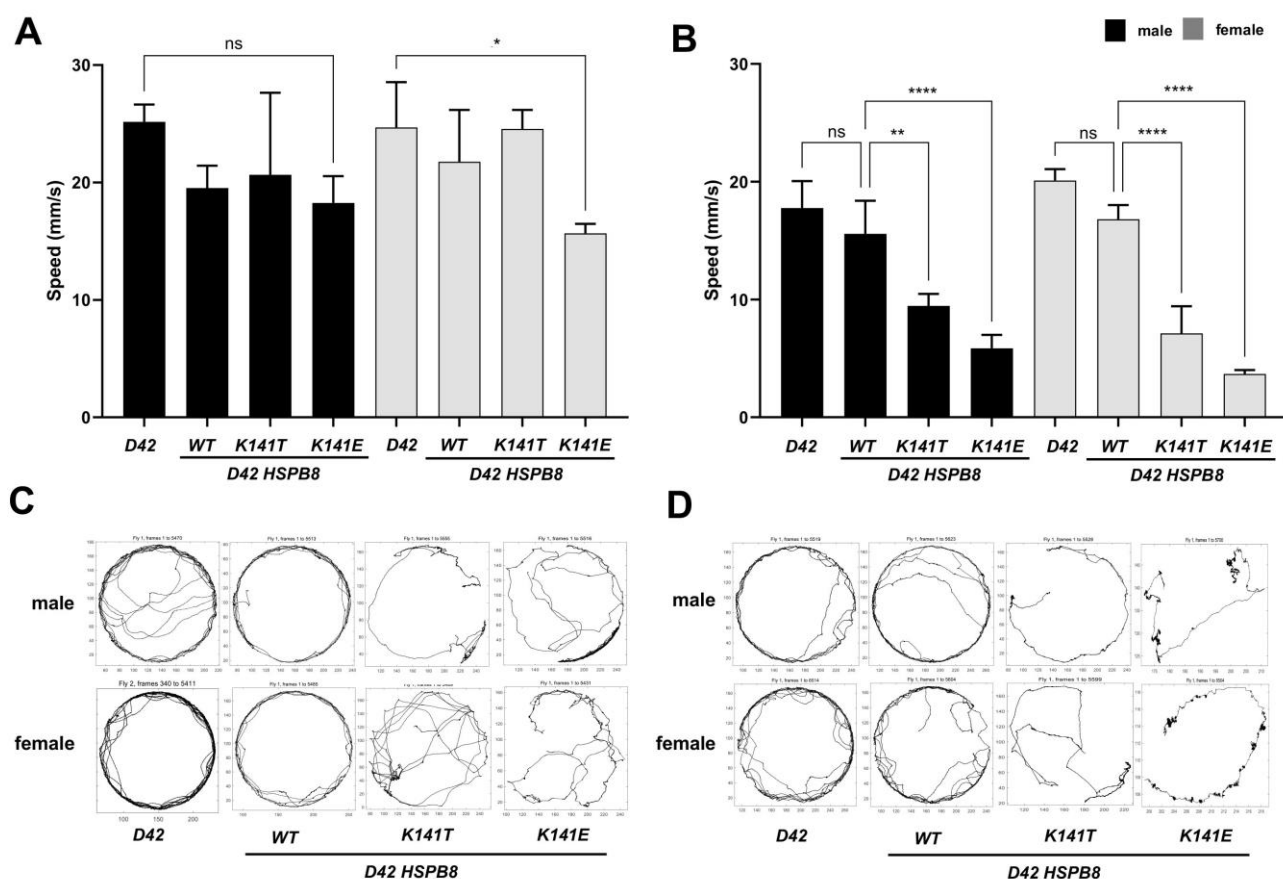
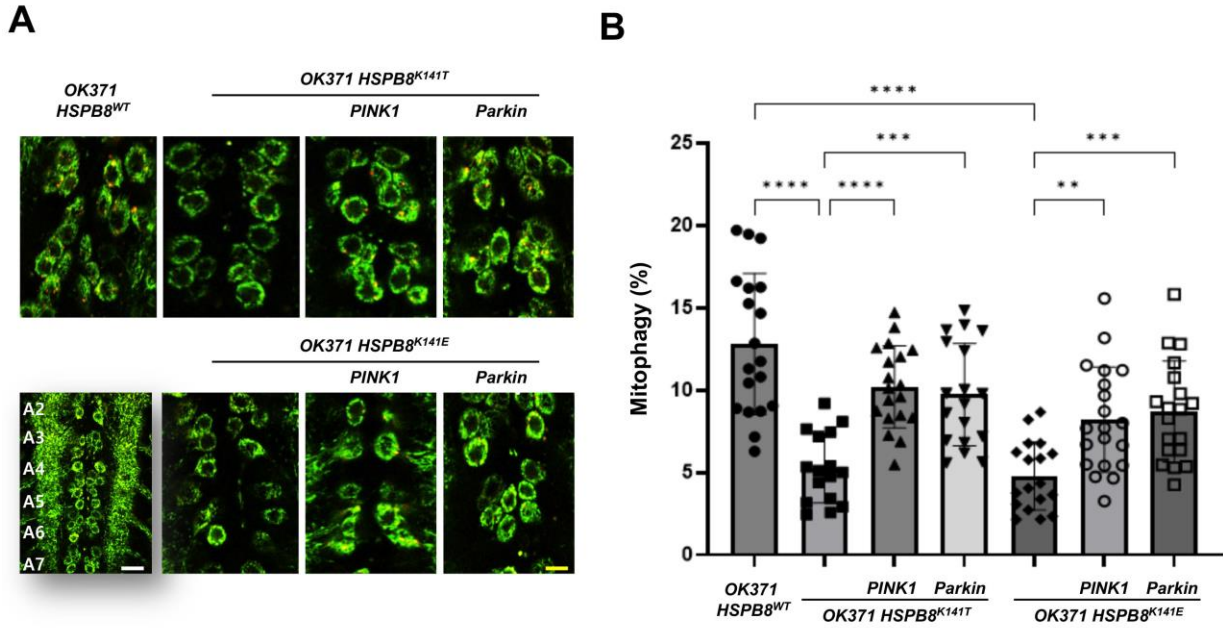


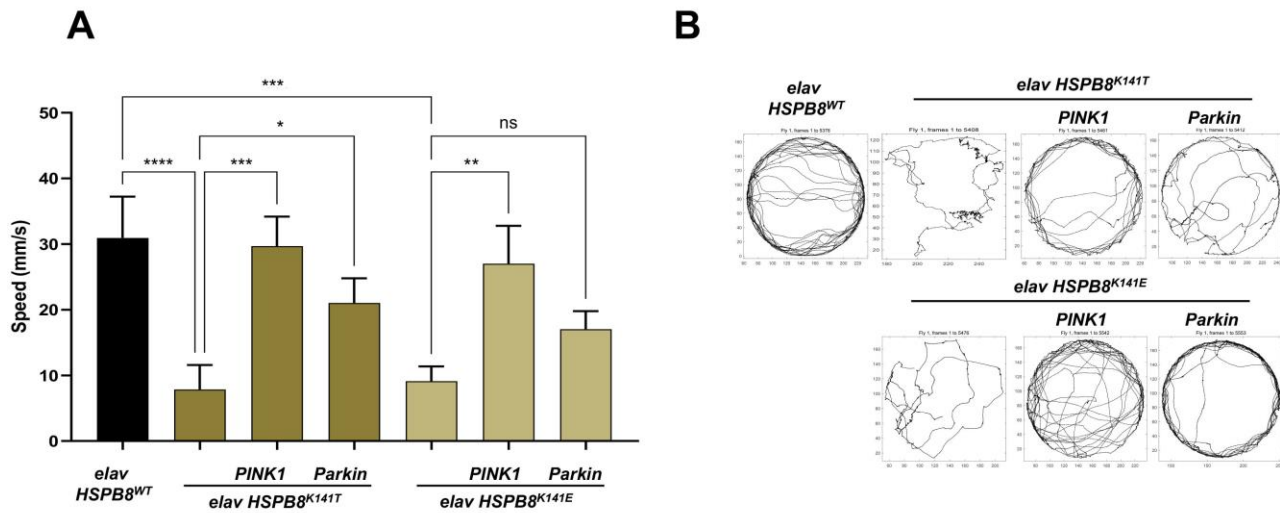
**Figure S1.** HSPB8 mutant genes decreased the climbing ability in *Drosophila*. (A) and (B) Comparison of climbing ability of 5- (A) and 15-day-old (B) *elav*, *elav HSPB8<sup>WT</sup>*, *elav HSPB8<sup>K141T</sup>* and *elav HSPB8<sup>K141E</sup>* flies ( $n = 4$  per group). (C) and (D) Comparison of climbing ability of 5- (C) and 15-day-old (D) flies expressing *D42*-GAL4 (*D42*), *HSPB8<sup>WT</sup>* (*D42 HSPB8<sup>WT</sup>*), *HSPB8<sup>K141T</sup>* (*D42 HSPB8<sup>K141T</sup>*) and *HSPB8<sup>K141E</sup>* (*D42 HSPB8<sup>K141E</sup>*) ( $n = 4$  per group). Significance was determined by one-way ANOVA with Sidak correction (\*,  $p < 0.05$ ; \*\*,  $p < 0.01$ ; \*\*\*\*,  $p < 0.0001$ ; NS, not significant). Error bars indicate the mean  $\pm$  SD.



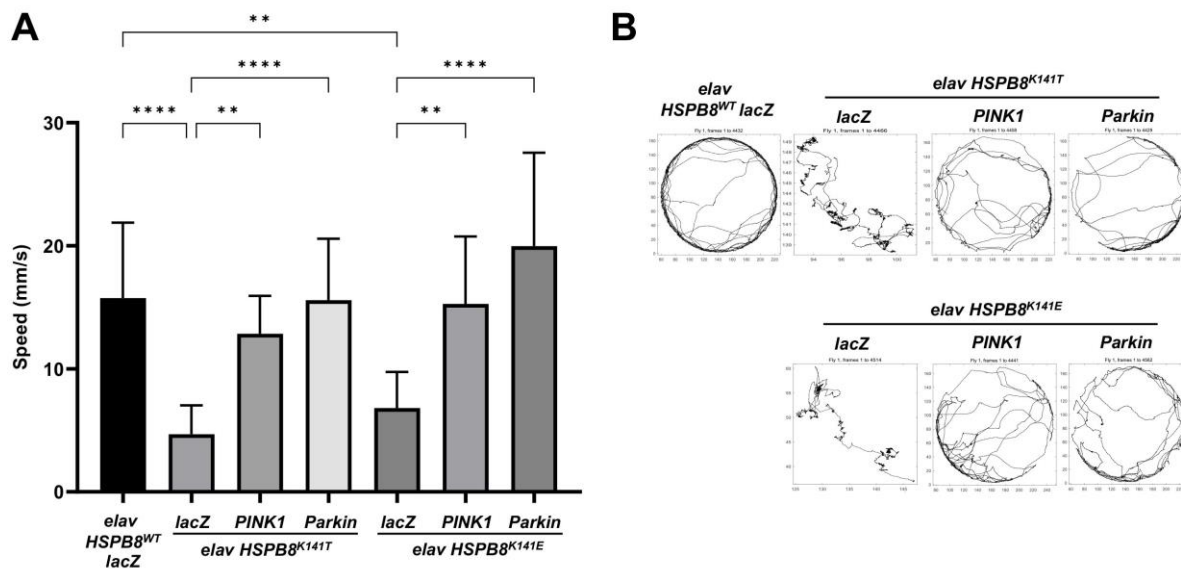
**Figure S2.** Expression of mutant HSPB8 in motor neurons induced motor defects in *Drosophila*. The following analyses were performed using 5- and 15-day-old flies expressing *D42*-GAL4 (*D42*), HSPB8<sup>WT</sup> (*D42 HSPB8*<sup>WT</sup>), HSPB8<sup>K141T</sup> (*D42 HSPB8*<sup>K141T</sup>) and HSPB8<sup>K141E</sup> (*D42 HSPB8*<sup>K141E</sup>): **(A)** and **(B)** Comparison of the mean walking speeds of **(A)** 5-day-old and **(B)** 15-day-old flies ( $n = 5$  per group). **(C)** and **(D)** Movement trajectories of **(C)** 5- and **(D)** 15-day-old flies. Significance was determined by one-way ANOVA with Sidak correction (\*,  $p < 0.05$ ; \*\*,  $p < 0.01$ ; \*\*\*\*,  $p < 0.0001$ ; NS, not significant). Error bars indicate the mean  $\pm$  SD.



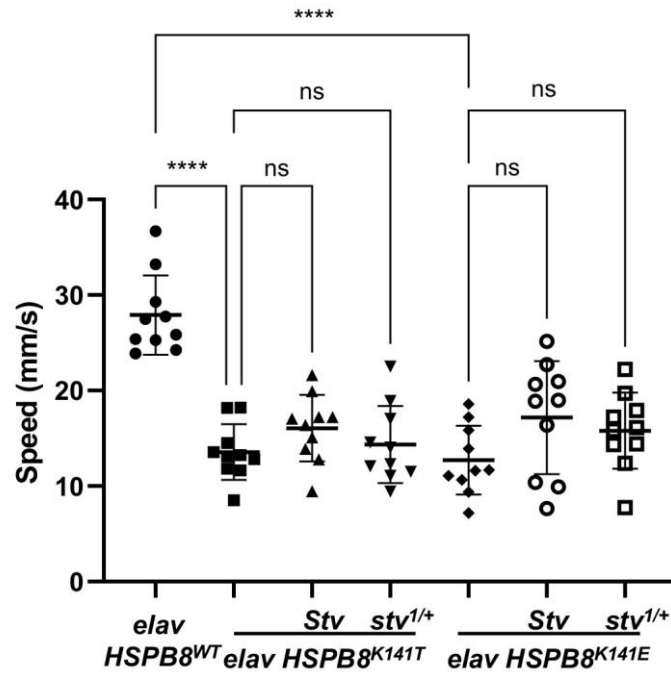
**Figure S3.** PINK1 and Parkin rescued decreased mitophagy levels in motor neurons expressing mutant HSPB8s. The following analyses were performed using wild-type HSPB8-expressing (*OK371 HSPB8<sup>WT</sup>*), mutant HSPB8-expressing (*OK371 HSPB8<sup>K141T</sup>*, *OK371 HSPB8<sup>K141E</sup>*), mutant HSPB8- and PINK1-expressing (*OK371 HSPB8<sup>K141T</sup> PINK1*, *OK371 HSPB8<sup>K141E</sup> PINK1*) and mutant HSPB8- and Parkin-expressing (*OK371 HSPB8<sup>K141T</sup> Parkin*, *OK371 HSPB8<sup>K141E</sup> Parkin*) flies: (A) Representative mt-Keima fluorescence images of motor neurons located in A4-5 regions of larval VNC. Scale bars: 25  $\mu$ m (white), 10  $\mu$ m (yellow). (B) Quantitative analysis of mitophagy in motor neurons located in A4-5 regions of larval VNC ( $n = 15$  per group). Significance was determined by one-way ANOVA with Sidak correction (\*,  $p < 0.01$ ; \*\*\*,  $p < 0.001$ ; \*\*\*\*,  $p < 0.0001$ ). Error bars indicate the mean  $\pm$  SD.



**Figure S4.** PINK1 and Parkin rescued motor defects in *HSPB8* mutants. The following analyses were performed using wild-type *HSPB8*-expressing (*elav HSPB8<sup>WT</sup>*), mutant *HSPB8*-expressing (*elav HSPB8<sup>K141T</sup>*, *elav HSPB8<sup>K141E</sup>*), mutant *HSPB8*- and PINK1-expressing (*elav HSPB8<sup>K141T</sup> PINK1*, *elav HSPB8<sup>K141E</sup> PINK1*) and mutant *HSPB8*- and Parkin-expressing (*elav HSPB8<sup>K141T</sup> Parkin*, *elav HSPB8<sup>K141E</sup> Parkin*) flies: **(A)** Comparison of the mean walking speeds of 15-day-old male flies ( $n = 5$  per group). **(B)** Movement trajectories of 15-day-old male flies. Significance was determined by one-way ANOVA with Sidak correction (\*,  $p < 0.05$ ; \*\*,  $p < 0.01$ ; \*\*\*,  $p < 0.001$ ; \*\*\*\*,  $p < 0.0001$ ; NS, not significant). Error bars indicate the mean  $\pm$  SD.



**Figure S5.** The expression of *lacZ* failed to rescue motor defects in *HSPB8* mutants. The following analyses were performed using wild-type *HSPB8*- and *lacZ*-expressing (*elav HSPB8<sup>WT</sup> lacZ*), mutant *HSPB8*- and *lacZ*-expressing (*elav HSPB8<sup>K141T</sup>*, *elav HSPB8<sup>K141E</sup>*), mutant *HSPB8*- and *PINK1*-expressing (*elav HSPB8<sup>K141T</sup> PINK1*, *elav HSPB8<sup>K141E</sup> PINK1*) and mutant *HSPB8*- and *Parkin*-expressing (*elav HSPB8<sup>K141T</sup> Parkin*, *elav HSPB8<sup>K141E</sup> Parkin*) flies: **(A)** Comparison of the mean walking speeds of 15-day-old male flies ( $n = 5$  per group). **(B)** Movement trajectories of 15-day-old male flies. Significance was determined by one-way ANOVA with Sidak correction (\*\*,  $p < 0.01$ ; \*\*\*\*,  $p < 0.0001$ ). Error bars indicate the mean  $\pm$  SD.



**Figure S6.** The expression and gene dosage reduction of *stv* failed to induce a meaningful change in motor activity in *HSPB8* mutants. Comparison of the mean walking speeds of 15-day-old male flies was performed using wild-type HSPB8-expressing (*elav HSPB8<sup>WT</sup>*), mutant HSPB8-expressing (*elav HSPB8<sup>K141T</sup>*, *elav HSPB8<sup>K141E</sup>*), mutant HSPB8- and *Stv*-expressing (*elav HSPB8<sup>K141T</sup> Stv*, *elav HSPB8<sup>K141E</sup> Stv*) and mutant HSPB8-expressing flies with a heterozygous *stv* mutation (*elav HSPB8<sup>K141T</sup> stv<sup>1/+</sup>*, *elav HSPB8<sup>K141E</sup> stv<sup>1/+</sup>*) ( $n = 10$  per group). Significance was determined by one-way ANOVA with Sidak correction (\*\*\*\*,  $p < 0.0001$ ; NS, not significant). Error bars indicate the mean  $\pm$  SD.