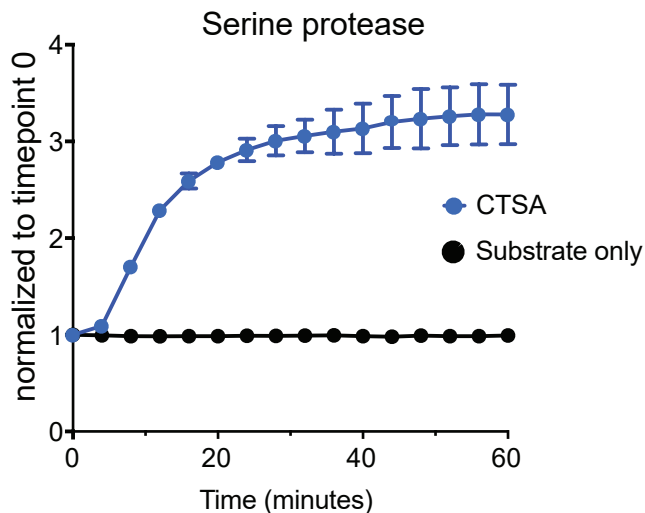
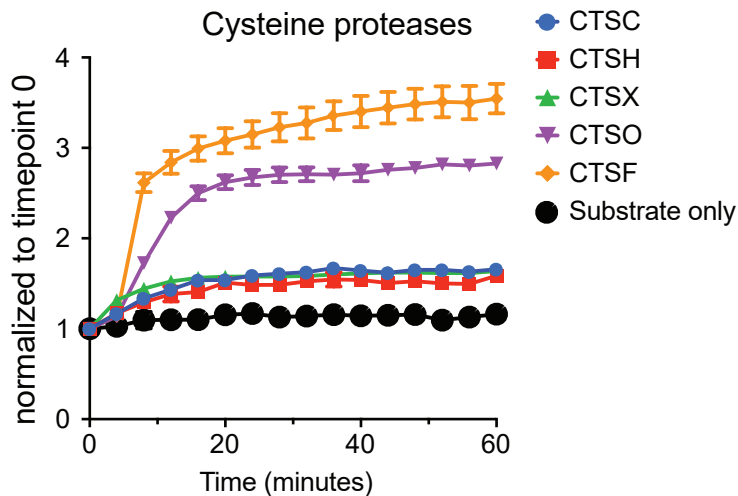


Figure S1



Alpha-synuclein

¹MDVFMKGLSKAKEGVVAAAEKTKQGVAAEAGKTKEGVLVVGSKTKEGVVHGVATVAEKTKEQVTNV
GGAVVTGVTAVAQKTVEGAGSIAAATGFVKKDQLGKNEEGAPQEGILEMPVDPDNEAYEMPSEEG
YQDYEPEA¹⁴⁰

Tau

¹MAEPRQEFEFVMEHDAGTYGLGDRKDQGGYT¹MHQD²QEGD³TDAGLKESPL⁴QTP⁵TEDGS⁶E⁷EP⁸G⁹SETSDA
KSTPTAEDVTAP¹⁰LVDEGAPGKQAAAQPHTEIPEGTTAAEEAGIGDTPSLEDEAAAGHVTQARMVSKSK
DGTGSDDKKAKGADGKTKIATPRGAAPPQKGQANATRIPAKTPPAPKTPP¹¹SSGEPPKSGDRSG¹²YS¹³
SP¹⁴GSP¹⁵GTPGSRSRTPSLPTPPTREP¹⁶PKKVAVVRTPPKSPSSAKSRLQTAPVPM¹⁷PDLKNVKSIGSTE
NLKHQ¹⁸P¹⁹GGGKVQ²⁰IINKKLD²¹LSNVQSKCGSKDNIKHVP²²GGGSVQ²³IVYKPV²⁴DL²⁵SKVTSKCGSLGNIHH
KP²⁶GGQVEVKSEKLD²⁷FKDRVQSKIGSLDNITHVP²⁸GGNKKIETHKLT²⁹FRENAKAKTDHGAEIVYKS
PVVSGDTS³⁰PRHL³¹SNVSSTGSIDMVDS³²PQLATLAD³³EV³⁴SASLAKQGL⁴⁴¹

TDP-43

¹MSEYIRVTEDE²ND³EP⁴IEI⁵PS⁶EDDGT⁷VL⁸LSTVTAQ⁹FP¹⁰GACGL¹¹RY¹²RNPVSQCM¹³RGVRLVEGIL¹⁴HAP¹⁵DA
GWGNL¹⁶VY¹⁷VVNYPKDNKRKMD¹⁸ETDASSAVKV¹⁹KRAVQ²⁰KTS²¹DLIVL²²GL²³LPWKTE²⁴QDLKEYFST²⁵FG²⁶EVLM
VQVKKDLKTGHSKGF²⁷GFVR²⁸TEYETQ²⁹VKVMSQR³⁰H³¹MIDGRWCDCKLPNSK³²QS³³QDEPLRSR³⁴KVFV³⁵GRC
TEDMTEDEL³⁶REF³⁷FSQ³⁸YGDVMDV³⁹FIPK⁴⁰PF⁴¹RAFAFVT⁴²FADDQIA⁴³QSLCGEDLI⁴⁴IKGISVHISNAEPKH
NSNRQLERSGRFGGNP⁴⁵GGFGN⁴⁶QGGFGNSRGGGAGL⁴⁷GNNQGS⁴⁸NMGGGMNF⁴⁹GAFSINPAM⁵⁰MAAQAAL
QSSWGMMLASQ⁵¹QN⁵²QSGPSGNNQ⁵³QGNMQREPNQAF⁵⁴GSGNNS⁵⁵YSGSNSGAAIGWGSASNAGSGG
FNGGF⁵⁶GSSMSDSKSSGWM⁴¹⁴

X Cleaved by CTSK after this residue (P1 position)

X Cleaved by CTSG after this residue (P1 position)

a

Asyn Vmax (nM/min)				
	WT	Mutant	WT	Mutant
	CTSD		CTSE	
A30P	0.0	0.0	0.0	0.0
E46K	0.0	0.0	0.0	0.0
G51D	0.0	0.0	0.0	0.0
A53T	0.0	0.0	45.2	17.2
	CTSB		CTSF	
A30P	149.7	116.7	0.0	0.0
E46K	172.2	176.1	0.0	0.0
G51D	152.4	144.1	0.0	0.0
A53T	149.3	175.7	0.0	0.0
	CTSL		CTSK	
A30P	130.6	184.6	59.1	109.7
E46K	30.9	45.0	2000.0	2000.0
G51D	34.0	12.2	2000.0	0.0
A53T	83.1	73.0	44.9	0.0
	CTSS		CTSV	
A30P	161.8	252.7	323.6	397.8
E46K	0.0	0.0	16.0	16.1
G51D	0.0	0.0	148.1	133.4
A53T	76.0	77.4	228.6	255.5
	AEP			
A30P	0.0	0.0		
E46K	0.0	0.0		
G51D	0.0	0.0		
A53T	0.0	0.0		

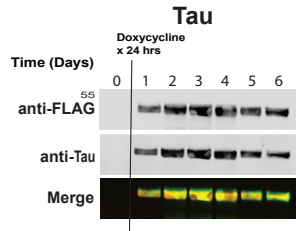
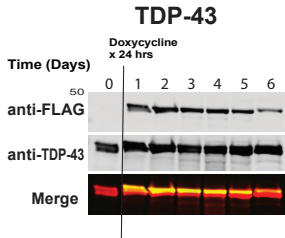
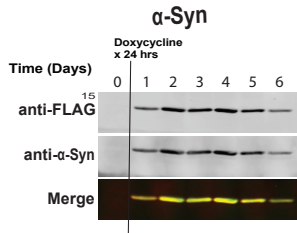
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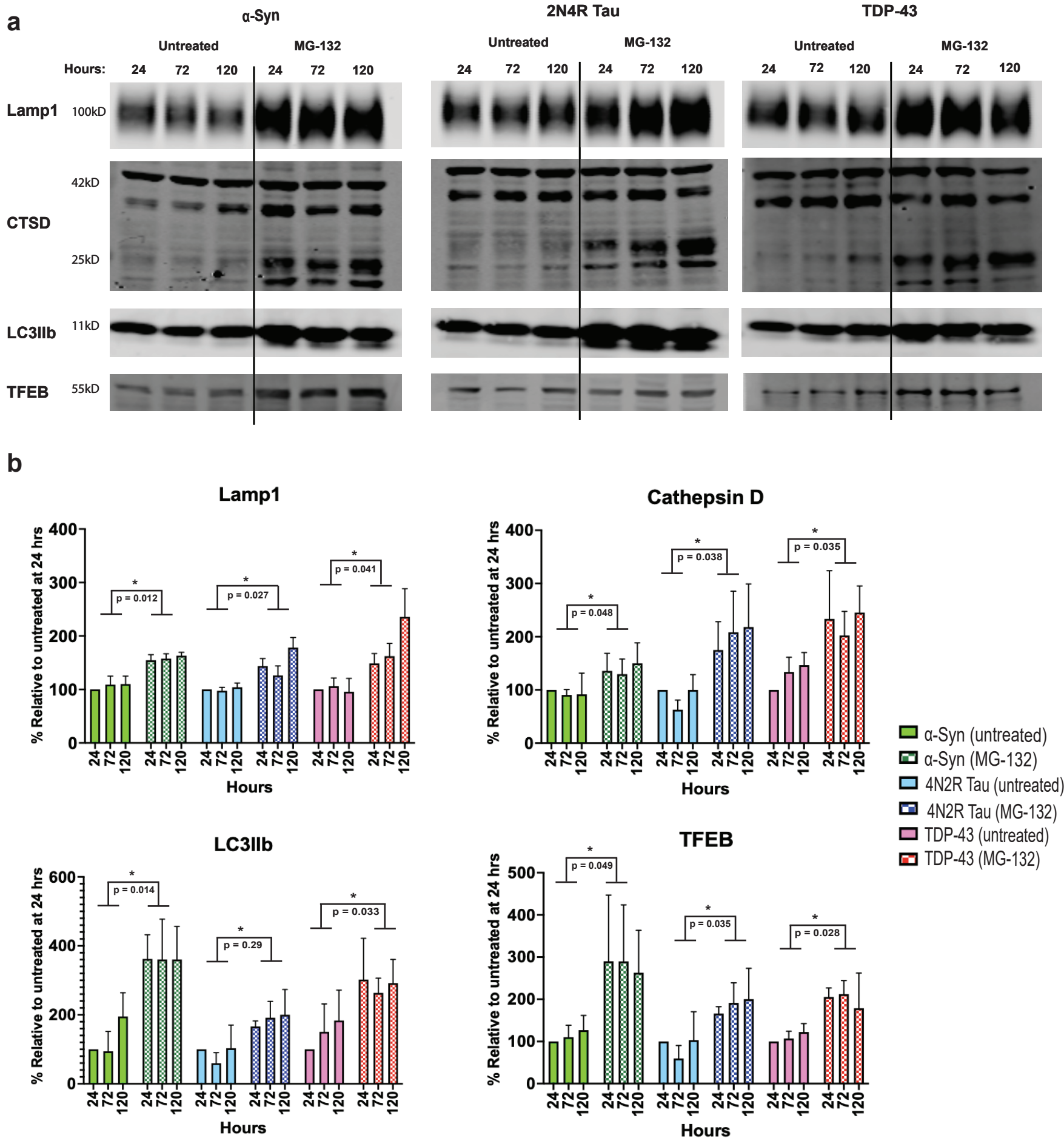
TDP-43 Vmax (nM/min)				
	WT		Mutant	
	CTSD		CTSE	
G298S	0.0	0.0	0.0	0.0
A315T	0.0	0.0	60.0	23.4
A321G	135.2	112.5	34.1	21.0
Q331K	0.0	0.0	0.0	0.0
M337V	0.0	0.0	0.0	0.0
	CTSB		CTSF	
G298S	61.2	45.0	0.0	0.0
A315T	131.0	333.4	0.0	0.0
A321G	945.3	1116.8	0.0	0.0
Q331K	181.0	0.0	0.0	0.0
M337V	45.4	90.0	0.0	0.0
	CTSL		CTSK (1nM)	
G298S	2000.0	2000.0	87.3	63.7
A315T	2000.0	2000.0	2000.0	2000.0
A321G	2000.0	2000.0	99.2	108.3
Q331K	220.0	0.0	133.3	2000.0
M337V	2000.0	2000.0	52.5	37.4
	CTSS		CTSV	
G298S	38.2	46.8	181.0	196.3
A315T	93.8	43.5	205.0	155.4
A321G	145.3	159.1	82.2	79.0
Q331K	306.5	2000.0	216.3	26.5
M337V	115.2	61.9	161.7	124.9
	AEP			
G298S	0.0	0.0		
A315T	0.0	0.0		
A321G	0.0	0.0		
Q331K	0.0	0.0		
M337V	0.0	0.0		

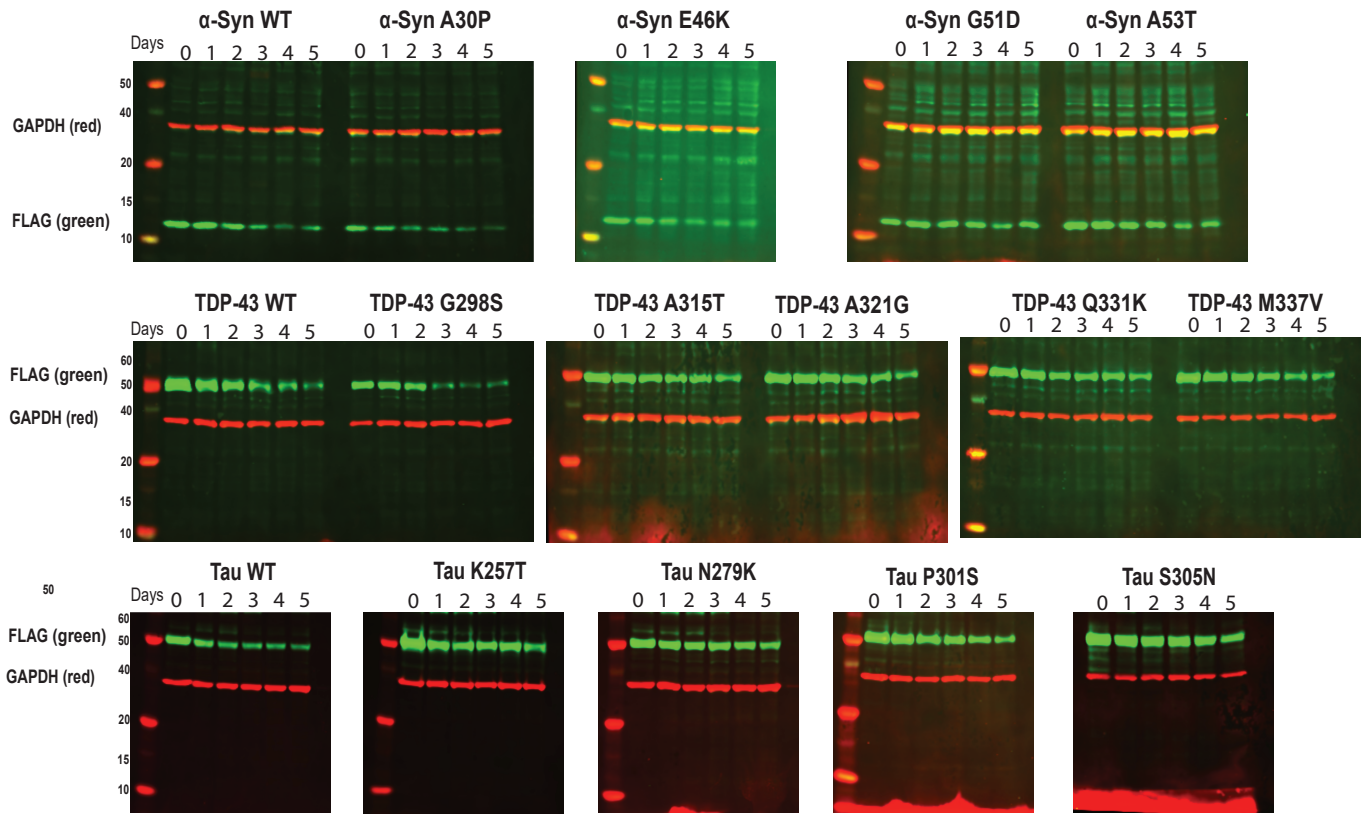
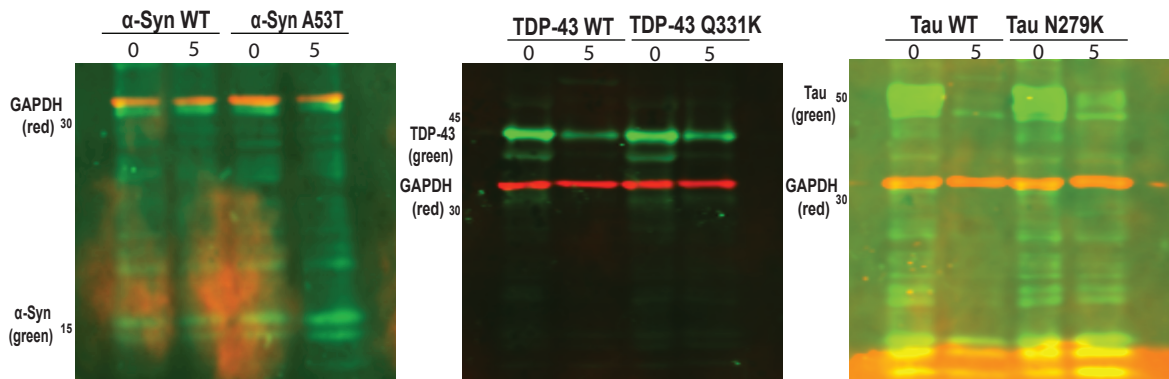
c

Tau Vmax (nM/min)				
	WT	Mutant	WT	Mutant
	CTSD		CTSE	
K257T	0.0	0.0	0.0	0.0
N279K	2000.0	2000.0	2000.0	2000.0
P301S	0.0	0.0	0.0	0.0
S305N	0.0	0.0	53.5	0.0
	CTSB		CTSF	
K257T	2000.0	2000.0	40.4	0.0
N279K	2000.0	2000.0	0.0	0.0
P301S	140.4	198.8	0.0	0.0
S305N	16.3	8.6	0.0	0.0
	CTSL		CTSK (1 nM)	
K257T	2000.0	2000.0	289.7	170.0
N279K	2000.0	2000.0	72.2	120.5
P301S	2000.0	2000.0	0.0	0.0
S305N	2000.0	2000.0	0.0	0.0
	CTSS		CTSV	
K257T	23.6	58.5	0.0	0.0
N279K	20.7	0.0	0.0	0.0
P301S	0.0	0.0	65.0	45.9
S305N	23.8	28.5	126.9	146.1
	AEP			
K257T	42.8	29.1		
N279K	95.7	0.0		
P301S	0.0	0.0		
S305N	0.0	0.0		

Figure S4





aSH-SY5Y cells**b**iNeurons

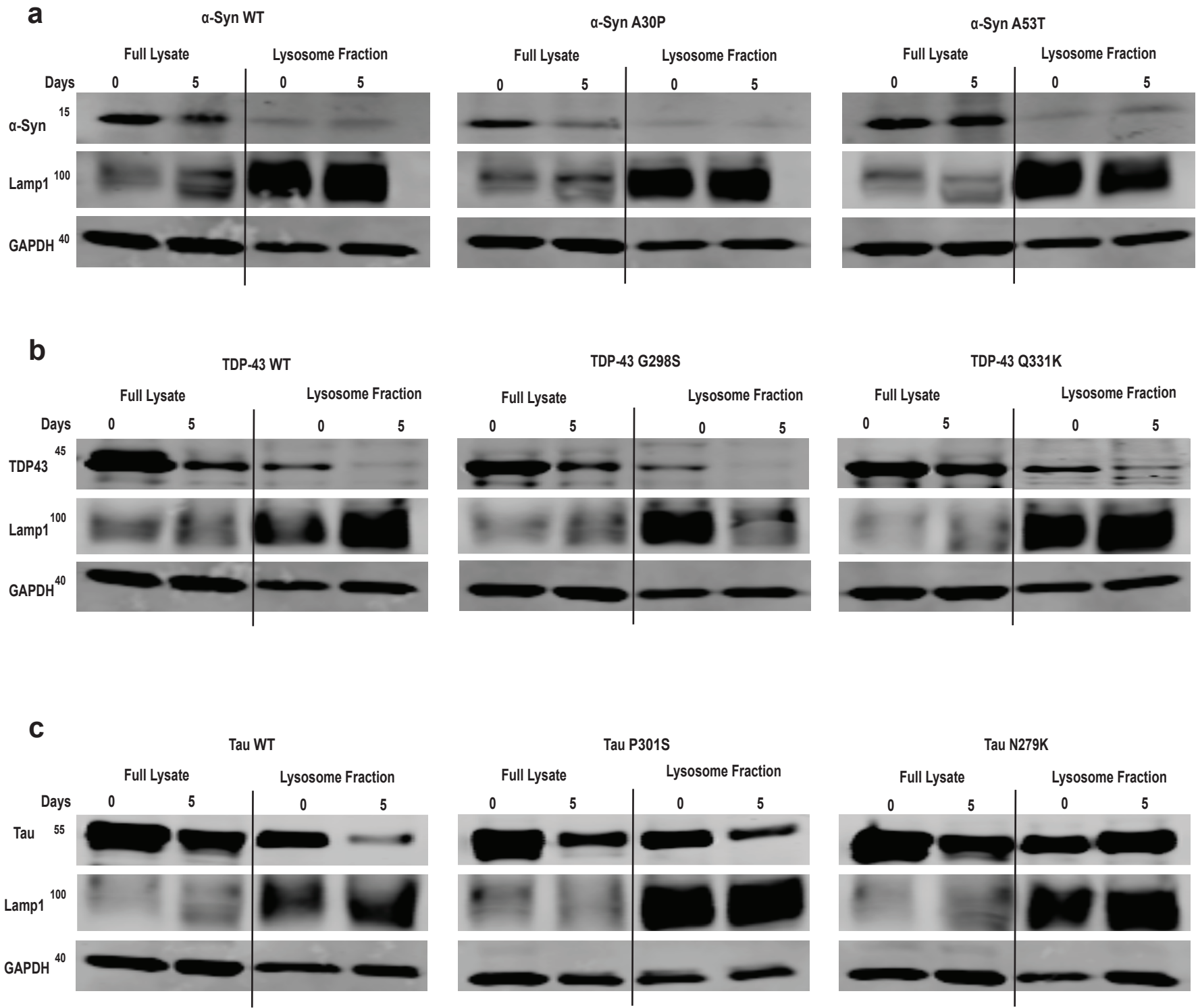


Table S1

Sequence Name	peptide sequence	Sequence Name	peptide sequence	Sequence Name	peptide sequence
alpha-syn_1-18	REGMDVFMKGLSKAKEGVVAAGGR	tau_1-18	RGGMAEPRQEFVEMDHAGTYGKR	tdp43_1-18	RKGMSEYIRVTEDENDIEIGKR
alpha-syn_14-31	RNKGVVAAAEKTKQGVAAEAGNGR	tau_14-31	RKGHAGTYGLGDRKDQGGYTMGSR	tdp43_14-31	RKGEPIEPSEDDGTVLLSTVGKR
alpha-syn_27-44	RDPAEAAGKTKEGVLYVGSKTGSR	tau_27-44	RKTGGYTMHQDQEGD TDAGLKQQR	tdp43_27-44_C39A	RDGLLSTVTAQFPGAAGLRYRGDK
alpha-syn_40-57	RDGVGSKTKEGVVHGVATVAEGNR	tau_40-57	RPGDAGLKESPLQTPTEDGSEGKR	tdp43_40-57_C50A	RDGGLRYRNPVSQAMRGVRLVGDR
alpha-syn_53-70	RGGATVAEKTKEQVTNVGGAVGKR	tau_53-70	RKGEDGSEEPGSETSDAKSTPGGR	tdp43_53-70	RSGGVRLVEGILHAPDAGWNGKK
alpha-syn_66-83	RKGVGGAVVTGVTAVAQKTVEGSR	tau_66-83	RKGAKSTPTAEDVTAPLVDEGGIR	tdp43_66-83	RDGAGWGNLVVYVNPKNKRGRDR
alpha-syn_79-96	RGGQKTVEGAGSIAATGFVKGSR	tau_79-96	RKGLVDEGAPGKQAAQPHTEGKR	tdp43_79-96	RDGKDNKRKMDETDASSAVKVGSR
alpha-syn_92-109	RGGTGFVKKDKLGKNEEGAPQGNR	tau_92-109	RKGQPHTEIPEGTTAEEAGIGGQR	tdp43_92-109	RDGSAVKVKRAVQKTSDLMLGSR
alpha-syn_105-118	RRKGEGAPQEGILEMPVGGKGR	tau_105-122	RKNEAGIGDTPSLEDEAAGHV GKR	tdp43_105-122	RKSDLMLGLPWKTTEQDLKEGSR
alpha-syn_114-125	RSRKGEDMPVDPDNEAYGKGR	tau_118-135	RDNAAGHVTQARMVSKSKDGTGGR	tdp43_118-135	RKGQDLKEYFSTFGEVLMVQVGNR
alpha-syn_121-132	RGRKGDNEAYEMPSEEGGGKGR	tau_131-148	RQGSKDGTGSDDKKAKGADGKGR	tdp43_131-148	RSDLMVQVKDLKTGHSGKFGGDR
alpha-syn_128-140	RNRGKPSEEGYQDYEP EAGGKR	tau_144-161	RDGGADGKTKIATPRGAAPP GGDR	tdp43_144-161	REPSKGFVFRFTEYETQVKVGGR
		tau_157-174	RESAAPPQGKQANATRIPAKGDR	tdp43_157-174_C173A	RDGTQVKVMSQRHMIDGRWADGGR
		tau_170-187	RDGRIPAKTPPAPKTPSSGEGNR	tdp43_170-187_CC173AA	RSSGRWADAKLPNSKQSQDEPGKR
		tau_183-200	RGGPSSGEPKSGDRSGYSSPGKR	tdp43_183-200_C198A	REGSQDEPLRSRKVFVGRATEGGR
		tau_196-213	RDGGYSSPGSPGTPGSRRTPGDR	tdp43_196-213_C198A	RKSGRATEDMTEDELREFFSQGGR
		tau_209-226	REDRSRTPSLPTPTREPKVGDR	tdp43_209-226	RNGEFFSQYGDVMDVFIPKPFGR
		tau_222-239	RDGEPKKVAVVRTPPKSPSSAGDR	tdp43_222-239	RKSIPKPFRAFAFVTFADDQIGSR
		tau_235-252	RDGSPSSAKSRLQTAPVPMPDGR	tdp43_235-252_C244A	RKIADDQIAQSLAGEDLIKGSR
		tau_248-265	REGVPMPDLKNVSKIGSTENGYR	tdp43_248-265	RNGLIKGISVHISNAEPKHNGSR
		tau_261-278	RNGGSTENLKHQPGGKVKIIGDR	tdp43_261-278	REGEPKHNSNRQLERSGRFGGTDR
		tau_274-291_C291A	RDGKVQIINKKLDLSNVQSKAGER	tdp43_274-291	RSDGRFGGNPGGFGNQGGFGNGGR
		tau_300-317	RDGVPGGGSVQVYKPVDSLKGDR	tdp43_287-304	RTDGGFGNSRGGGAGLGNQGGDR
		tau_313-330_C322A	RSGVDLSKVTSKAGSLGNIHHSR	tdp43_300-317	RDGGNNQGSNMGGGMNFGAFSGGR
		tau_326-343	REGGNIHHKPGGGQVEVKSEKGR	tdp43_313-330	RDGFGAFSINPAMMAAAQAALGNR
		tau_339-356	RGGVKSEKLD FKDRVQSKIGSGDR	tdp43_326-343	RSGAQAAQLQSSWGMMGLASQGKR
		tau_352-369	REGSKIGSLDNITHVPGGNGKTR	tdp43_339-356	RNKMLASQQNQSGPSGNNQNGGSR
		tau_365-382	RSDGGGNKKIETHKLT FRENAGSR	tdp43_352-369	RKGNNQNQGNMQREPNAFGSGSR
		tau_378-395	RDGFRENAKAKTDHGA EIVYKGR	tdp43_365-382	RKGQAFGSGNNSYSGSNSGAAGKR
		tau_391-408	RMGEIVYKSPVVS GDTSPRHLGSR	tdp43_378-395	RKGNSGAAGWGSASNAGSGSGKR
		tau_404-421	RDGSPRHLSNVSS TGSIDMVDGSR	tdp43_391-408	RKGAGSGSGFNGGFGSSMDSKGNR
		tau_417-434	RKGIDMVDSPQLATLADEV SAGKK	tdp43_404-414	RNGSMDSKSSGWGMGGR
		tau_430-441	RPKDEV SASLAKQGLGSR		

Cathepsins	Pre-activation:	Protease concentrations and pHs used for mass spectrometry:	Mass spectrometry time points:
CTSA	Trans-activated with CTSL following the manufacturer's recommendation. The irreversible cysteine protease inhibitor E-64 was then added for an additional 30 min at 37 °C to allow for inhibition of CTSL.	41 nM in pH 4.5	1h, 4h
CTSB	N/A	20 nM in pH 4.5 and 5.5	1h, 4h
CTSC	Trans-activated with CTSL following the manufacturer's recommendation. The irreversible cysteine protease inhibitor E-64 was then added for an additional 30 min at 37 °C to allow for inhibition of CTSL.	N/A	1h, 4h
CTSD	N/A	10 nM in pH 3.4 and 4.5	1h, 4h
CTSE	N/A	10 nM in pH 3.4 and 4.5	1h, 4h
CTSF	N/A	50 nM in pH 4.5 buffer	2h, 6h
CTSG	N/A	N/A	1h, 4h
CSTH	Trans-activated with thermolysin following the manufacturer's recommendation. Phosphoramidon was then added to these preparations for an additional 30 min at 37 °C to allow for inhibition of thermolysin	N/A	1h, 4h
CTSK	N/A	1 nM at pH 4.5	15min, 2h
CTSL	N/A	8.0 nM at pH 4.5 and 5.5	1h, 4h
CTSO	N/A	25 nM at pH 5.5	1h, 4h
CTSS	N/A	10 nM at pH 4.5 and 5.5	1h, 4h
CTSV	N/A	20 nM at pH 3.4 and 4.5	1h, 4h
CTSX	Preincubated with dithiothreitol (DTT) following the manufacturer's recommendation.	50 nM at pH 3.4 and 4.5	1h, 4h
AEP	N/A	20 nM at pH 4.5 and 5.5	1h, 4h

α -Syn**a**

Mutation	Peptide sequence	P1 site	CTSB	P1 site	CTSK	P1 site	CTSL	P1 site	CTSS	P1 site	CTSD	P1 site	CTSE
A53T	51-GV A TV A E KT-59	57	5 (-79.35)	56	2 (-31.80)	57	7 (-79.20)	57	5 (-37.13)	55	2 (-70.91)	55	9 (-83.46)
G51D	47-GV V H GV A TV-55	53	19 (-100.47)	-	-	53	44 (-115.01)	53	29 (-63.75)	52	15 (-97.34)	48	20 (-101.85)
E46K	40-VG S KT K E GV-48	42	28 (-107.33)	41	28 (-71.35)	42	22 (-96.04)	44	43 (-75.58)	45	48 (-127.55)	45	48 (-137.62)
A30P	24-QG V A EA A GK-32	25	37 (-115.57)	27	6 (-45.70)	27	23 (-98.26)	27	20 (-55.19)	26	21 (-106.02)	26	13 (-91.31)

TDP-43**b**

Mutation	Peptide sequence	P1 site	CTSB	P1 site	CTSK	P1 site	CTSL	P1 site	CTSS	P1 site	CTSD	P1 site	CTSE
A321G	318-IN P A M MAAA-326	321	8 (-60.10)	324	11 (-37.03)	324	8 (-56.12)	321	3 (0)	322	7 (-39.72)	322	6 (-46.79)
A315T	314-G A FSIN PA M-322	321	8 (-60.10)	-	-	319	30 (-74.32)	321	3 (0)	321	32 (-49.76)	321	13 (-58.00)
Q331K	327-Q AAL Q SSWG-335	327	25 (-79.99)	331	2 (-10.24)	331	1 (-24.72)	331	9 (-20.45)	-	-	330	11 (-57.00)
M337V	336-M M GM LAS Q Q-344	337	26 (-80.55)	342	17 (-45.01)	-	-	343	41 (-44.74)	339	4 (-33.79)	339	8 (-49.36)
G298S	294-GGG A GLNN-302	-	-	-	-	-	-	-	-	-	-	-	-

Tau**c**

Mutation	Peptide sequence	P1 site	CTSB	P1 site	CTSK	P1 site	CTSL	P1 site	CTSS	P1 site	CTSD	P1 site	CTSE
N279K	274-KVQI I N KKL-282	279	8 (-73.99)	279	47 (-55.87)	279	4 (-51.57)	279	8 (-28.70)	277	5 (-65.12)	277	4 (-42.20)
S305N	304-G S VQI V YKP-312	308	13 (-81.71)	308	24 (-43.58)	309	10 (-65.66)	-	-	308	6 (-68.34)	308	7 (-49.00)
K257T	253-LKN V K S KI G-261	258	36 (-94.04)	257	4 (-25.09)	257	6 (-62.82)	-	-	260	18 (-77.40)	260	22 (-82.14)
P301S	299-HV P GGGSVQ-307	-	-	-	-	-	-	-	-	300	29 (-83.48)	-	-

a	α-Syn	
	A30P	25-QGVAAEAAGK-33
		25-QGVAE P AGK-33
	E46K	40-VGSKTKEGV-48
		40-VGSKTK K GV-48
	G51D	47-GVVHGVATV-55
		47-GVVH D VATV-55
	A53T	51-GVATVAEKT-59
		51-GV T TVAEKT-59
b	Tau	
	K257T	253-LKNVSKSIG-261
		253-LKNV T SKIG-261
	N279K	274-KVQIINKKL-282
		274-KVQII K KKL-282
	P301S	299-HVPGGGSVQ-307
		299-HV S GGGSVQ-307
	S305N	304-GSVQIVYKP-312
		304- G NVQIVYKP-312
c	TDP-43	
	G298S	294-GGGAGLGNN-302
		294-GGGAS L GNN-302
	A315T	314-GAFSINPAM-322
		314-G T FSINPAM-322
	A321G	318-INPAMMAAA-326
		318-INP G MMAAA-326
	Q331K	327-QAALQSSWG-335
		327-QAAL K SSWG-335
	M337V	336-MMGMLASQQ-344
		336-M V GMLASQQ-344