

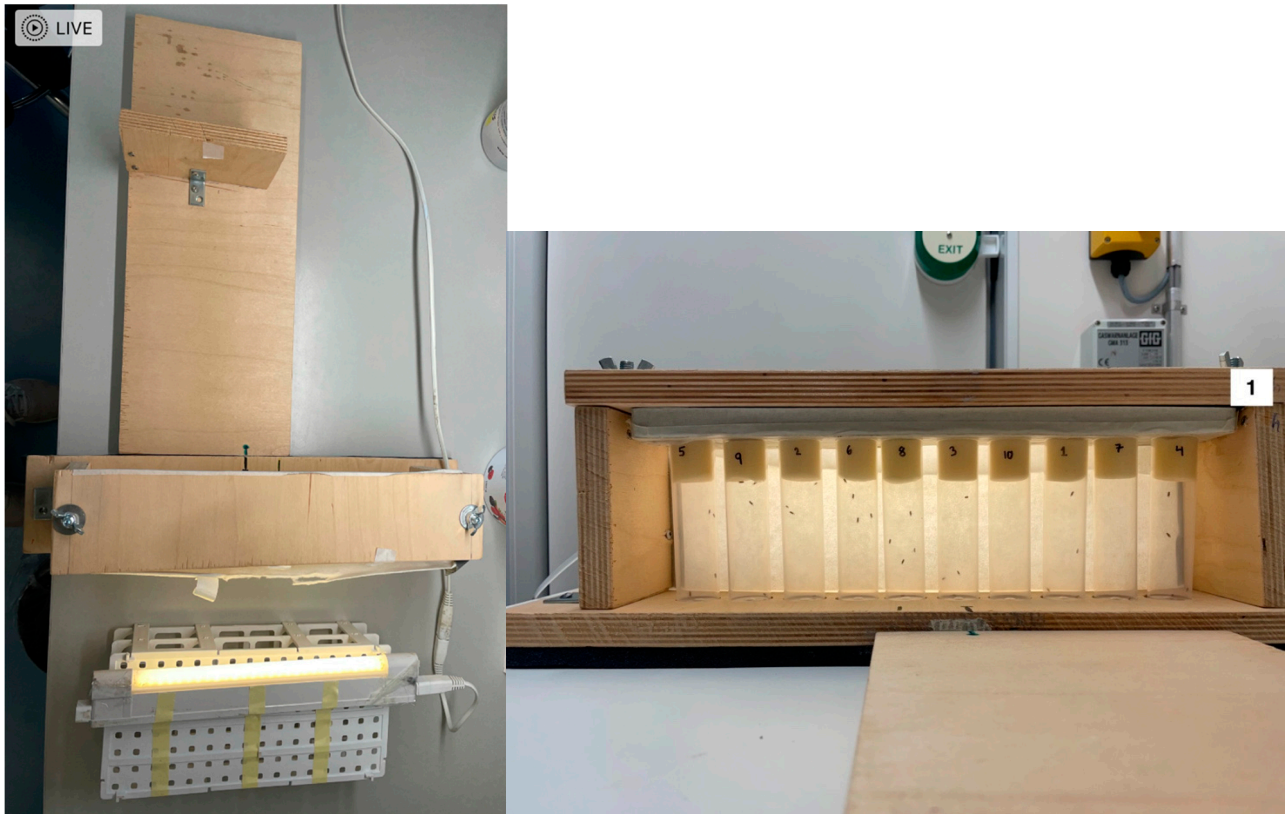
This file encompasses the below supplementary information associated with the manuscript.

Supplementary Figures

Figure S1 The rapid iterative negative geotaxis (RING) apparatus.

Supplementary Tables

Table S1	Overview PD associated genes and their <i>Drosophila</i> orthologs. [Excel file]
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Supplementary Figure S1 | Overview of the rapid iterative negative geotaxis (RING) system. The RING system consists of a wooden box with an open face and a removable lid held in place with hinges on each side, fastened using a bolt and a nut. The floor of the apparatus supports ten 46.63 mL vials (25 mm x 95 mm) (VWR™, PA) with a flat bottom. The lid is lined with foam to ensure that each tube is fastened in the apparatus. Adjacent to the face of the apparatus is a wooden board with a vertical board attached, allowing placement of a camera apparatus 30 cm in front of the RING apparatus. The camera apparatus was fastened to the wooded board using masking tape. To provide uniform lightning of the RING apparatus, a light source was placed behind a white opaque filter attached to the backside of the apparatus.

Supplementary Table S2 | Overview of the 11 selected PD risk genes with DIOPT score ≥ 12 and where a homozygous viable UAS line was available from VDRC. Complete overview of all PD associated genes can be seen in Supplementary Table S2.

PD risk gene	Reference	Fly gene symbol	VDRC strain	DIOPT score	UAS genotype
<i>CLCN3</i>	Nalls et al. 2019	<i>Clc-c</i>	6465	12	w ¹¹¹⁸ ; P{GD1663}v6465
<i>SLC44A1</i>	Nalls et al. 2019 Chang et al. 2017	<i>Ctl1</i>	12242	14	w ¹¹¹⁸ ; P{GD3579}v12242
<i>HIP1R</i>	Iwaki et al. 2019	<i>Hip1</i>	16137	12	w ¹¹¹⁸ ; P{GD7152}v16137
<i>ATP6V0A1</i>	Chang et al. 2017	<i>Vha100-1</i>	22924	13	w ¹¹¹⁸ ; P{GD12710}v22924
<i>SH3GL2</i>	Nalls et al. 2019 Chang et al. 2017	<i>EndoA</i>	24617	12	w ¹¹¹⁸ ; P{GD7681}v24617
<i>MCCC1</i>	Nalls et al. 2019 Foo et al. 2020 Chang et al. 2017	<i>Mccc1</i>	25406	15	w ¹¹¹⁸ ; P{GD9729}v25406
<i>RPS6KL1</i>	Nalls et al. 2019	<i>CG7156</i>	26035	12	w ¹¹¹⁸ ; P{GD10657}v26035
<i>MICU3</i>	Chang et al. 2017	<i>MICU3</i>	41259	14	w ¹¹¹⁸ ; P{GD5587}v41259
<i>FAM49B</i>	Nalls et al. 2019	<i>CG32066</i>	44825	12	w ¹¹¹⁸ ; P{GD8705}v44825
<i>CTSB</i>	Nalls et al. 2019	<i>CtsB1</i>	45345	14	w ¹¹¹⁸ ; P{GD6407}v45345
<i>SLC41A1</i>	Nalls et al. 2019 Chang et al. 2017 Iwaki et al. 20019	<i>CG33181</i>	47868	13	w ¹¹¹⁸ ; P{GD16951}v47868
-	-	Control	60000	NA	w ¹¹¹⁸

Supplementary Table S3 | QC metrics from BGI. After sequencing, the raw reads were filtered. Data filtering includes removing adaptor sequences, contamination, and low-quality reads from raw reads. Clean reads: Total clean reads number. Clean bases: Total clean bases number. Q20: The number of nucleotide with quality higher than 20/nucleotide(clean read1,read2).

VDRC number	Fly symbol	Human symbol	RNA concentration (ng/μl)	Volume (μl)	Total Mass (μg)	Clean reads	Clean bases	Q20(%)
6465	<i>ClC-c</i>	<i>CLCN3</i>	1.39	40	0.06	51,853,150	5,185,315,000	95.68%
12242	<i>Ctl1</i>	<i>SLC44A1</i>	10.3	40	0.41	52,239,002	5,223,900,200	95.94%
16137	<i>Hip1</i>	<i>HIP1R</i>	24.1	40	0.96	51,662,710	5,166,271,000	95.52%
22924	<i>Vha100-1</i>	<i>ATP6V0A1</i>	3.69	40	0.15	47,561,146	4,756,114,600	95.83%
24617	<i>EndoA</i>	<i>SH3GL2</i>	26.9	40	1.08	51,933,872	5,193,387,200	95.44%
25406	<i>Mccc1</i>	<i>MCCC1</i>	4.31	40	0.17	49,913,570	4,991,357,000	95.58%
26035	<i>CG7156</i>	<i>RPS6KL1</i>	10.3	40	0.41	50,451,936	5,045,193,600	95.58%
41259	<i>MICU3</i>	<i>MICU3</i>	11.6	40	0.46	54,369,786	5,436,978,600	95.72%
44825	<i>CG32066</i>	<i>FAM49B</i>	31.6	40	1.26	52,050,708	5,205,070,800	95.95%
45345	<i>CtsB1</i>	<i>CTSB</i>	7.87	40	0.31	52,103,894	5,210,389,400	95.78%
47868	<i>CG33181</i>	<i>SLC41A1</i>	16.3	40	0.65	51,782,270	5,178,227,000	95.38%
60000	Control line	NA	22	40	0.88	51,963,058	5,196,305,800	95.74%

Supplementary Table S4 | Within-gene read counts with between sample normalization using trimmed mean of M-values (TMM) of the 11 selected putative risk genes for sporadic PD. The column “TMM_{UAS-GAL4}” shows the TMM of the respective gene knockdown of the UAS-GAL4 lines, whereas the column “TMM_{W1118-GAL4}” shows the corresponding gene’s TMM for the common control line. The column “Ratio” is the ratio between TMM_{UAS-GAL4} and TMM_{W1118-GAL4}.

Fly gene symbol	TMM_{UAS-GAL4}	TMM_{W1118-GAL4}	Ratio	log2(Ratio)
<i>Ctsb1</i>	23.0225	84.3228	0.27	-1.87
<i>CG7156</i>	12.608	26.2477	0.48	-1.058
<i>Mccc1</i>	25.4293	43.815	0.58	-0.78
<i>Hip1</i>	31.8947	45.8818	0.70	-0.52
<i>CG33181</i>	126.467	176.293	0.72	-0.48
<i>Vha100.1</i>	129.217	177.945	0.73	-0.46
<i>CG32066</i>	50.3101	66.5491	0.76	-0.40
<i>Ctl1</i>	61.1542	69.6493	0.88	-0.19
<i>MICU3</i>	59.8264	65.331	0.92	-0.13
<i>ClCc</i>	140.981	131.018	1.08	0.11
<i>EndoA</i>	388.711	358.581	1.08	0.12

Supplementary Table S5 | Statistics from analysis of variance (ANOVA) within each *Drosophila* line investigating whether an effect of ageing on flight response was observed. The table contains for each test day the mean activity (Mean), standard deviation of the mean (SD), the number of observations (n, mean of five pictures for five flies within test vial), the regression coefficient (Beta), and the associated *P*-value from ANOVA.

Fly line	Day 5			Day 10			Day 15			Beta	<i>P</i> -value
	Mean	SD	n	Mean	SD	n	Mean	SD	n		
<i>Ctl1</i>	2.34	1.26	45	1.76	1.03	45	1.92	1.34	45	-0.04	0.07
<i>Hip1</i>	2.86	1.14	59	2.63	1.13	60	3.1	1.08	60	0.03	0.18
<i>Vha100-1</i>	2.4	1.24	55	2.06	1.16	50	1.62	1.33	20	-0.07	0.03
<i>Mccc1</i>	2.84	0.97	69	2.82	1.19	73	2.75	1.14	70	-0.01	0.65
<i>CG7156</i>	2.67	1.24	69	2.49	1.19	70	2.04	0.96	65	-0.06	0.0018
<i>MICU3</i>	1.84	1.02	74	1.78	1.08	67	1.71	1.1	65	-0.01	0.4
<i>CG32066</i>	2.63	0.88	75	2.25	1.19	80	2.44	1.03	75	-0.02	0.28
<i>CtsB1</i>	2.84	1.12	84	2.17	1.24	80	1.17	1.1	45	-0.16	1.97×10^{-13}
<i>CG33181</i>	2.76	1.1	74	2.34	1.06	75	2.03	0.98	75	-0.07	4.6×10^{-6}
<i>Control</i>	2.73	1.03	84	2.82	1.21	90	2.63	1.13	85	-0.01	0.53

Supplementary Table S6 | Statistics from analysis of variance (ANOVA) comparing each *Drosophila* knockdown line with the common control line (w¹¹¹⁸-GAL4) at test day 5. The table contains the mean activity (Mean), standard deviation of the mean (SD), the number of observations (n, mean of five pictures for five flies within test vial), the difference between knockdown line and control, the standard error of the difference in activity (computed as $\sqrt{\frac{SD_{UAS-GAL4}}{n_{UAS-GAL4}} + \frac{SD_{W^{1118}-GAL4}}{n_{W^{1118}-GAL4}}}$), the %-reduction in activity compared to the control line, the associated *P*-value from ANOVA, and the false-discovery rate adjusted (FDR) *P*-value.

Test day	Fly line	Human gene	Mean	SD	n	Difference to w ¹¹¹⁸	SE on difference	%-reduction to w ¹¹¹⁸	<i>P</i> -value	FDR
5	<i>MICU3</i>	<i>MICU3</i>	1.84	1.02	74	-0.89	0.16	32.75	2.94E-07	2.65E-06
5	<i>Ctl1</i>	<i>SLC44A1</i>	2.34	1.26	45	-0.39	0.20	14.18	9.11E-02	4.10E-01
5	<i>Vha100-1</i>	<i>ATP6V0A1</i>	2.40	1.24	55	-0.33	0.19	11.95	1.85E-01	5.54E-01
5	<i>Hip1</i>	<i>HIP1R</i>	2.86	1.14	59	0.13	0.18	-4.65	5.83E-01	7.50E-01
5	<i>CtsB1</i>	<i>CTSB</i>	2.84	1.12	84	0.11	0.16	-4.17	4.04E-01	7.50E-01
5	<i>Mccc1</i>	<i>MCCC1</i>	2.84	0.97	69	0.10	0.16	-3.83	5.38E-01	7.50E-01
5	<i>CG32066</i>	<i>FAM49B</i>	2.63	0.88	75	-0.10	0.15	3.82	4.17E-01	7.50E-01
5	<i>CG7156</i>	<i>RPS6KL1</i>	2.67	1.24	69	-0.06	0.17	2.21	9.00E-01	9.00E-01
5	<i>CG33181</i>	<i>SLC41A1</i>	2.76	1.10	74	0.03	0.16	-0.97	8.14E-01	9.00E-01

Supplementary Table S7 | Statistics from analysis of variance (ANOVA) comparing each *Drosophila* knockdown line with the common control line (w¹¹¹⁸-GAL4) at test day 10. The table contains the mean activity (Mean), standard deviation of the mean (SD), the number of observations (n, mean of five pictures for five flies within test vial), the difference between knockdown line and control, the standard error of the difference in activity (computed as $\sqrt{\frac{SD_{UAS-GAL4}}{n_{UAS-GAL4}} + \frac{SD_{W^{1118}-GAL4}}{n_{W^{1118}-GAL4}}}$), the %-reduction in activity compared to the control line, the associated *P*-value from ANOVA, and the false-discovery rate (FDR) adjusted *P*-value.

Test day	Fly line	Human gene	Mean	SD	n	Difference to w ¹¹¹⁸	SE on difference	%-reduction to w ¹¹¹⁸	<i>P</i> -value	FDR
10	<i>Ctl1</i>	<i>SLC44A1</i>	1.757	1.031	45	-1.06	0.19	37.61	3.76E-07	1.69E-06
10	<i>MICU3</i>	<i>MICU3</i>	1.776	1.076	67	-1.04	0.17	36.93	1.04E-07	9.38E-07
10	<i>Vha100-1</i>	<i>ATP6V0A1</i>	2.056	1.159	50	-0.76	0.19	26.97	1.96E-03	4.42E-03
10	<i>CtsB1</i>	<i>CTSB</i>	2.171	1.244	80	-0.65	0.17	22.91	6.20E-05	1.86E-04
10	<i>CG32066</i>	<i>FAM49B</i>	2.251	1.186	80	-0.57	0.17	20.07	4.53E-03	8.16E-03
10	<i>CG33181</i>	<i>SLC41A1</i>	2.340	1.060	75	-0.48	0.17	16.92	2.17E-02	3.26E-02
10	<i>CG7156</i>	<i>RPS6KL1</i>	2.485	1.190	70	-0.33	0.17	11.74	4.33E-01	4.87E-01
10	<i>Hip1</i>	<i>HIP1R</i>	2.634	1.127	60	-0.18	0.18	6.47	4.06E-01	4.87E-01
10	<i>Mccc1</i>	<i>MCCC1</i>	2.825	1.187	73	0.01	0.17	-0.32	7.21E-01	7.21E-01

Supplementary Table S8 | Statistics from analysis of variance (ANOVA) comparing each *Drosophila* knockdown line with the common control line (w¹¹¹⁸-GAL4) at test day 15. The table contains the mean activity (Mean), standard deviation of the mean (SD), the number of observations (n, mean of five pictures for five flies within test vial), the difference between knockdown line and control, the standard error of the difference in activity (computed as $\sqrt{\frac{SD_{UAS-GAL4}}{n_{UAS-GAL4}} + \frac{SD_{W^{1118}-GAL4}}{n_{W^{1118}-GAL4}}}$), the %-reduction in activity compared to the control line, the associated *P*-value from ANOVA, and the false-discovery rate (FDR) adjusted *P*-value.

Test day	Fly line	Human gene	Mean	SD	n	Difference to w ¹¹¹⁸	SE on difference	%-reduction to w ¹¹¹⁸	<i>P</i> -value	FDR
15	<i>CtsB1</i>	<i>CTSB</i>	1.17	1.10	45	-1.46	0.19	55.59	1.11E-12	9.96E-12
15	<i>Vha100-1</i>	<i>ATP6V0A1</i>	1.62	1.33	20	-1.01	0.28	38.46	1.15E-01	1.47E-01
15	<i>MICU3</i>	<i>MICU3</i>	1.71	1.10	65	-0.92	0.17	34.90	1.31E-06	5.91E-06
15	<i>Ch1</i>	<i>SLC44A1</i>	1.92	1.34	45	-0.70	0.21	26.77	9.11E-03	1.37E-02
15	<i>CG33181</i>	<i>SLC41A1</i>	2.03	0.98	75	-0.60	0.16	22.82	7.91E-04	1.78E-03
15	<i>CG7156</i>	<i>RPS6KL1</i>	2.04	0.96	65	-0.58	0.17	22.17	5.65E-03	1.02E-02
15	<i>Hip1</i>	<i>HIP1R</i>	3.10	1.08	60	0.48	0.18	-18.11	5.72E-04	1.72E-03
15	<i>CG32066</i>	<i>FAM49B</i>	2.44	1.03	75	-0.18	0.16	6.89	5.42E-01	5.42E-01
15	<i>Mccc1</i>	<i>MCCC1</i>	2.75	1.14	70	0.13	0.17	-4.87	1.68E-01	1.89E-01

Supplementary Table S9 | Average survival from day 5 to day 10, and average survival from day 5 to day 15. The %-survival was computed as proportion of flies that survived from first test day (i.e., day 5) to test day 2 and test day 3 and is reported as the mean of the mean within replicate (i.e., across technical replicates).

Line	Gene	Mean %-survival from	
		Day 5 - 10	Day 5 - 15
41259	<i>MICU3</i>	100	100
60000	<i>control</i>	100	100
44825	<i>CG32066</i>	100	100
6465	<i>ClC-c</i>	25	23
16137	<i>Hip1</i>	100	100
22924	<i>Vha100-1</i>	62	52
12242	<i>Ctl1</i>	100	99
45345	<i>CtsB1</i>	92	72
47868	<i>CG33181</i>	100	100
25406	<i>Mccc1</i>	100	99
26035	<i>CG7156</i>	99	99