

**Supplementary Material:**

**A human huntingtin SNP alters post-translational modification and pathogenic proteolysis of the protein causing Huntington disease**

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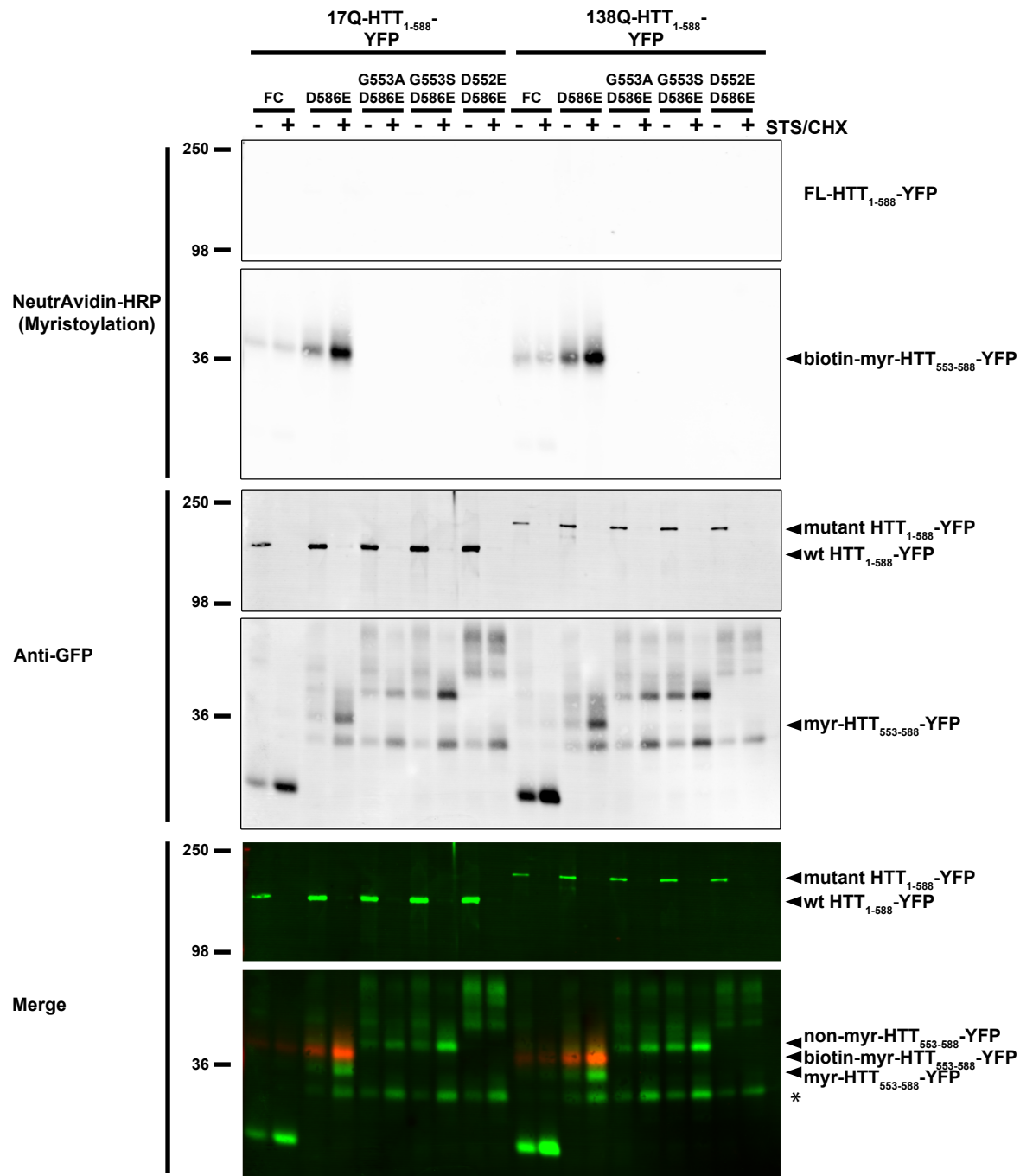
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**Supplemental Table 1** – List of established PTMs<sup>1,2</sup> and novel missense mutations. Amino acid positions refer to NP\_002102.4.

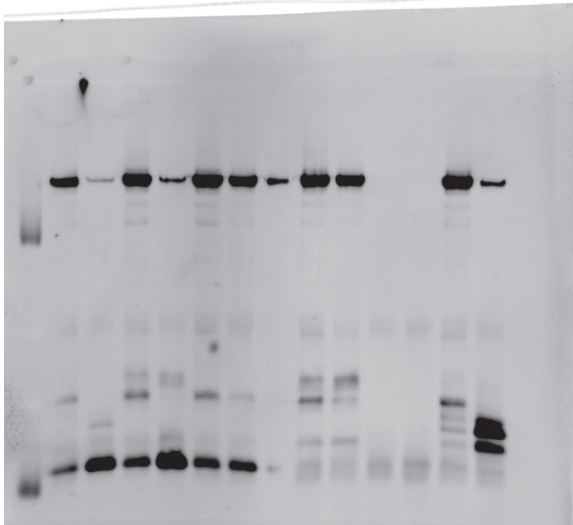
PTM Position	Amino acid	PTM	Missense mutation position	Original AA	Effect
3	T	phosphorylation			
6	K	Sumoylation			
6	K	ubiquitination			
9	K	Sumoylation			
9	K	ubiquitination			
13	S	phosphorylation			
15	K	Sumoylation			
15	K	ubiquitination			
16	S	phosphorylation			
81-129		proteolysis			
104-114		proteolysis			
124		proteolysis			
167	R	proteolysis			
205-214		proteolysis			
214	C	palmitoylation	233	A	V
402	G	proteolysis			
421	S	phosphorylation			
431	S	phosphorylation			
432	S	phosphorylation			
434	S	phosphorylation			
437	L	proteolysis			
444	K	acetylation			
469	T	proteolysis			
513	D	proteolysis			
536	S	proteolysis			
552	D	proteolysis			
553	G	myristoylation	553	G	E
586	D	proteolysis	627	Q	R
			698	G	E
			789	V	M
			895	G	E
			996	Y	R
			1066	V	I
			1084	D	H
			1093	I	M
			1175	T	A
1181	S	phosphorylation			
1201	S	phosphorylation	1262	T	M
			1387	N	H
			1710	C	R
			1722	T	N
2076	S	phosphorylation	2076	S	P
			2311	Y	H
			2446	E	D
			2645	D	del
			2647	D	E
2653	S	phosphorylation			
2657	S	phosphorylation	2788	V	I



**Supplementary Figure 1 – Orthogonal detection of post-translational myristoylation in HTT<sub>1-588</sub>-YFP.** HTT<sub>1-588</sub>-YFP was immunoprecipitated from HeLa cells incubated with alkyne-myristate. The myristate analog was covalently linked to azido-biotin through Click chemistry for detection. Biotinylated myristoylated cleaved HTT (biotin-myr-HTT<sub>553-588</sub>-YFP) migrated at an intermediate molecular weight between unlabeled myristoylated cleaved HTT (myr-HTT<sub>553-588</sub>-YFP) and non-myristoylated cleaved HTT (non-myr-HTT<sub>553-588</sub>-YFP). Both biotin-myr-HTT<sub>553-588</sub>-YFP and myr-HTT<sub>553-588</sub>-YFP could not be detected when the essential N-terminal glycine was substituted to an alanine (G553A) or serine (G553S). Non-myr-HTT<sub>553-588</sub>-YFP migrates a higher molecular weight than myr-HTT<sub>553-588</sub>-YFP. Cleavage bands correlating to myristoylated and non-myristoylated HTT could not be detected when caspase cleavage was blocked by substituting D552 with glutamate. \*- denotes unknown band.

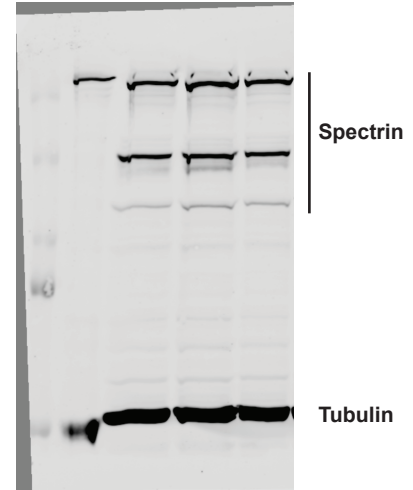
Uncropped gel from Figure 3B

STS	FC		G553E		G553E		Mock		D586E	
	-	+	-	+	-	+	-	+	-	+



Uncropped gel from Figure 3D

Edge Control	FC	G553E	G553E/D513E
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## Supplemental References

1. Ehrnhoefer, D. E., Sutton, L. & Hayden, M. R. Small Changes, Big Impact: Posttranslational Modifications and Function of Huntingtin in Huntington Disease. *Neurosci.* **17**, 475–492 (2011).
2. Martin, D. D. O., Ladha, S., Ehrnhoefer, D. E. & Hayden, M. R. Autophagy in Huntington disease and huntingtin in autophagy. *Trends Neurosci.* **38**, 26–35 (2015).