**Supplementary Materials**

# Ik2/TBK1 and Hook/Dynein, an adaptor complex for early endosome transport, are genetic modifiers of FTD-associated mutant CHMP2B toxicity in *Drosophila*

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**Supplementary 1. Genetic aberrations of alleles used in this study.**

**Supplementary 2. Motor function defect in *CHMP2B$^{Intron5}$* Larvae**

Motor neuronal expression of *UAS-CHMP2B$^{Intron5}$* by *OK6-Gal4* results in a significant decrease in median crawling speed in 3rd instar wandering larvae compared to wild-type (Canton S, *$w^{1118}$* outcross) and driver only (*OK6-Gal4*/+) larvae. Co-expression of *UAS-ik2* shows no significant difference to *UAS-CHMP2B$^{Intron5}$* larvae, indicating other pathways also contribute to motor dysfunction induced by mutant CHMP2B. ANOVA with Tukey's post-hoc multiple comparison to wild-type **: $p < 0.01$, ***: $p < 0.001$.

**Supplementary 3. Partial loss of Spn-F activity does not induce retinal degeneration phenotype.**

A genetic allele and UAS-RNAi were used to knockdown Spn-F in the eye. The image was taken with a Nikon DS-Fi1 camera on a Nikon SMZ1500 stereomicroscope using the NIS-Element BR software version 3.10. This software requires purchase and is not freely available. However, a simpler software, NIS-Elements Viewer, is freely available ( https://www.microscope.healthcare.nikon.com/en_AOM/products/software/nis-elements/viewer).

**Supplementary 4. Genotypes of the flied used in this study.**

**Supplementary 5. Fly lines and reagents used in this study and their sources.**

**Supplementary 6. Values of all the statistical test results.**

## Methods

## Motor function assay

Larval locomotor assays were performed as described previously [1]. Briefly, female third instar wandering larvae, raised at 18 °C, of the appropriate genotype were selected and washed in HL3 (70 mM NaCl, 5 mM KCl, 1 mM $CaCl_2 \cdot 2H_2O$, 10 mM $NaHCO_3$, 5 mM trehalose, 115 mM sucrose and 5 mM BES in $dH_2O$). Larvae were filmed from above for 120 s (0.2 frames $s^{-1}$) using VirtualDub software whilst crawling on a petri dish filled with 1% agar, 2-3 at a time. Videos were analyzed using imageJ and custom macros using the MTrack2 plugin as described [1] .

## References


1. Ahmad, S. T., Sweeney, S. T., Lee, J. A., Sweeney, N. T. & Gao, F. B. Genetic screen identifies serpin5 as a regulator of the toll pathway and CHMP2B toxicity associated with frontotemporal dementia. *Proceedings of the National Academy of Sciences of the United States of America* **106**, 12168-12173 (2009).
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4. Kramer, H. & Phistry, M. Genetic analysis of hook, a gene required for endocytic trafficking in drosophila. *Genetics* **151**, 675-684 (1999).

5. West RJH, Ugbode C, Gao FB, Sweeney ST. The pro-apoptotic JNK scaffold POSH/SH3RF1 mediates CHMP2BIntron5-associated toxicity in animal models of frontotemporal dementia. Hum Mol Genet 27: 1382-1395 (2018).
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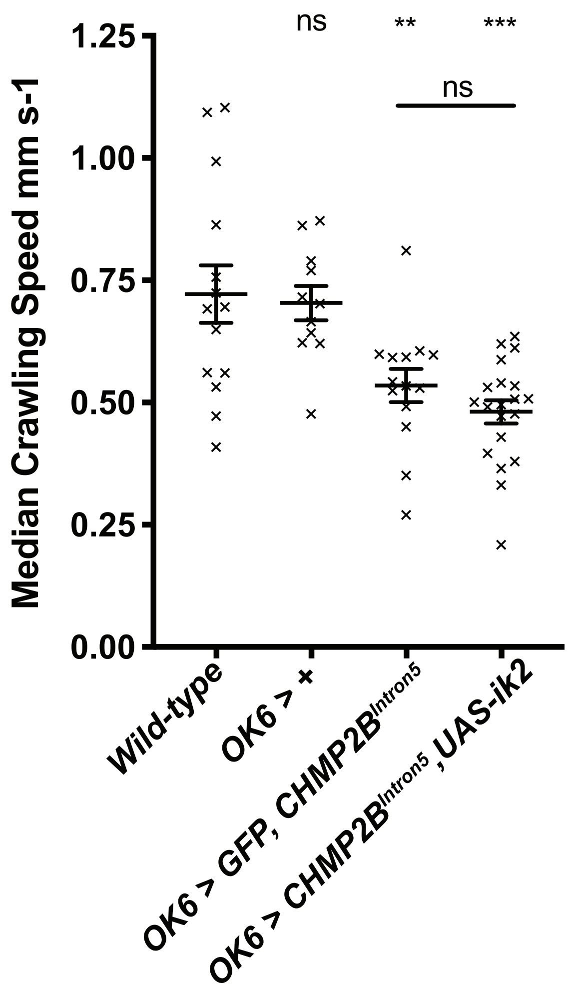
| Fly mutants | Mutation information |
|---|---|
| *Ik2*$^{Alice}$ | Point mutation in the kinase domain (E297**I**) |
| *Ird5*$^{KG08072}$ | P element intertion mutation |
| *Ter94*$^{K15502}$ | P element intertion mutation |
| *Spn-F*$^{2}$ | A 67 nucleotide deletion starting at base 726 (where +1 is the A of the initiator Met) |
| *Hook*$^{7}$ | Point mutation, resulting in a 185aa polypeptide. |
| *Hook*$^{11}$ | Point mutation, resulting in a 60aa polypeptide. |

**Supplementary 1**. Genetic aberrations of alleles used in this study.




**Supplementary 2**. Motor function defect in CHMP2BIntron5 Larvae
Motor neuronal expression of *UAS-CHMP2BIntron5* by *OK6-Gal4* results in a significant decrease in median crawling speed in 3rd instar wandering larvae compared to wild-type ( Canton S, *w1118* outcross) and driver only (*OK6-Gal4*/+) larvae. Co-expression of *UAS-ik2* shows no significant difference to UAS-CHMP2BIntron5 larvae, indicating other pathways also contribute to motor dysfunction induced by mutant CHMP2B. ANOVA with Tukey's post-hoc multiple comparison to wild-type **: $p < 0.01$, ***: $p < 0.001$.

*GMR-Ga4;UAS-Spn-F RNAi*

**Supplementary 3**. Partial loss of Spn-F activity does not induce retinal degeneration phenotype. A UAS-RNAi was used to knockdown Spn-F in the eye.

| Associated figures | Genotypes |
|---|---|
| **Figure 1** | +/$w^{1118}$; GMR-Gal4/+;+/+ |
| | +/$w^{1118}$; GMR-Gal4, UAS-GFP/+;+/+ |
| | +/$w^{1118}$; GMR-Gal4, UAS-GFP/$Ik2^{Alice}$;+/+ |
| | +/$w^{1118}$; GMR-Gal4, UAS-GFP/+;UAS-Ik235266/+ |
| | +/$w^{1118}$; GMR-Gal4, UAS-$CHMP2B^{Intron5}$/+;+/+ |
| | +/$w^{1118}$; GMR-Gal4, UAS-$CHMP2B^{Intron5}$/$Ik2^{Alice}$;+/+ |
| | +/$w^{1118}$; GMR-Gal4, UAS-$CHMP2B^{Intron5}$/+; UAS-Ik2 $RNAi^{235266}$/+ |
| | +/$w^{1118}$; GMR-Gal4, UAS-$CHMP2B^{Intron5}$/+; $Ird5^{KG08072}$/+ |
| **Figure 2** | +/$w^{1118}$;+/+;+/+ (Outcrossed Wild-type) |
| | +/$w^{1118}$;OK6-Gal4/+;+/+ |
| | +/$w^{1118}$;OK6-Gal4/UAS-$CHMP2B^{Intron5}$;+/+ |
| | +/$w^{1118}$;OK6-Gal4/+;UAS-ik2/+ |
| | +/$w^{1118}$;OK6-Gal4/UAS-$CHMP2B^{Intron5}$;UAS-ik2/+ |
| **Figure 3** | +/$w^{1118}$; GMR-Gal4, UAS-$CHMP2B^{Intron5}$/+;+/+ |
| | +/$w^{1118}$; GMR-Gal4, UAS-$CHMP2B^{Intron5}$/+;Spn-$F^{2}$/+ |
| | +/$w^{1118}$; GMR-Gal4, UAS-$CHMP2B^{Intron5}$/UAS-GFP;+/+ |
| | +/$w^{1118}$; GMR-Gal4, UAS-$CHMP2B^{Intron5}$/UAS-Spn-F $RNAi^{65226}$;+/+ |
| **Figure 4** | +/$w^{1118}$; GMR-Gal4, UAS-GFP/+;+/+ |
| | +/$w^{1118}$; GMR-Gal4, UAS-GFP/$Hook^{7}$;+/+ |
| | +/$w^{1118}$; GMR-Gal4, UAS-GFP/$Hook^{11}$;+/+ |
| | +/$w^{1118}$; GMR-Gal4, UAS-GFP/$+^{1}$;UAS-Hook $RNAi^{35483}$/+ |
| | +/$w^{1118}$; GMR-Gal4, UAS-GFP/$+^{1}$;UAS-Hook $RNAi^{35485}$/+ |
| | +/$w^{1118}$; GMR-Gal4, UAS-$CHMP2B^{Intron5}$/+;+/+ |
| | +/$w^{1118}$; GMR-Gal4, UAS-$CHMP2B^{Intron5}$/$Hook^{7}$;+/+ |
| | +/$w^{1118}$; GMR-Gal4, UAS-$CHMP2B^{Intron5}$/$Hook^{11}$;+/+ |
| | +/$w^{1118}$; GMR-Gal4, UAS-$CHMP2B^{Intron5}$/$+^{1}$;UAS-Hook $RNAi^{35483}$/+ |
| | +/$w^{1118}$; GMR-Gal4, UAS-$CHMP2B^{Intron5}$/$+^{1}$;UAS-Hook $RNAi^{35483}$/+ |
| **Figure 5** | +/$w^{1118}$; GMR-Gal4, UAS-GFP/+;+/+ |
| | +/$w^{1118}$; GMR-Gal4, UAS-GFP/UAS-$Ctp^{43115}$;+/+ |
| | +/$w^{1118}$; GMR-Gal4, UAS-GFP/UAS-$Ctp^{109084}$;+/+ |
| | +/$w^{1118}$; GMR-Gal4, UAS-$CHMP2B^{Intron5}$/+;+/+ |
| | +/$w^{1118}$; GMR-Gal4, UAS-$CHMP2B^{Intron5}$/UAS-$Ctp^{43115}$;+/+ |
| | +/$w^{1118}$; GMR-Gal4, UAS-$CHMP2B^{Intron5}$/UAS-$Ctp^{109084}$;+/+ |

**Supplementary 4**. Genotypes of the flied used in this study.

| **Reagents** | **Sources** |
|---|---|
| **Flies** | |
| *GMR-Gal4* | Ahmad et al., 2009 |
| *UAS-GFP* | RRID:BDSC_5431 |
| *GMR-Gal4, UAS-GFP* | This study |
| *GMR-Gal4, UAS-CHMP2B$^{Intron5}$* | Ahmad et al., 2009 |
| *Ik2$^{Alice}$* | RRID:BDSC_31035 |
| *UAS-Ik2* RNAi | RRID: BDSC_35266 (Bergalet et al., 2020) |
| *Ird5$^{KG08072}$* | RRID: BDSC_14684 |
| *UAS-Spn-F* RNAi | RRID:BDSC_65226 |
| *Spn-F$^{2}$* | Lin et al, 2015 |
| *UAS-GFP-Spn-F* | Lin et al, 2015 |
| *Hook$^{7}$* and *Hook$^{11}$* | Kramer et al, 1998 |
| *UAS-Hook* RNAi lines | VDRC: 35483 AND 35485 |
| *UAS-Ctp* RNAi lines | VGCR: 43115 and 109084 (Zaytseva et al., 2014) |
| *UAS-CHMP2B$^{Intron5}$* | Ahmad et al., 2009 |
| *UAS-ik2* | F001016, FlyORF |
| *CS* | RRID:BDSC_64349 |
| *W$^{1118}$* | RRID:BDSC_5905 |
| **Antibodies** | |
| anti-synaptotagmin | RRID:AB_2713991 |
| Cy3-Conjugated anti-HRP | RRID:AB_2338959 |
| anti-Rabbit IgG (H+L) Alexa Fluor 488 | RRID:AB_2576217 |

**Supplementary 5.** Fly lines and reagents used in this study and their sources.

| Figure 1 | *Chi-squared test for categorical data* | *Summary* | *P value* |
|---|---|---|---|
| | *GMR-Gal4,UAS-CHMP2B$^{Intron5}$/Ik2$^{Alice}$ vs. GMR-Gal4,UAS-CHMP2B$^{Intron5}$* | *** | 7.58403E-09 |
| | *GMR-Gal4,UAS-CHMP2B$^{Intron5}$/+; UAS-Ik2 RNAi$^{235266}$/+ vs. GMR-Gal4,UAS-CHMP2B$^{Intron5}$* | *** | 5.9822E-21 |
| | *GMR-Gal4,UAS-CHMP2B$^{Intron5}$/+; Ird5$^{KG08072}$/+ vs. GMR-Gal4,UAS-CHMP2B$^{Intron5}$* | ns | 0.105646194 |
| Figure 2B | Tukey's multiple comparisons test | Summary | P Value |
| | Wildtype vs. OK6 > CHMP2B$^{Intron5}$ | ** | 0.0013 |
| | Wildtype vs. OK6 > mCD8-GFP, CHMP2B$^{Intron5}$ | ** | 0.0024 |
| | Wildtype vs. OK6 > CHMP2B$^{Intron5}$, UAS-TBK1 | ns | 0.5239 |
| | OK6 > CHMP2B$^{Intron5}$ vs. OK6 > mCD8-GFP, CHMP2B$^{Intron5}$ | ns | 0.9873 |
| | OK6 > CHMP2B$^{Intron5}$ vs. OK6 > CHMP2B$^{Intron5}$, UAS-TBK1 | ns | 0.0543 |
| | OK6 > mCD8-GFP, CHMP2B$^{Intron5}$ vs. OK6 > CHMP2B$^{Intron5}$, UAS-TBK1 | ns | 0.0633 |
| Figure 2C | Tukey's multiple comparisons test | Summary | P Value |
| | Wild type vs. OK6 > CHMP2B$^{Intron5}$ | * | 0.0144 |
| | Wild type vs. OK6 > mCD8-GFP, CHMP2B$^{Intron5}$ | * | 0.0429 |
| | Wild type vs. OK6 > CHMP2B$^{intron5}$, UAS-TBK1 | ns | 0.5674 |
| | OK6 > CHMP2B$^{Intron5}$ vs. OK6 > mCD8-GFP, CHMP2B$^{Intron5}$ | ns | 0.9708 |
| | OK6 > CHMP2B$^{Intron5}$ vs. OK6 > CHMP2B$^{intron5}$, UAS-TBK1 | ns | 0.1777 |
| | OK6 > mCD8-GFP, CHMP2B$^{Intron5}$ vs. OK6 > CHMP2B$^{intron5}$, UAS-TBK1 | ns | 0.3813 |
| Figure 2D | Tukey's multiple comparisons test | Summary | P Value |
| | Wild type vs. OK6 > CHMP2B$^{Intron5}$ | **** | <0.0001 |
| | Wild type vs. OK6 > mCD8-GFP, CHMP2B$^{Intron5}$ | **** | <0.0001 |
| | Wild type vs. OK6 > CHMP2B$^{intron5}$, UAS-TBK1 | **** | <0.0001 |
| | OK6 > CHMP2B$^{Intron5}$ vs. OK6 > mCD8-GFP, CHMP2B$^{Intron5}$ | ns | 0.7216 |
| | OK6 > CHMP2B$^{Intron5}$ vs. OK6 > CHMP2B$^{intron5}$, UAS-TBK1 | ns | 0.5821 |
| | OK6 > mCD8-GFP, CHMP2B$^{Intron5}$ vs. OK6 > CHMP2B$^{intron5}$, UAS-TBK1 | ns | 0.0665 |
| Figure 2E | Tukey's multiple comparisons test | Summary | P Value |
| | Wild type vs. OK6 > CHMP2B$^{Intron5}$ | **** | <0.0001 |
| | Wild type vs. OK6 > mCD8-GFP, CHMP2B$^{Intron5}$ | **** | <0.0001 |
| | Wild type vs. OK6 > CHMP2B$^{intron5}$, UAS-TBK1 | * | 0.0129 |
| | OK6 > CHMP2B$^{Intron5}$ vs. OK6 > mCD8-GFP, CHMP2B$^{Intron5}$ | ns | 0.9479 |
| | OK6 > CHMP2B$^{Intron5}$ vs. OK6 > CHMP2B$^{intron5}$, UAS-TBK1 | ** | 0.0051 |
| | OK6 > mCD8-GFP, CHMP2B$^{Intron5}$ vs. OK6 > CHMP2B$^{intron5}$, UAS-TBK1 | *** | 0.0004 |
| Figure 2F | Tukey's multiple comparisons test | Summary | P Value |
| | Wild type vs. OK6 > CHMP2B$^{Intron5}$ | **** | <0.0001 |
| | Wild type vs. OK6 > mCD8-GFP, CHMP2B$^{Intron5}$ | **** | <0.0001 |
| | Wild type vs. OK6 > CHMP2B$^{intron5}$, UAS-TBK1 | ** | 0.0034 |
| | OK6 > CHMP2B$^{Intron5}$ vs. OK6 > mCD8-GFP, CHMP2B$^{Intron5}$ | ns | 0.8572 |
| | OK6 > CHMP2B$^{Intron5}$ vs. OK6 > CHMP2B$^{intron5}$, UAS-TBK1 | ns | 0.0785 |
| | OK6 > mCD8-GFP, CHMP2B$^{Intron5}$ vs. OK6 > CHMP2B$^{intron5}$, UAS-TBK1 | ** | 0.0063 |
| Figure 3 | *Chi-squared test for categorical data* | *Summary* | *P value* |
| | *GMR-Gal4,UAS-CHMP2B$^{Intron5}$/+;Spn-F$^{2}$/+ vs. GMR-Gal4,UAS-CHMP2B$^{Intron5}$/+;* | *** | 6.2127E-22 |
| | *GMR-Gal4, UAS-CHMP2B$^{Intron5}$/UAS-Spn-F RNAi$^{65226}$ vs. GMR-Gal4,UAS-CHMP2B$^{Intron5}$/UAS-GFP* | *** | 1.97234E-39 |
| | *GMR-Gal4,UAS-CHMP2B$^{Intron5}$/Hook$^{7}$ vs. GMR-Gal4,UAS-CHMP2B$^{Intron5}$* | *** | 0.00001981 |
| | *GMR-Gal4,UAS-CHMP2B$^{Intron5}$/Hook$^{11}$ vs. GMR-Gal4,UAS-CHMP2B$^{Intron5}$* | ** | 0.005270609 |
| Figure 4 | *Chi-squared test for categorical data* | *Summary* | *P value* |

| | | | |
|---|---|---|---|
| | *GMR-Gal4,UAS-CHMP2B$^{Intron5}$/Hook$^{11}$ vs. GMR-Gal4,UAS-CHMP2B$^{Intron5}$* | ** | 0.005270609 |
| | *GMR-Gal4,UAS-CHMP2B$^{Intron5}$/+;UAS-Hook RNAi$^{35483}$/+ vs. GMR-Gal4,UAS-CHMP2B$^{Intron5}$/UAS-GFP* | *** | 9.70605E-10 |
| | *GMR-Gal4,UAS-CHMP2B$^{Intron5}$/+;UAS-Hook RNAi$^{35485}$/+ vs. GMR-Gal4,UAS-CHMP2B$^{Intron5}$/UAS-GFP* | ** | 2.02058E-12 |
| Figure 5 | *Chi-squared test for categorical data* | *Summary* | *P value* |
| | *GMR-Gal4, UAS-CHMP2B$^{Intron5}$/UAS-Ctp$^{4311\ 5}$ vs. GMR-Gal4,UAS-CHMP2B$^{Intron5}$/UAS-GFP* | *** | 0.0000000307 |
| | *GMR-Gal4, UAS-CHMP2B$^{Intron5}$/UAS-Ctp$^{109084}$ vs. GMR-Gal4,UAS-CHMP2B$^{Intron5}$/UAS-GFP* | ** | 0.0079128247 |
| Supplementary 2 | Tukey's multiple comparisons test | Summary | P value |
| | Wild-type vs. OK6 > + | ns | 0.9897 |
| | Wild-type vs. OK6 > GFP, CHMP2B$^{Intron5}$ | ** | 0.0076 |
| | Wild-type vs. OK6 > CHMP2B$^{Intron5}$, UAS-IK2 | *** | 0.0001 |
| | OK6 > + vs. OK6 > GFP, CHMP2B$^{Intron5}$ | * | 0.0310 |
| | OK6 > + vs. OK6 > CHMP2B$^{Intron5}$, UAS-IK2 | *** | 0.0010 |
| | OK6 > GFP, CHMP2B$^{Intron5}$ vs. OK6 > CHMP2B$^{Intron5}$, UAS-IK2 | ns | 0.7182 |

**Supplementary 6.** Values of all the statistical test results.