Science Advances
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Supplementary Materials for

Deubiquitinase CYLD acts as a negative regulator of dopamine neuron survival in Parkinson's disease

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Published 1 April 2022, *Sci. Adv.* **8**, eabh1824 (2022)
DOI: 10.1126/sciadv.abh1824

The PDF file includes:

Other Supplementary Material for this manuscipt includes the following:

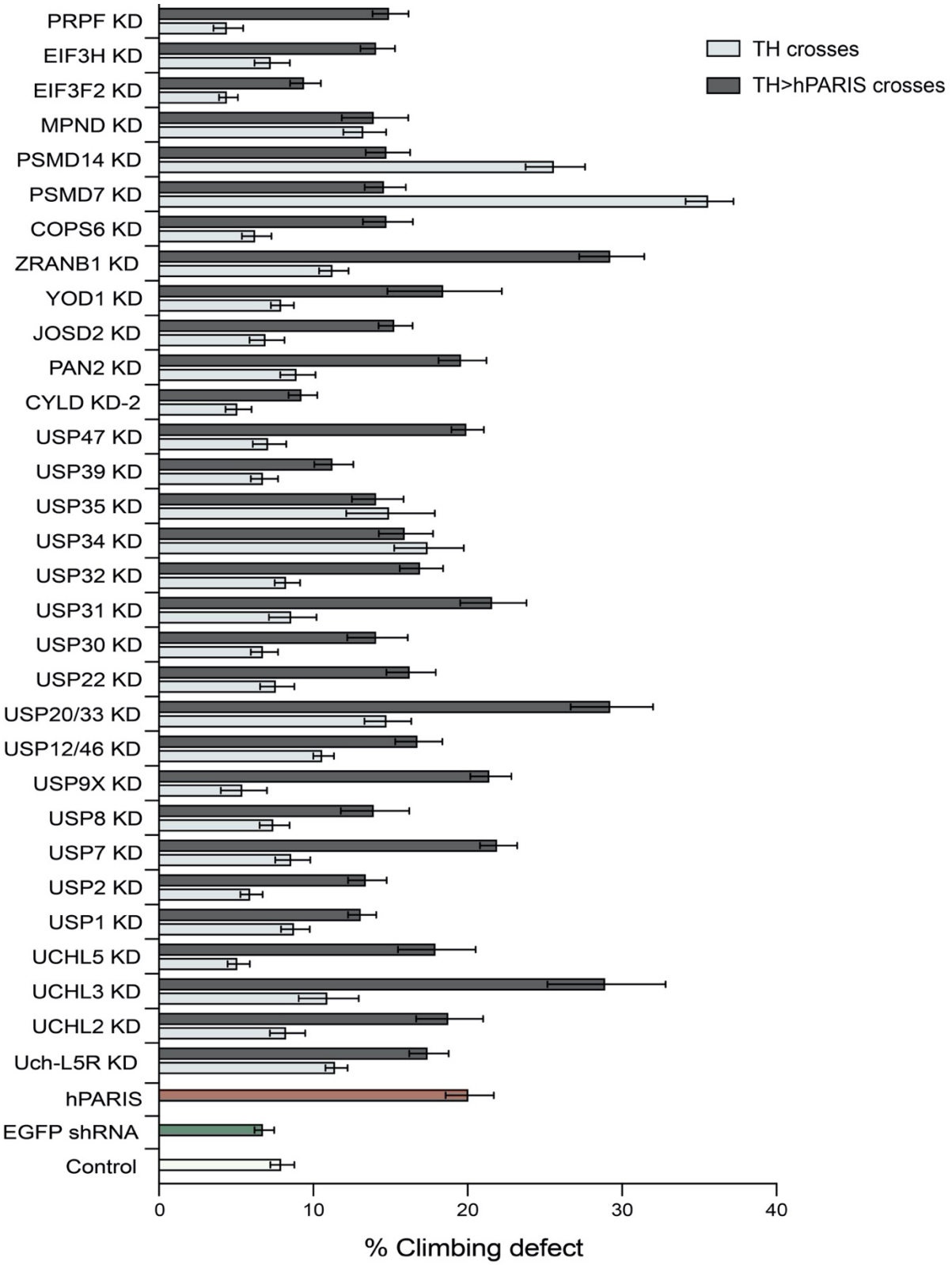
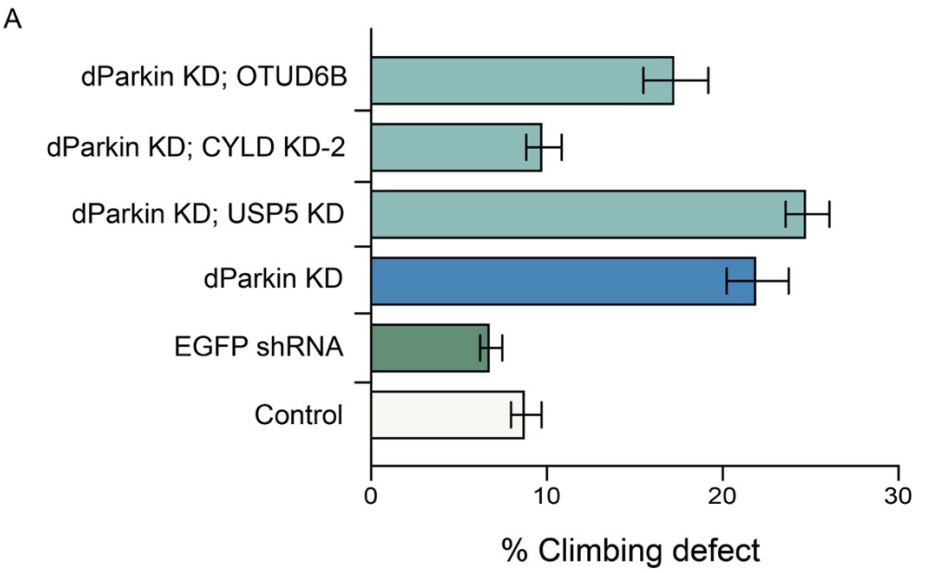


Fig S1: Genome-wide RNAi screen in *Drosophila* for DUBs that modify hPARIS induced climbing deficits. Summary of DUBs that modified climbing defects induced by dopaminergic accumulation of hPARIS. TH-Gal4/+ flies served as control. TH-Gal4 mediated EGFP shRNA

induction served as non-target control for shRNA response. N=60 flies per group at 20-days of age. See also Table S2.



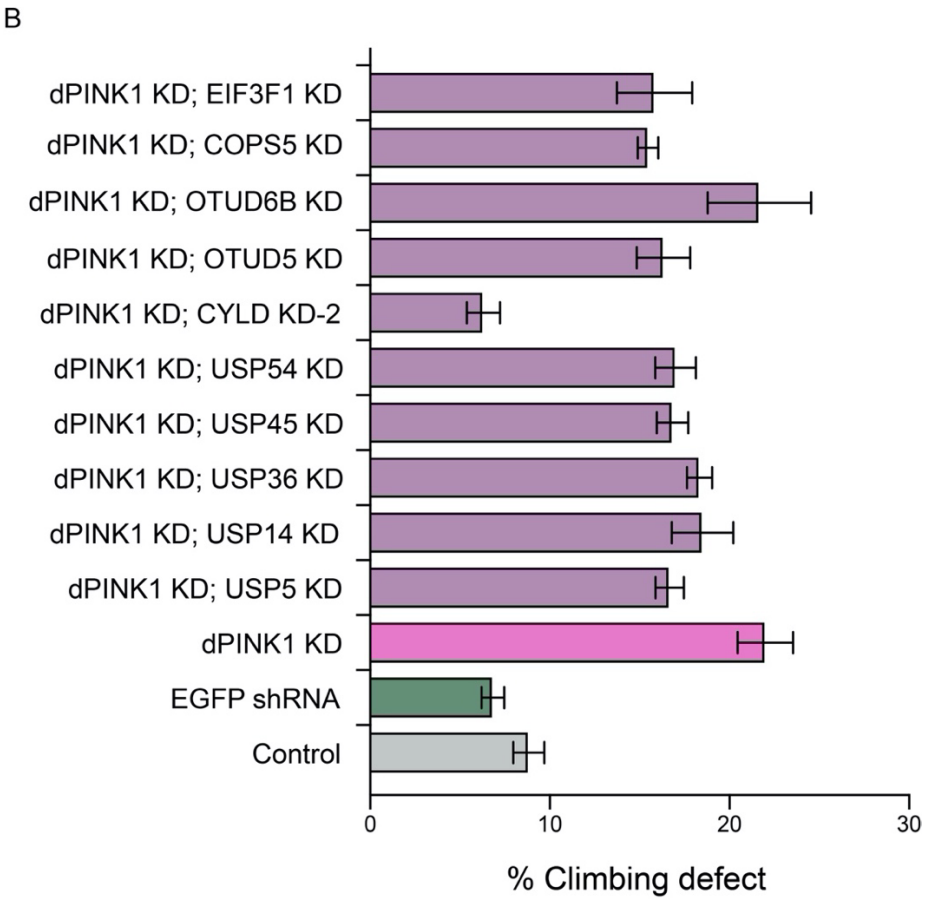


Fig S2: Secondary screens under conditions of parkin or PINK1 KD to identify modifiers of climbing deficits. A) Summary of DUB candidates from primary screen scored for suppression of climbing defects induced by dopaminergic KD of parkin. B) Summary of DUB candidates

from primary screen scored in PINK1 KD flies for their modifying effect on climbing deficits. TH-Gal4/+ flies served as control. TH-Gal4 mediated EGFP shRNA induction served as non-target control for shRNA response. N=60 flies per group at 20-days of age. See also Table S3.

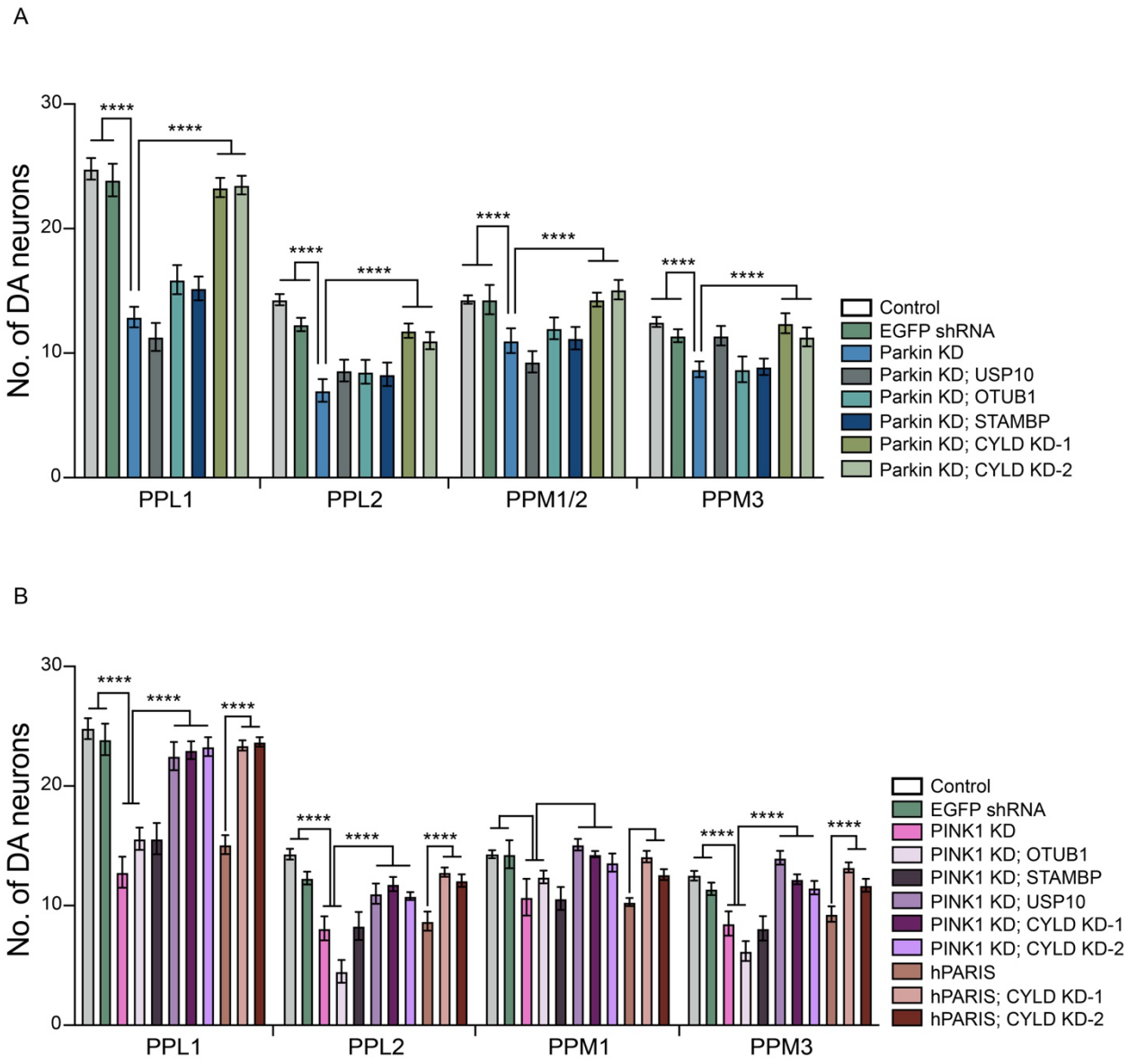


Fig S3: CYLD knockdown suppresses dopaminergic neurodegeneration in *Drosophila* under conditions of parkin or PINK1 insufficiency. A) Quantification of DA neuron number in PPL1, PPL2, PPM1/2, and PPM3 dopaminergic clusters in the indicated genotypes under conditions of parkin KD. B) Summary of dopamine neuron quantification in the same dopaminergic clusters as (A) under conditions of PINK1 KD in the indicated genotypes. TH-Gal4/+ flies served as control. TH-Gal4 mediated EGFP shRNA induction served as non-target control for shRNA response. N=10 flies per group at 20-days of age. Quantitative data = mean ± SEM. One-way ANOVA *p < 0.05, **p < 0.01, *** p < 0.001, ****p < 0.0001.

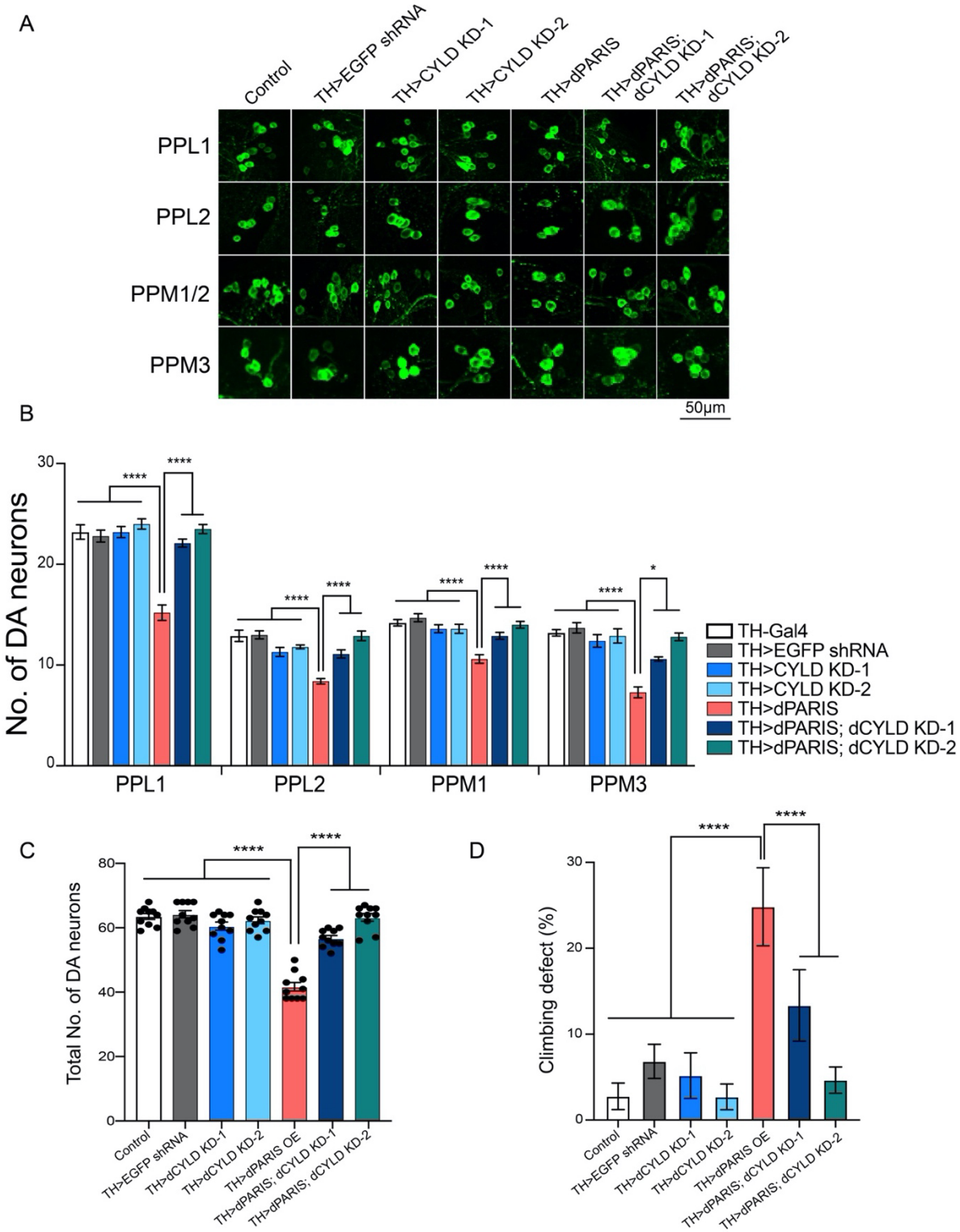


Fig S4: CYLD knockdown suppresses dPARIS induced dopaminergic neurotoxicity in *Drosophila*. A) Representative confocal images of individual dopamine neurons in PPL1, PPL2, PPM1/2, and PPM3 DA neuron clusters in the indicated genotypes at 30-days of age. Scale = 500 µM. B) Quantification of dopamine neuron numbers in the individual PPL1, PPL2, PPM1/2, and PPM3 clusters for the indicated genotypes on day 30. Observed rescue effects verified using

two independent shRNA fly lines (KD-1 and KD-2) targeting dCYLD. C) Quantification of total dopamine neuron numbers in the indicated genotypes, age group same as B. N=10 flies per indicated genotype. D) Climbing performance in the indicated genotypes on day 30. N=45-60 flies per group. TH-Gal4/+ flies served as control. TH-Gal4 mediated GFP shRNA induction served as non-target control for shRNA response. Quantitative data = mean ± SEM. One-way ANOVA *$p < 0.05$, **$p < 0.01$, *** $p < 0.001$, ****$p < 0.0001$.

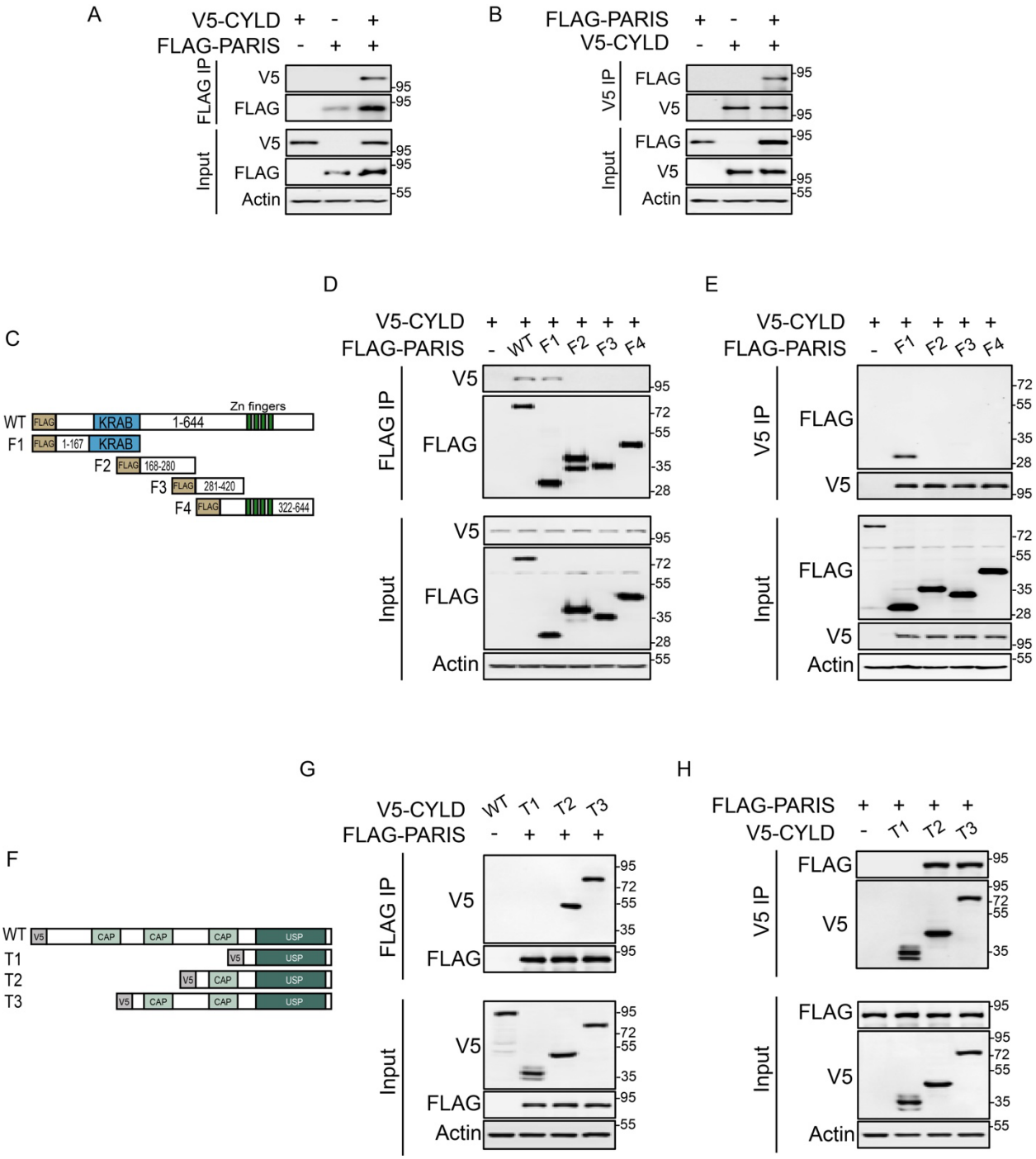


Fig S5: Mapping of protein interaction domains in PARIS and CYLD. A) Co-immunoprecipitation of N-terminally V5-tagged CYLD by N-terminally FLAG-tagged PARIS in SH-SY5Y cells using anti-FLAG antibodies. Similar results observed in three independent experiments. B) Reciprocal co-immunoprecipitation using anti-V5 antibodies verify interaction between PARIS and CYLD in SH-SY5Y cells transfected with indicated transgenes. N=3 independent experiments. C) Schematic of N-terminally FLAG tagged truncated fragments of PARIS used for mapping CYLD binding region in PARIS. D) Co-immunoprecipitation

experiments using the indicated truncated fragments of PARIS show that PARIS interacts with CYLD at its N-terminus (Fragment F1). N=3. E) Schematic of N-terminally V5 tagged truncated fragments of CYLD used for mapping PARIS binding region in CYLD. E) Co-immunoprecipitation experiments using the indicated truncated fragments of CYLD show that CYLD binds PARIS at its C-terminus in the region spanning its third CAP domain (Fragment F1). N=3.

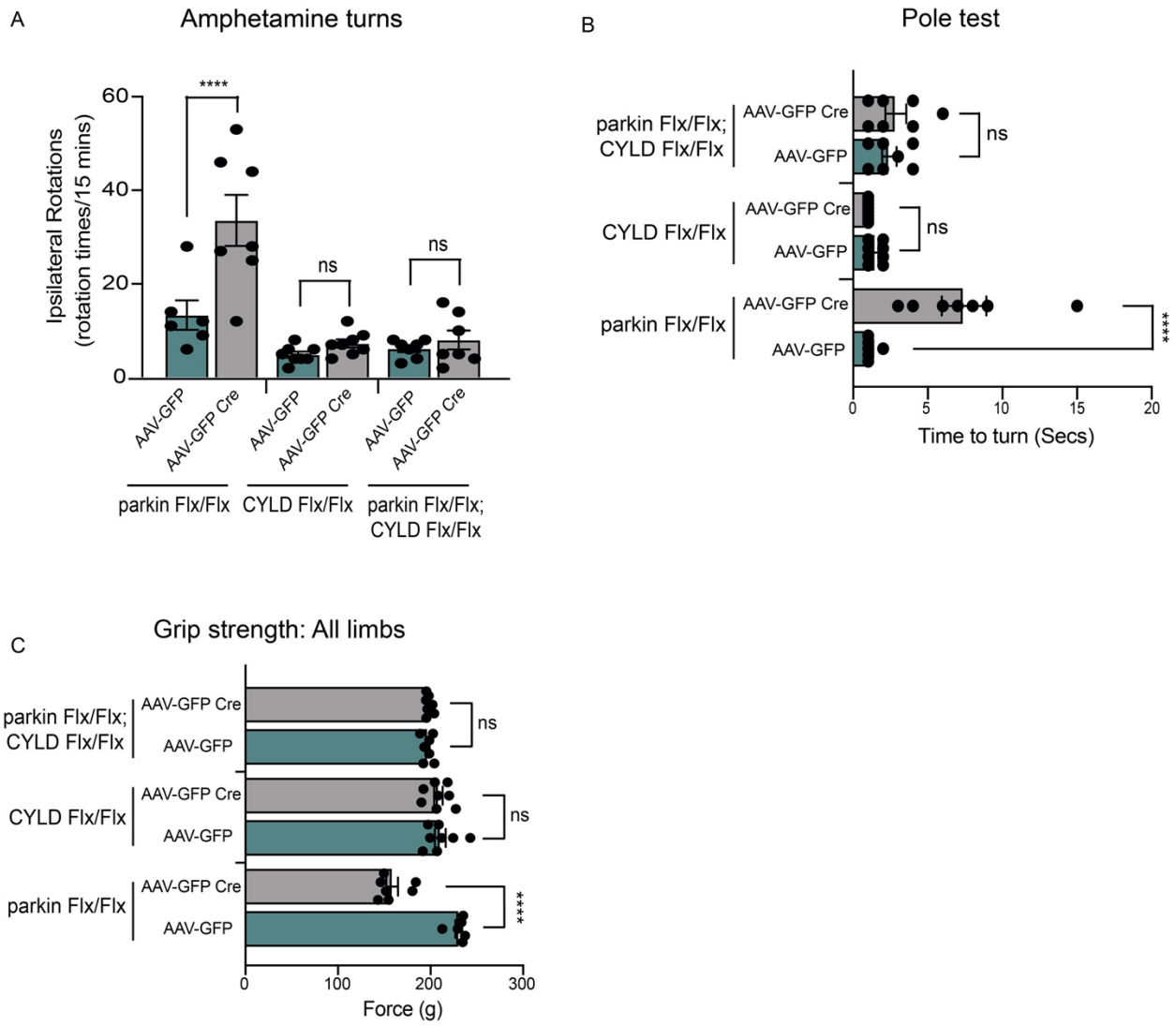


Fig S6: CYLD knockdown ameliorates behavioral deficits under conditions of parkin insufficiency. A) Amphetamine induced ipsilateral turns in indicated groups. B) Pole test measuring time to turn in indicated groups. C) Grip strength test measuring force in all limbs in indicated injection groups. Quantitative data = mean ± SEM with Tukey post-hoc test. One-way ANOVA *$p < 0.05$, **$p < 0.01$, *** $p < 0.001$, ****$p < 0.0001$.

Table S1. List of DUB RNAi lines used in genome-wide RNAi screen

No.	Flybase ID	Human homolog (Gene Symbol)	DUB family	Fly line used	Source
1	FBgn0030370	Uch-L5R	Ubiquitin C-terminal Hydrolase	330593	VDRC
2	FBgn0262166	UCHL2		v107757	VDRC
3	FBgn0010288	UCHL3		26468	VDRC
4	FBgn0011327	UCHL5		35433	BDSC
5	FBgn0028476	USP1	Ubiquitin specific Protease	28356	BDSC
6	FBgn0031187	USP2		104382	VDRC
7	FBgn0035402	USP5		31886	BDSC
8	FBgn0030366	USP7		34708	BDSC
9	FBgn0038862	USP8		38982	BDSC
10	FBgn0005632	USP9X		35728	BDSC
11	FBgn0052479	USP10		36897	BDSC
12	FBgn0039025	USP12/46		35110 (MiMIC)	BDSC
13	FBgn0032216	USP14		53262	BDSC
14	FBgn0033916	USP20/33		42609	VDRC
15	FBgn0013717	USP22		28725	BDSC
16	FBgn0029819	USP30		110616	VDRC
17	FBgn0050421	USP31		33726	VDRC
18	FBgn0036913	USP32		18981	VDRC
19	FBgn0039214	USP34		27517	VDRC
20	FBgn0033738	USP35		28960	VDRC
21	FBgn0260936	USP36		33406	BDSC
22	FBgn0030969	USP39		110535	VDRC
23	FBgn0029763	USP45		110286	VDRC
24	FBgn0016756	USP47		44645	BDSC
25	FBgn0000542	USP54		106671	VDRC
26	FBgn0032210	CYLD		101414; 40840	VDRC; BDSC
27	FBgn0033352	PAN2		53249	BDSC
28	FBgn0029853	JOSD2	Machado Joseph Disease Domain Protease	108379	VDRC
29	FBgn0003023	No ortholog	Otubain Proteases	NA	NA
30	FBgn0031622	No ortholog		NA	NA
31	FBgn0035593	YOD1		21893	VDRC
32	FBgn0032214	OTUB1		35615	BDSC
33	FBgn0036180	OTUD5		109912	VDRC
34	FBgn0026738	OTUD6B		105469	VDRC
35	FBgn0037734	ZRANB1		24030	VDRC
36	FBgn0039773	STAMBP	JAMM Domain Proteases	108622	VDRC

37	FBgn0027053	COPS5		42781	BDSC
38	FBgn0028837	COPS6		36073	BDSC
39	FBgn0002787	PSMD7		35411	BDSC
40	FBgn0028694	PSMD14		33662	BDSC
41	FBgn0032348	MPND		45530	VDRC
42	FBgn0037270	EIF3F1		33980	BDSC
43	FBgn0033069	EIF3F2		108169	VDRC
44	FBgn0022023	EIF3H		55603	BDSC
45	FBgn0033688	PRPF		34622	BDSC

Table S2. Statistical comparison of climbing performance of DUB knockdowns in primary screen

No.	Targeted DUB	TH crosses			TH; hPARIS crosses		
		Significance	Summary	Adjusted p-value	Significance	Summary	Adjusted p-value
1	Uch-L5R	No	ns	0.6557	No	ns	0.998
2	UCHL2	No	ns	0.9998	No	ns	0.9993
3	UCHL3	No	ns	0.8656	Yes	**	0.0044
4	UCHL5	No	ns	0.9174	No	ns	0.9986
5	USP1	No	ns	0.9994	No	ns	0.0641
6	USP2	No	ns	0.9982	No	ns	0.0943
7	USP5	No	ns	0.2191	Yes	****	<0.0001
8	USP7	No	ns	0.9996	Yes	**	0.0016
9	USP8	No	ns	0.9997	No	ns	0.1607
10	USP9X	No	ns	0.9796	No	ns	0.9992
11	USP10	No	ns	0.6557	Yes	****	<0.0001
12	USP12/46	No	ns	0.9552	No	ns	0.9591
13	USP14	No	ns	0.9984	Yes	****	<0.0001
14	USP20/33	No	ns	0.0077	Yes	**	0.0025
15	USP22	No	ns	0.9998	No	ns	0.8481
16	USP30	No	ns	0.9991	No	ns	0.1895
17	USP31	No	ns	0.9996	No	ns	0.9991
18	USP32	No	ns	0.9998	No	ns	0.999
19	USP34	No	ns	<0.0001	No	ns	0.7387
20	USP35	No	ns	0.0997	No	ns	0.1895
21	USP36	No	ns	0.0055	Yes	**	0.0037
22	USP39	No	ns	>0.9999	Yes	**	0.0049
23	USP45	No	ns	0.9991	Yes	****	<0.0001
24	USP47	No	ns	0.9994	No	ns	>0.9999
25	USP54	No	ns	0.2191	Yes	***	0.0009
26	CYLD	No	ns	0.9986	Yes	***	0.0005
27	PAN2	No	ns	0.9993	No	ns	0.9997
28	JOSD2	No	ns	0.9993	No	ns	0.499
29	YOD1	No	ns	>0.9999	No	ns	0.999
30	OTUB1	No	ns	>0.9999	0.9986	****	<0.0001
31	OTUD5	No	ns	0.9996	Yes	****	<0.0001
32	OTUD6B	No	ns	0.0028	Yes	***	0.0009
33	ZRANB1	No	ns	0.7312	Yes	**	0.0025
34	STAMBP	No	ns	0.5068	Yes	****	<0.0001

35	COPS5	No	ns	0.9996	Yes	***	0.0007
36	COPS6	No	ns	0.9986	No	ns	0.343
37	PSMD7	No	ns	<0.0001	No	ns	0.2986
38	PSMD14	No	ns	<0.0001	No	ns	0.343
39	MPND	No	ns	0.0932	No	ns	0.1607
40	EIF3F1	No	ns	0.9982	Yes	****	<0.0001
41	EIF3F2	No	ns	0.6557	Yes	***	0.0002
42	EIF3H	No	ns	0.999	No	ns	0.1895
43	PRPF	No	ns	0.6557	No	ns	0.3914

One-way ANOVA analysis with Tukey's post-hoc multiple correction comparing climbing performance of the DUB knockdowns in the primary screen employing dopaminergic overexpression of PARIS. **p < 0.01, *** p < 0.001, ****p < 0.0001, ns – not significant

Table S3. Statistical comparison of climbing performance of DUB knockdowns in secondary screen.

No.	Targeted DUB	TH>Parkin KD crosses			TH>PINK1 KD crosses		
		Significance	Summary	Adjusted p-value	Significance	Summary	Adjusted p-value
1	USP5	No	ns	0.6711	No	ns	0.0609
2	USP10	Yes	****	<0.0001	Yes	****	<0.0001
3	USP14	Yes	****	<0.0001	No	ns	0.4597
4	USP36	Yes	****	<0.0001	No	ns	0.3994
5	USP45	Yes	****	<0.0001	No	ns	0.0763
6	USP54	Yes	****	<0.0001	No	ns	0.0947
7	CYLD	Yes	***	0.0004	Yes	****	<0.0001
8	OTUB1	Yes	****	<0.0001	Yes	****	<0.0001
9	OTUD5	Yes	****	<0.0001	Yes	*	0.038
10	OTUD6B	No	ns	0.1065	No	ns	0.9998
11	STAMBP	Yes	****	<0.0001	Yes	****	<0.0001
12	COPS5	Yes	****	<0.0001	Yes	**	0.0097
13	EIF3F1	Yes	***	0.0003	Yes	*	0.0177

One-way ANOVA with Tukey's post-hoc multiple correction comparing climbing performance of DUB knockdowns in the secondary screen employing dopaminergic knockdown of parkin or PINK1. *p<0.05, **p < 0.01, *** p < 0.001, ****p < 0.0001, ns – not significant

Supplemental Videos

Video 1: Rescue of climbing deficits in human PARIS (hPARIS) flies by two independent dCYLD knockdown lines

Video 2: Rescue of climbing deficits in Drosophila PARIS (dPARIS) flies by two independent dCYLD knockdown lines

Video 3: Representative videos of pole test analysis in CYLD Flx/Flx, parkin Flx/Flx, and parkin Flx/Flx; CYLD Flx/Flx mice stereotactically injected with AAV-GFP or AAV-GFP Cre as indicated.