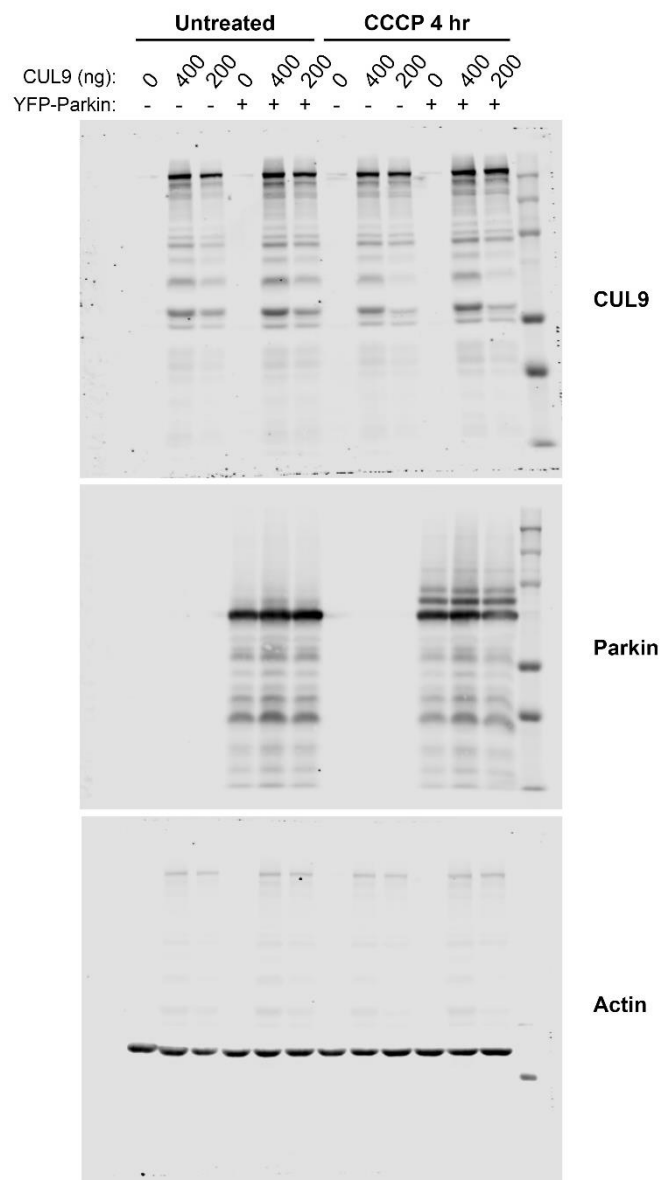


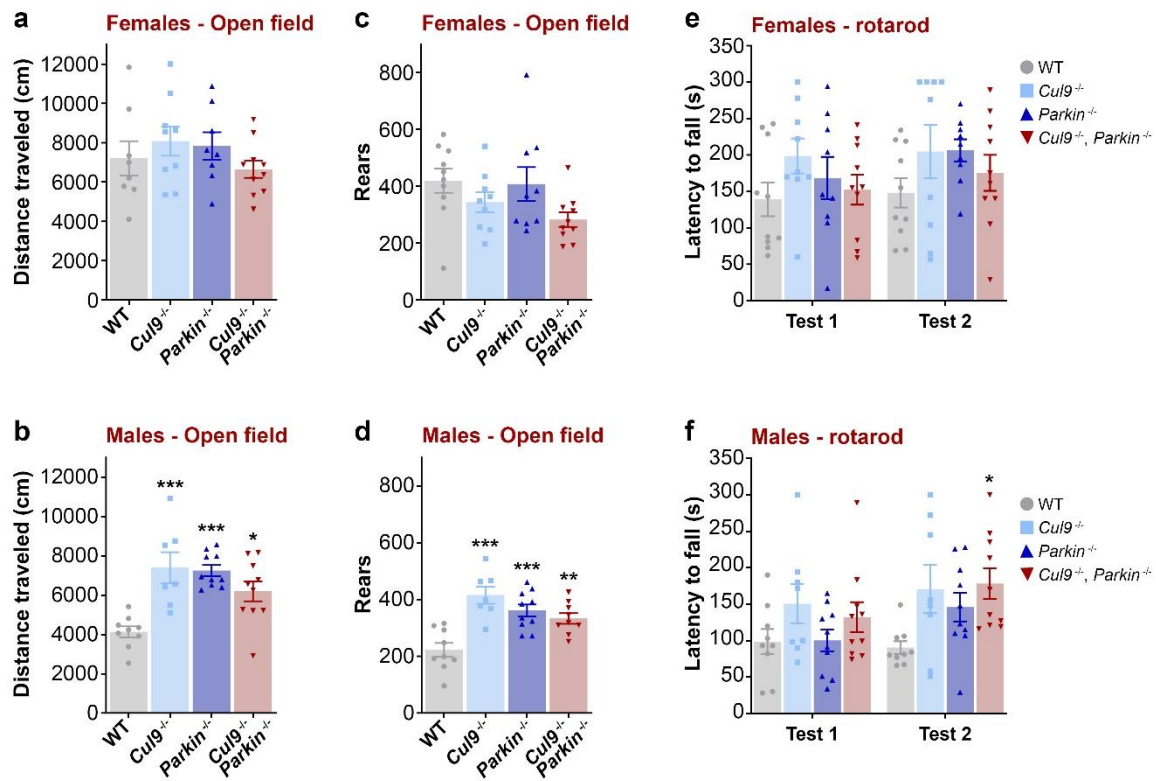
Supplementary Information

Characterization of a *Cul9* – *Parkin* double knockout mouse model for Parkinson's disease

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Supplementary Figure S1. Overexpression of CUL9 and Parkin in HeLa cells. HeLa cells were transfected with the indicated amount of plasmid encoding CUL9 along with YFP-Parkin construct (200 ng). Cells were treated with CCCP (10 μ M) for 4 hr and whole-cell lysates were analysed by immunoblotting. Full-length immunoblots of blots presented in Fig. 1c.



Supplementary Figure S2. Locomotor function characterization of aged *Cul9*^{-/-}, *Parkin*^{-/-} mice. **(a, b)** Total distance travelled and **(c, d)** total number of rears in the open field (1 hr) of *Cul9* and *Parkin* single and double knockout mice (13-14 months old), (females distance: $n = 8-10$; males distance: $n = 7-10$; females rears: $n = 9-10$; males rears: $n = 7-10$). One-way ANOVA with post-hoc Tukey's multiple comparisons test (males distance: WT vs. *Cul9*^{-/-}, *** $p = 0.0003$; WT vs. *Parkin*^{-/-}, *** $p = 0.0002$; WT vs. *Cul9*^{-/-}, *Parkin*^{-/-}, * $p = 0.0141$; males rearing: WT vs. *Cul9*^{-/-}, *** $p < 0.0001$; WT vs. *Parkin*^{-/-}, *** $p = 0.0008$; WT vs. *Cul9*^{-/-}, *Parkin*^{-/-}, ** $p = 0.0100$). **(e, f)** Latency to fall from an accelerating rotarod (5 minute test, 48 hr between test 1 and test 2) of *Cul9* and *Parkin* single and double knockout mice (11-12 months old), (females: $n = 9-10$; males: $n = 8-10$). Two-way repeated measures ANOVA with post-hoc Tukey's multiple comparisons test (males test 2: WT vs. *Cul9*^{-/-}, *Parkin*^{-/-}, * $p = 0.0173$). Means \pm SEM are shown, n represents the number of independent animals.

	age (months)	Females			Males				
		Wild-type (n)	<i>Cul9</i> KO (n)	<i>Parkin</i> KO (n)	<i>Cul9, Parkin</i> KO (n)	Wild-type (n)	<i>Cul9</i> KO (n)	<i>Parkin</i> KO (n)	<i>Cul9, Parkin</i> KO (n)
Locomotor function									
Distance travelled (cm)	3-4	7687 ± 573 (7)	9723 ± 939 (9)	5911 ± 504 (9)	5913 ± 392 (10)	5751 ± 494 (9)	6030 ± 105 (7)	7225 ± 469 (10)	5688 ± 404 (10)
Vertical activity (rears)	3-4	356 ± 39 (10)	303 ± 38 (9)	245 ± 27 (9)	213 ± 27 (10)	317 ± 77 (9)	313 ± 30 (9)	388 ± 35 (10)	263 ± 20 (10)
Rotarod test 2, latency to fall (s)	4-5	217.6 ± 21.8 (10)	246.0 ± 19.2 (9)	269.0 ± 10.6 (9)	264.1 ± 16.1 (9)	153.3 ± 16.5 (9)	261.6.3 ± 19.4 (9)	260.6 ± 23.0 (10)	262.2 ± 16.4 (10)
Distance travelled (cm)	13-14	7202 ± 876 (8)	8085 ± 753 (9)	7840 ± 696 (8)	6655 ± 444 (10)	4139 ± 275 (9)	7403 ± 789 (7)	7261 ± 273 (10)	6205 ± 509 (10)
Vertical activity (rears)	13-14	420 ± 43 (10)	344 ± 35 (9)	408 ± 60 (9)	283 ± 26 (10)	223 ± 25 (9)	416 ± 31 (7)	362 ± 21 (10)	334 ± 19 (9)
Rotarod test 2, latency to fall (s)	11-12	148.2 ± 20.3 (10)	204.9 ± 36.7 (9)	206.3 ± 15.2 (9)	175.5 ± 24.7 (10)	90.4 ± 8.7 (9)	170.8 ± 33.1 (8)	145.9 ± 19.6 (10)	178.3 ± 20.9 (10)
Sensory function									
Hot plate, latency (s)	6-7	27.0 ± 1.0 (10)	30.0 ± 0.1 (9)	29.0 ± 0.8 (9)	27.0 ± 1.0 (10)	28.0 ± 1.0 (9)	29.0 ± 0.6 (8)	28.0 ± 1.0 (10)	28.0 ± 0.8 (10)
Buried food, latency (s)	4-5	47 ± 11 (8)	122 ± 57 (8)	80 ± 23 (8)	109 ± 20 (10)	128 ± 46 (9)	144 ± 30 (9)	107 ± 32 (9)	62 ± 9 (10)
Visible platform day 1, latency (s)	4-5	21.3 ± 2.8 (10)	20.6 ± 2.3 (9)	24.1 ± 2.3 (9)	22.9 ± 3.3 (9)	23.1 ± 3.7 (9)	18.6 ± 3.1 (8)	17.5 ± 2.8 (9)	18.5 ± 2.4 (9)
Cognitive function									
Spatial learning day 6, latency (s)	5-7	18.1 ± 2.5 (10)	13.6 ± 1.8 (9)	16.7 ± 4.3 (9)	18.3 ± 3.3 (10)	17.4 ± 4.4 (9)	13.4 ± 2.1 (9)	13.6 ± 1.8 (10)	11.2 ± 1.4 (10)
Reversal learning day 7, latency (s)	5-7	21.9 ± 4.8 (10)	12.1 ± 2.1 (9)	20.0 ± 2.9 (9)	17.9 ± 4.6 (10)	16.8 ± 5.5 (9)	12.3 ± 2.0 (8)	9.6 ± 1.5 (10)	12.5 ± 2.5 (10)

Supplementary Table S1. Summary of behavioural tests results for *Cul9* and *Parkin* single and double knockout mice.

	age (months)	Females			Males				
		Wild-type (n)	<i>Cul9</i> KO (n)	<i>Parkin</i> KO (n)	<i>Cul9, Parkin</i> KO (n)	Wild-type (n)	<i>Cul9</i> KO (n)	<i>Parkin</i> KO (n)	<i>Cul9, Parkin</i> KO (n)
Stereology									
TH-positive cells	17-19	4170 ± 143 (4)	5355 ± 342 (4)	4717 ± 464 (3)	5046 ± 381 (4)	4261 ± 116 (5)	4904 ± 270 (3)	5056 ± 391 (6)	4926 ± 378 (5)
Nissl-positive cells	17-19	6692 ± 1017 (4)	8607 ± 976 (4)	8028 ± 289 (3)	8433 ± 538 (4)	6771 ± 818 (5)	9513 ± 951 (3)	8718 ± 848 (6)	7445 ± 941 (5)

Supplementary Table S2. Stereological analysis of the SNpc of *Cul9* and *Parkin* single and double knockout mice.