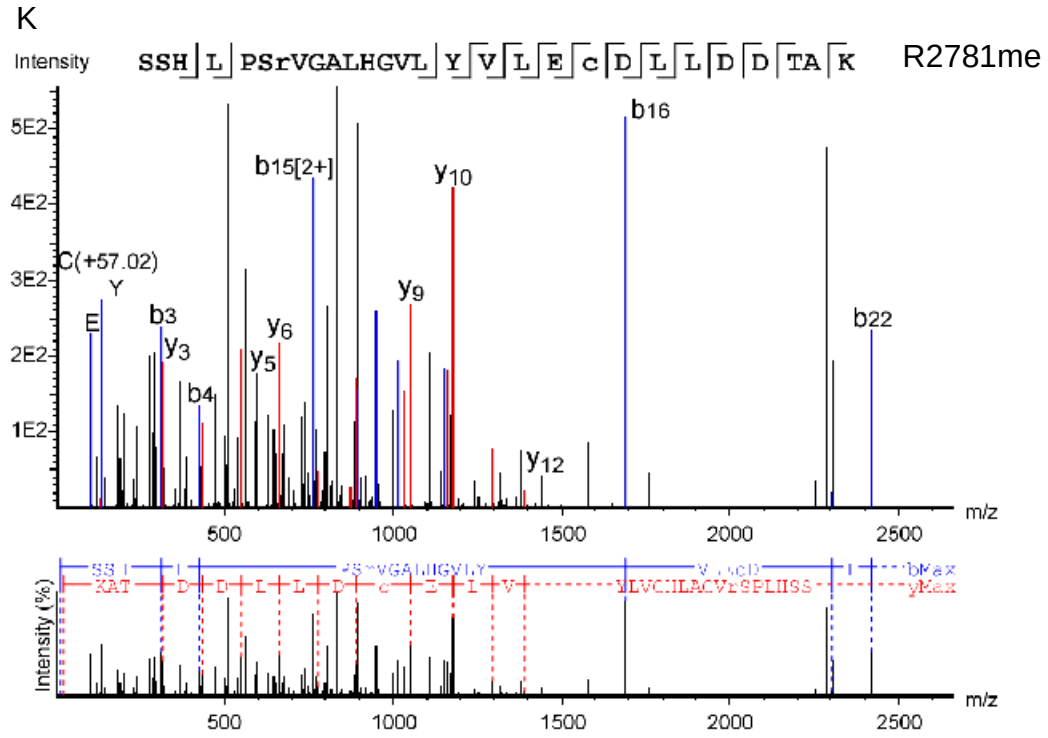

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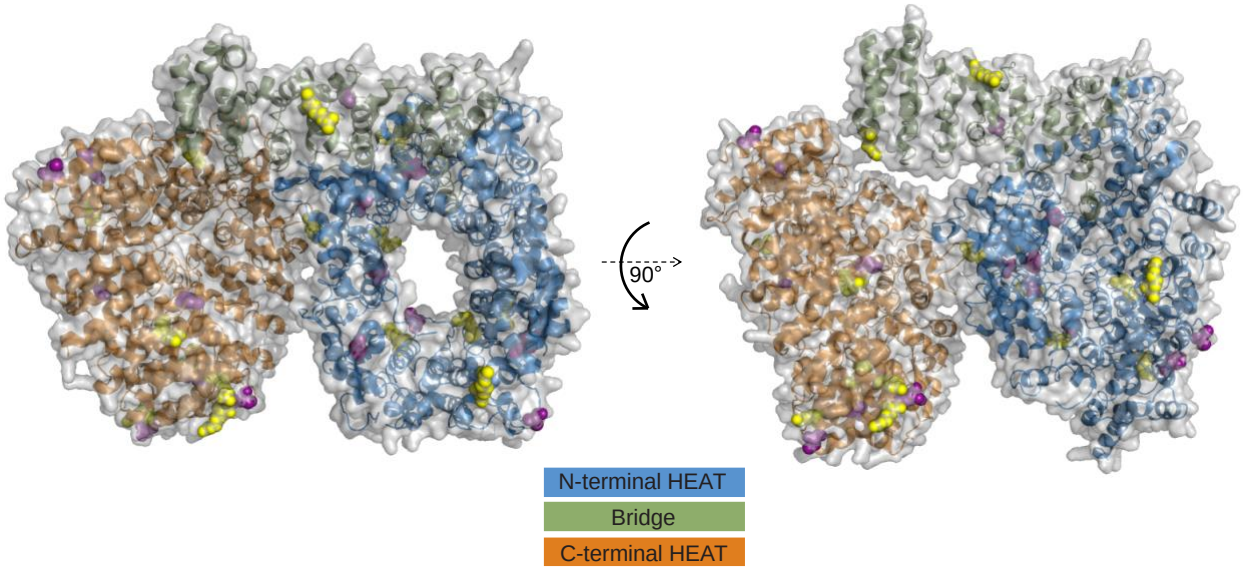

Figure S7. Mapping HTT posttranslational modifications identified from HTT$^{1\text{-}3144}$ samples from Sf9 and EXPI293F cells onto the HTT structure.
HTT is viewed top-down looking through the void in the N-terminal HEAT domain on the right hand side. Phosphorylation sites are shown in pink and all other modification sites are shown in green. As these samples were expressed in the absence of the stabilising HAP40 protein, it is likely that the more conformationally flexible apo HTT protein molecule would have greater exposure of different domain surfaces that would permit more sites to be modified than might be estimated from assessing the HTT-HAP40 molecule.

REFERENCES

1. Mellacheruvu, D., Wright, Z., Couzens, A. L., Lambert, J.-P., St-Denis, N., Li, T., Miteva, Y. V., Hauri, S., Sardiu, M. E., Low, T. Y., Halim, V. A., Bagshaw, R. D., Hubner, N. C., al-Hakim, A., Bouchard, A., Faubert, D., Fermin, D., Dunham, W. H., Goudreault, M., Lin, Z.-Y., Badillo, B. G., Pawson, T., Durocher, D., Coulombe, B., Aebersold, R., Superti-Furga, G., Colinge, J., Heck, A. J. R., Choi, H., Gstaiger, M., Mohammed, S., Cristea, I. M., Bennett, K. L., Washburn, M. P., Raught, B., Ewing, R. M., Gingras, A.-C., and Nesvizhskii, A. I. (2013) The CRAPome: a Contaminant Repository for Affinity Purification Mass Spectrometry Data. *Nat. Methods*. **10**, 730–736
2. Jones, P., Côté, R. G., Martens, L., Quinn, A. F., Taylor, C. F., Derache, W., Hermjakob, H., and Apweiler, R. (2006) PRIDE: a public repository of protein and peptide identifications for the proteomics community. *Nucleic Acids Res.* **34**, D659–D663