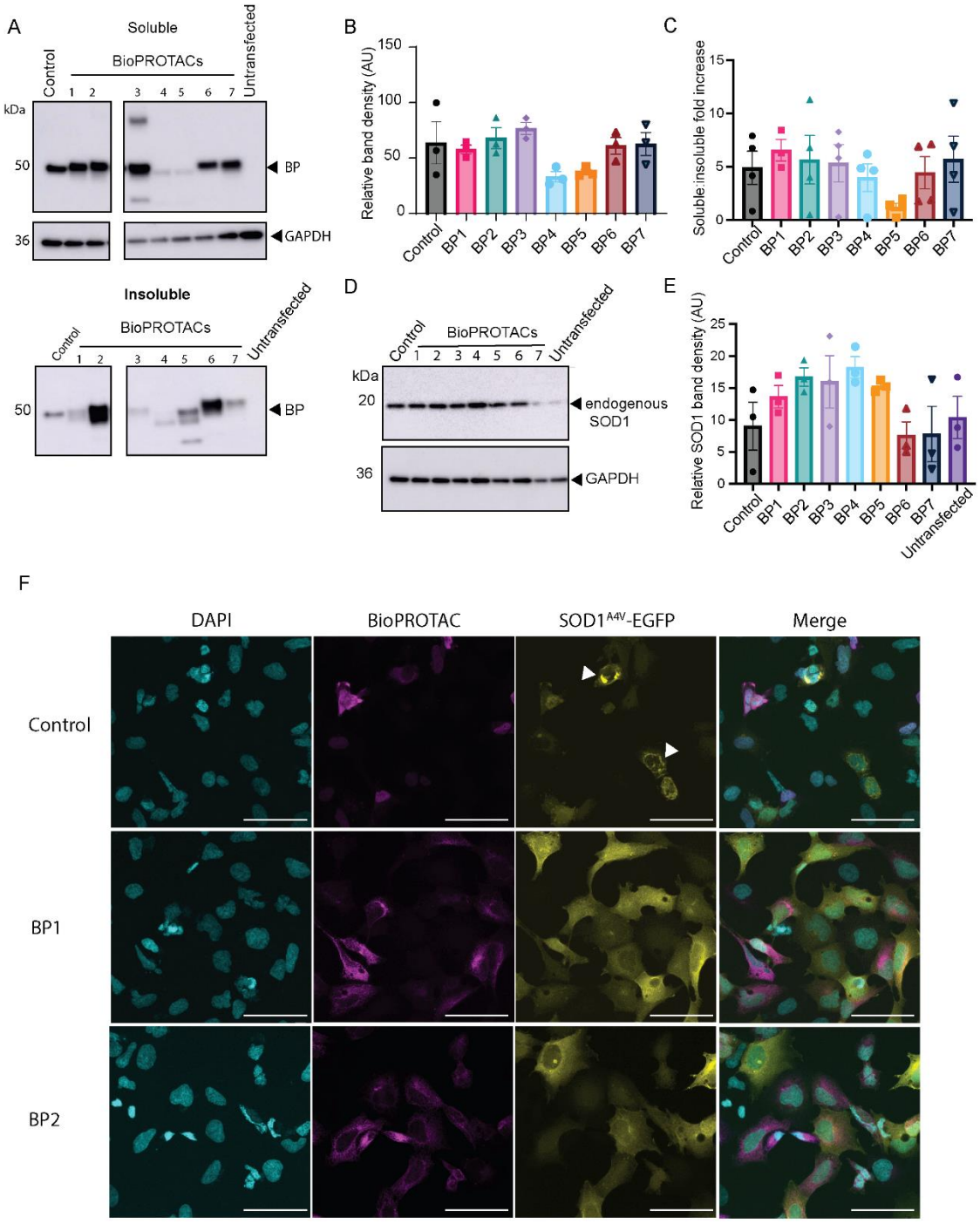
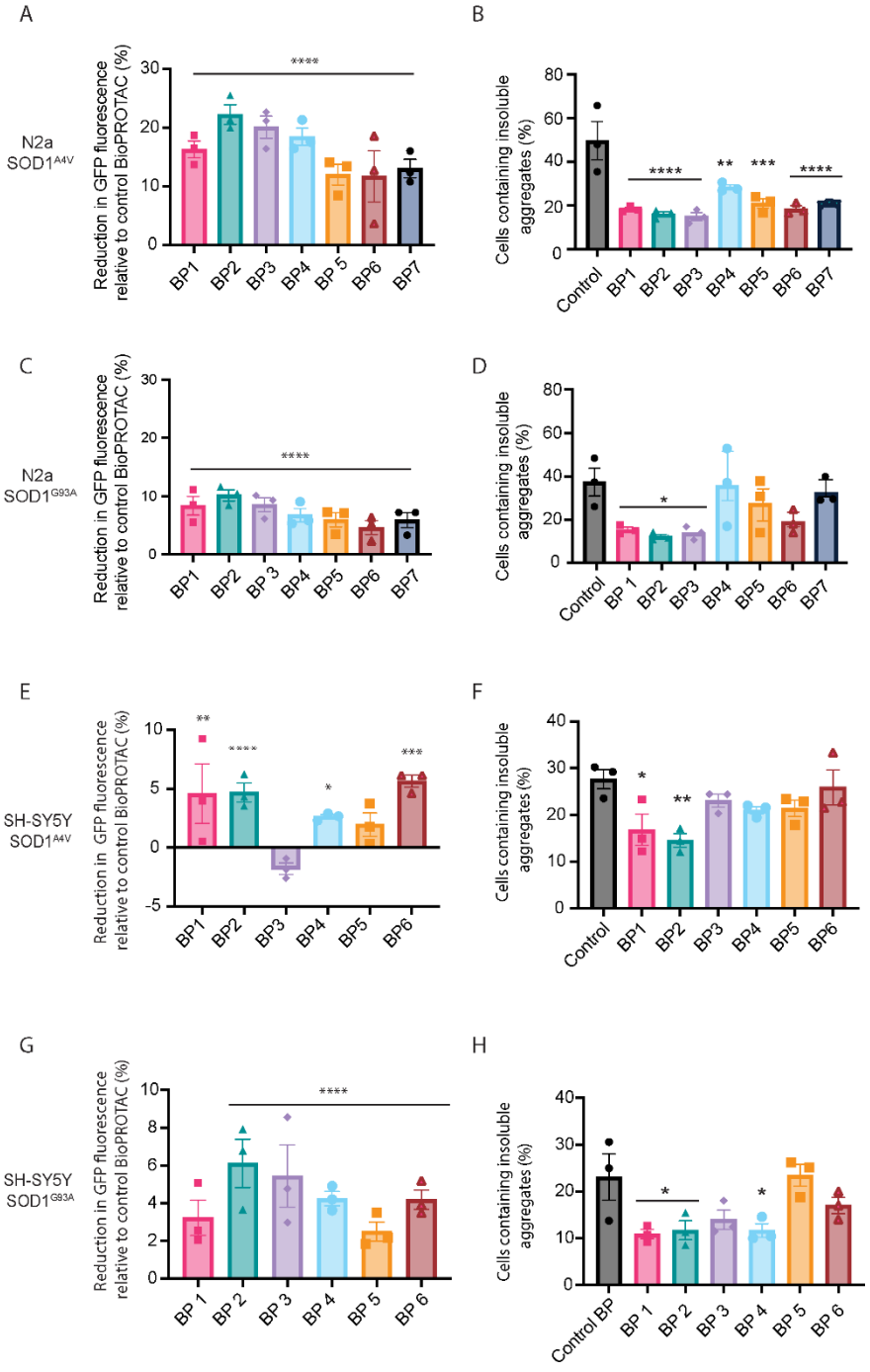
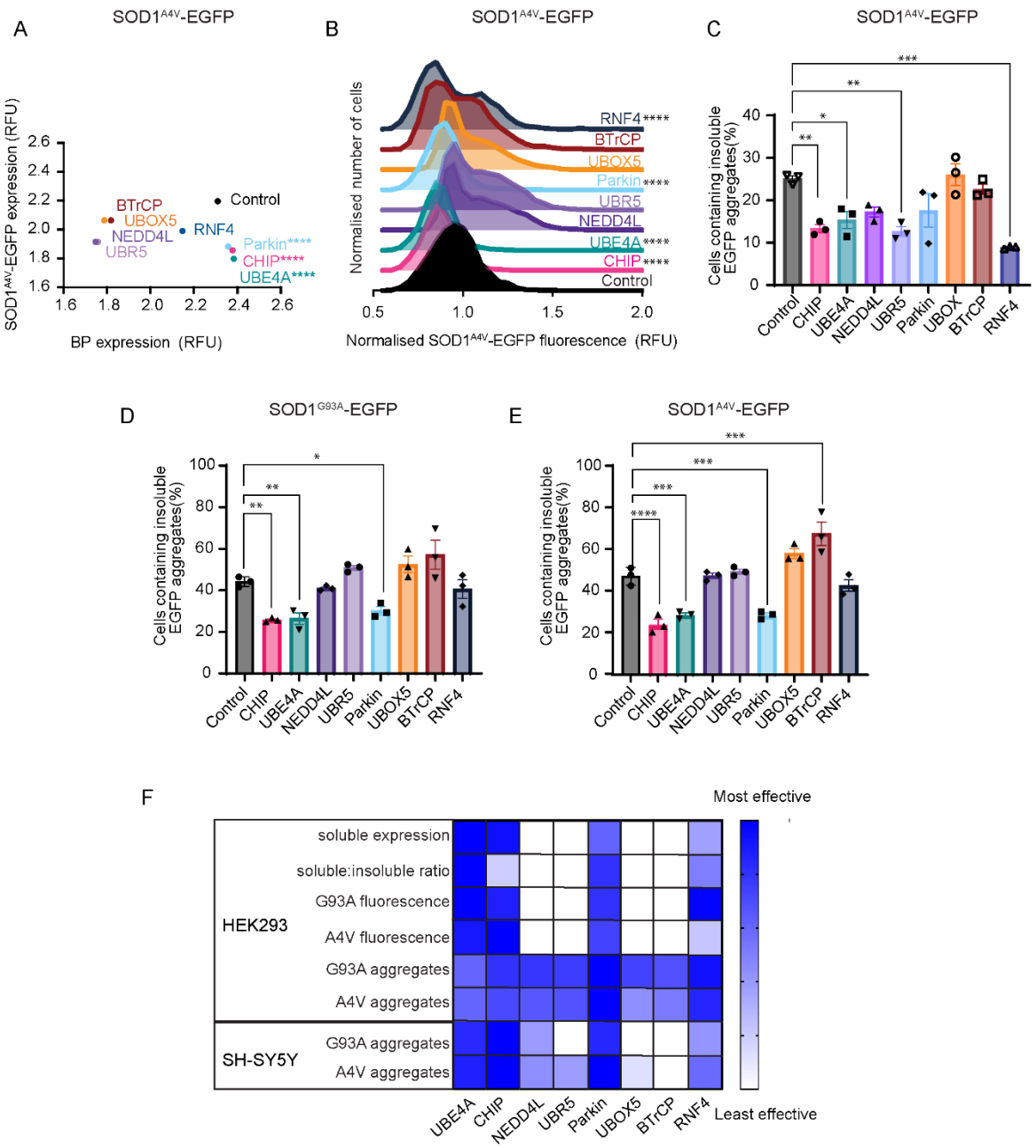
Supplementary Information



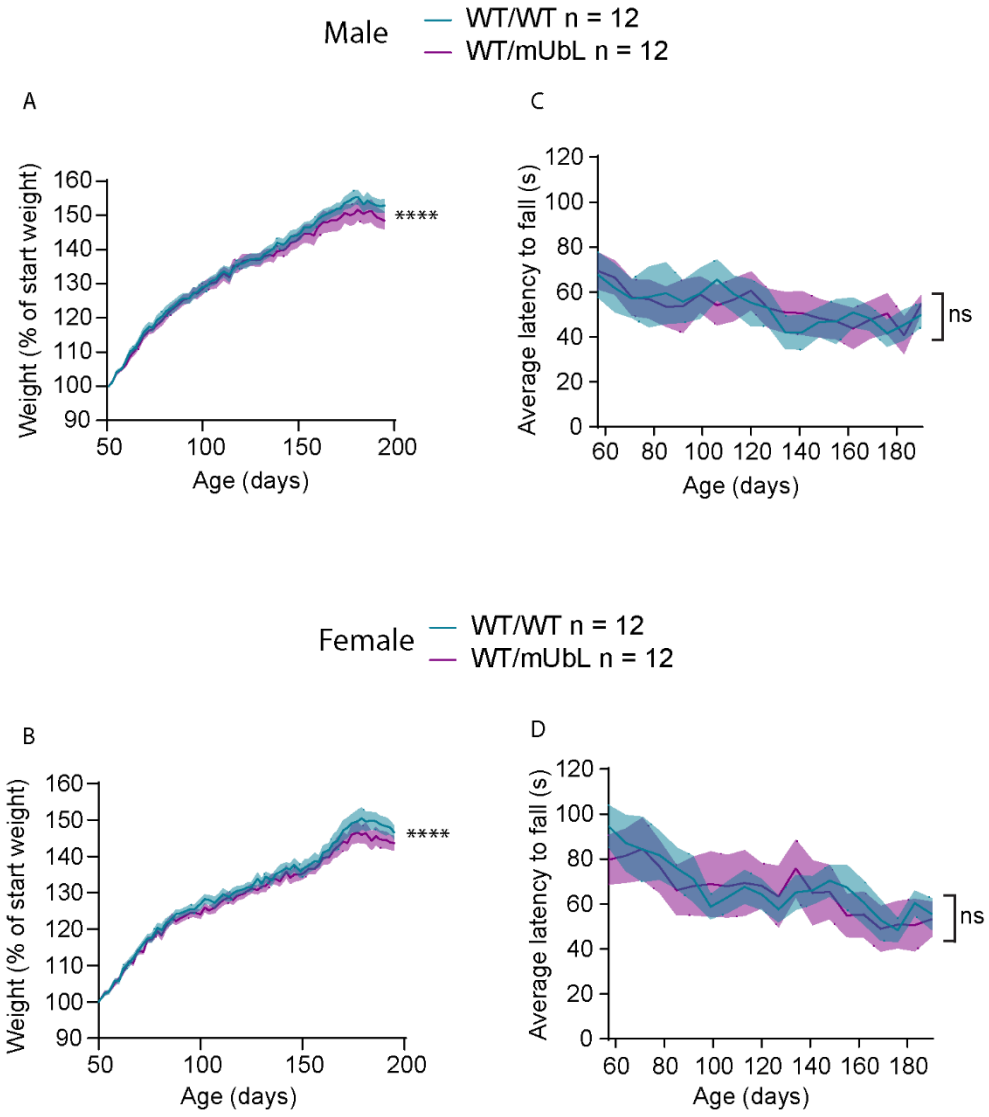
Supplementary Figure 1. BioPROTACs are expressed in the soluble fraction of the nucleus and cytoplasm, and do not reduce endogenous SOD1. (A and B) BioPROTAC expression was assessed in the soluble and insoluble fractions from HEK293 lysates. **(C)** Fold increase in expression of soluble to insoluble fraction was calculated for each BioPROTAC. **(D and E)** The soluble fraction was also assessed for SOD1 levels to determine effect on endogenous SOD1. **(F)** Immunocytochemistry was used to assess BioPROTAC expression levels. Representative images for BP1 and BP2 and the control in HEK293 cells transiently transfected to express BioPROTACs (magenta) and SOD1^{A4V}-EGFP (yellow). Scale bars represent 50 µm. White arrows show aggregates. For all graphs, bars represent mean ± SEM. Statistical significance was determined using **(B and C)** ordinary one-way ANOVA paired with Tukey's multiple comparisons test or **(E)** repeated measures one-way ANOVA paired with Dunnett's multiple comparisons test. All statistical values are shown in Supplementary Table 2. Blots are representative from at least 3 independent experiments. Raw data and complete western blots and total protein images are shown in source data file.



Supplementary Figure 2. BioPROTACs degrade SOD1 variants in multiple cell types. Total **(A)** SOD1^{A4V}-EGFP and **(C)** SOD1^{G93A}-EGFP fluorescence in Neuro2A cells expressing BioPROTACs relative to cells co-transfected with the control over 48 hr. The number of cells containing insoluble **(B)** SOD1^{A4V}-EGFP and **(D)** SOD1^{G93A}-EGFP aggregates in Neuro2A cells expressing BioPROTACs relative to cells co-transfected with the control was quantified using the saponin permeability assay. Total **(E)** SOD1^{A4V}-EGFP and **(G)** SOD1^{G93A}-EGFP fluorescence in SH-SY5Y cells expressing BioPROTACs relative to cells co-transfected with the control over 48 hr. The number of cells containing insoluble **(F)** SOD1^{A4V}-EGFP and **(H)** SOD1^{G93A}-EGFP aggregates in SH-SY5Y cells expressing BioPROTACs relative to cells co-transfected with the control was quantified using the saponin permeability assay. For all graphs, bars represent mean ± SEM (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$). Statistical significance was determined using **(A, C and E-H)** repeated measures or **(B and D)** ordinary one-way ANOVA paired with Dunnett's multiple comparisons test. All statistical values are shown in Supplementary Table 2. Raw data are shown in source data file.



Supplementary Figure 3. Reduction of misfolded SOD1 is ligase dependent and consistent across cell lines and SOD1 mutants. (A) Immunocytochemistry was used to assess BioPROTAC and SOD1^{A4V}-EGFP expression in HEK293 cells. **(B)** The reduction in SOD1^{A4V}-EGFP fluorescence compared to control was determined when expression levels of the BioPROTACs were normalised. **(C)** The number of cells containing insoluble SOD1^{A4V}-EGFP aggregates in HEK293 cells expressing BioPROTACs relative to cells co-transfected with the control was quantified using the saponin permeability assay. **(D)** The number of cells containing insoluble SOD1^{G93A}-EGFP aggregates in SH-SY5Y cells expressing BioPROTACs relative to cells co-transfected with the control. **(E)** The number of cells containing insoluble SOD1^{A4V}-EGFP aggregates in SH-SY5Y cells expressing BioPROTACs relative to cells co-transfected with the control. **(F)** A weighted heat map comparing the relative efficacy of BioPROTACs in the various assays. For all graphs, bars represent mean ± SEM (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$). Statistical significance was determined using **(A and B)** Kruskal-Wallis one-way ANOVA paired with Dunn's multiple comparisons test or **(C-E)** ordinary one-way ANOVA paired with Dunnett's multiple comparisons test. All statistical and error values are shown in Supplementary Table 2 and 3, respectively. Raw data are shown in source data file.



Supplementary Figure 4. Phenotypic differences across the lifespan of non-transgenic WT/WT and WT/misfoldUbL mice. (A-D) Phenotypic data comparing WT/WT (n = 12 male and n = 12 female) and WT/MisfoldUbL control mice (n = 12 male and n = 12 female) are shown. Control groups were obtained as described in Figure 5D. Male and female mice were assessed for **(A and B)** weight gain and **(C and D)** motor function via latency to fall on the rotarod. Results represent mean ± SEM (shading) (**** $P < 0.0001$). **(A-D)** Statistical significance was determined using repeated measures one-way ANOVA paired with Tukey's multiple comparisons test. All statistical values are shown in Supplementary Table 2. Raw data are shown in source data file.

Supplementary Table 1. Antibodies used to investigate Development of a targeted BioPROTAC degrader selective for misfolded SOD1

Antibody	**Conjugate**	**Antibody type**	**Company**	Catalogue **number**	**Dilution**	**Clonality**	**Application**
anti-6X His	-	primary	Abcam	ab18184	1:1,000	monoclonal	immunocytochemistry, co-immunoprecipitation
anti-mouse IgG	Alexa Fluor 647	secondary	Abcam	ab150115	1:1,000	polyclonal	immunocytochemistry
anti-FLAG	-	primary	Sigma	F1804	1:1,000	monoclonal	immunoblotting
anti-6X His	-	primary	Abcam	ab9108	1:1,000	polyclonal	immunoblotting
anti-SOD1	-	primary	Abcam	ab13498	1:5,000	polyclonal	immunoblotting
anti-GAPDH	-	primary	Sigma	G8795	1:5,000	monoclonal	immunoblotting
anti-Hsp70	-	primary	Abcam	ab47455	1:1,000	monoclonal	immunoblotting
anti-mouse IgG	HRP	secondary	Agilent	P044701-2	1:1,000	polyclonal	immunoblotting
anti-rabbit IgG	HRP	secondary	Agilent	P044801-2	1:1,000	polyclonal	immunoblotting
anti-synaptophysin	-	primary	Abcam	ab32594	1:800	polyclonal	NMJ immunostaining
anti-neurofilament heavy polypeptide	-	primary	Abcam	ab8135	1:1,000	polyclonal	NMJ immunostaining
anti-rabbit IgG	Alexa Fluor 647	secondary	Abcam	ab150079	1:500	polyclonal	NMJ immunostaining
alpha-bungarotoxin	Alexa Fluor 488	secondary	Thermo Fisher Scientific	B13422	1:2,000	-	NMJ immunostaining

GAPDH, glyceraldehyde 3-phosphate dehydrogenase; His, histidine; HRP, horseradish peroxidase; Hsp70, heat shock protein 70; IgG, immunoglobulin G; NMJ, neuromuscular junction; SOD1, superoxide dismutase 1.

Supplementary Table 3. Descriptive statistics for Figure 2E, Figure 3D and Supplementary Figure 3A

Figure and Panel	Sample	BP expression (RFU)			SOD1-EGFP expression (RFU)		
		n	Mean	SEM	*n*	Mean	SEM
Figure 2E	Control BP	3445	1	0.012	3445	64.55	0.922
	BP 1	2420	1.322	0.018	2420	27.3	0.406
	BP 2	3429	0.905	0.009	2665	13.97	0.125
	BP 3	1867	8.452	0.192	1867	4.633	0.053
	BP 4	2665	3.722	0.048	3429	47.1	0.47
	BP 5	3169	2.126	0.027	3169	23.13	0.221
	BP 6	2830	1.002	0.013	2830	42.79	0.449
	BP 7	3304	1.279	0.014	3304	52.23	0.587
Figure 3D	Control	2565	2.311	0.007	2565	2.056	0.002
	CHIP	3069	2.374	0.005	3069	1.821	0.002
	UBE4A	2911	2.385	0.005	2911	1.853	0.002
	NEDD4L	6408	1.757	0.002	6408	2.346	0.002
	UBR5	6216	1.766	0.002	6216	2.367	0.002
	Parkin	2945	2.372	0.006	2945	1.876	0.002
	UBOX5	5593	1.822	0.002	5593	2.381	0.002
	βTrCP	3044	1.85	0.006	3044	2.175	0.003
	RNF4	3204	2.333	0.005	3204	1.828	0.002
Supp. Figure 3A	Control	2477	2.31	0.007	2477	2.196	0.008
	CHIP	2806	2.379	0.005	5960	1.857	0.004
	UBE4A	3073	2.384	0.005	3729	1.799	0.008
	NEDD4L	6326	1.747	0.002	5901	1.915	0.005
	UBR5	5901	1.759	0.003	2943	1.99	0.006
	Parkin	3051	2.357	0.005	6326	1.883	0.005
	UBOX5	5960	1.787	0.002	3073	2.065	0.006
	BTrCP	3729	1.819	0.005	2806	2.074	0.006
	RNF4	2943	2.148	0.007	6147	1.985	0.005

BP, BioPROTAC; βTrCP, beta-transducin repeat-containing protein; CHIP, C-terminus of Hsc70 interacting protein; N, sample size; NEDD4L, neural precursor cell expressed developmentally downregulated 4-like; RNF4, RING finger protein 4; RFU, relative fluorescence units; SEM, standard error of the mean; SOD1-EGFP, superoxide dismutase 1-enhanced green fluorescent protein; Supp., supplementary; UBE4A, ubiquitination factor E4A; UBOX5, U-Box domain containing 5; UBR5, ubiquitin protein ligase E3 component N-recognin 5.

Supplementary Video 1: $SOD1^{G93A}$/mUbL mouse at 182 days and end-stage due to 20% weight loss criteria.

Supplementary Video 2: $SOD1^{G93A}$/WT mouse at 174 days and end-stage due to reaching a neurological score of 4.