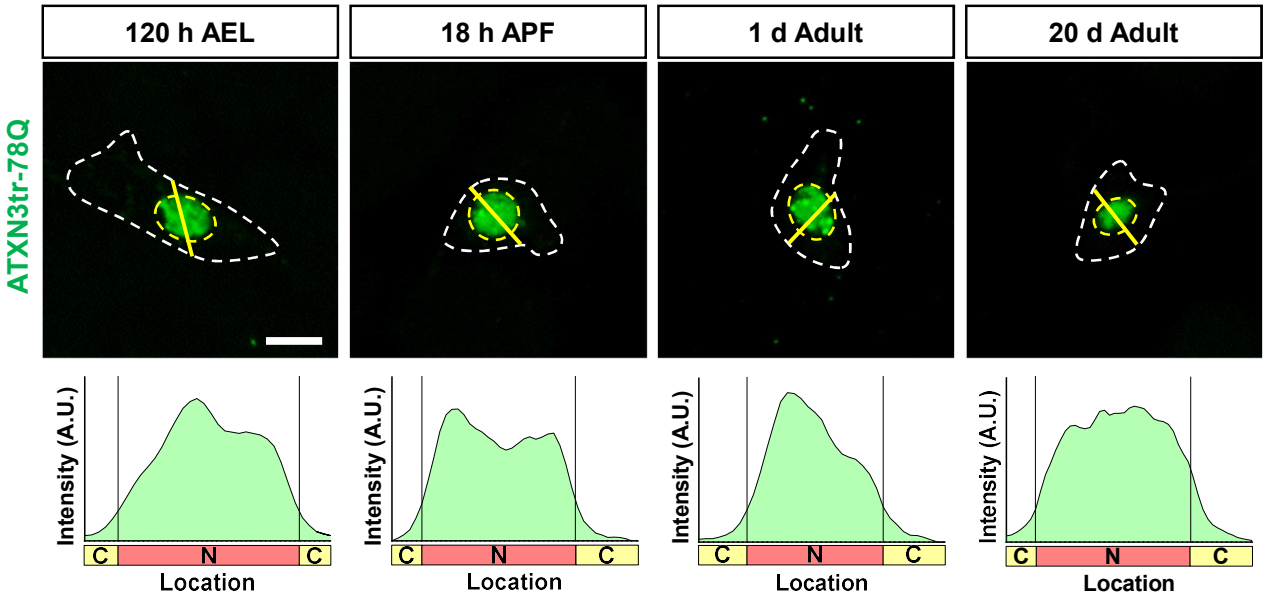
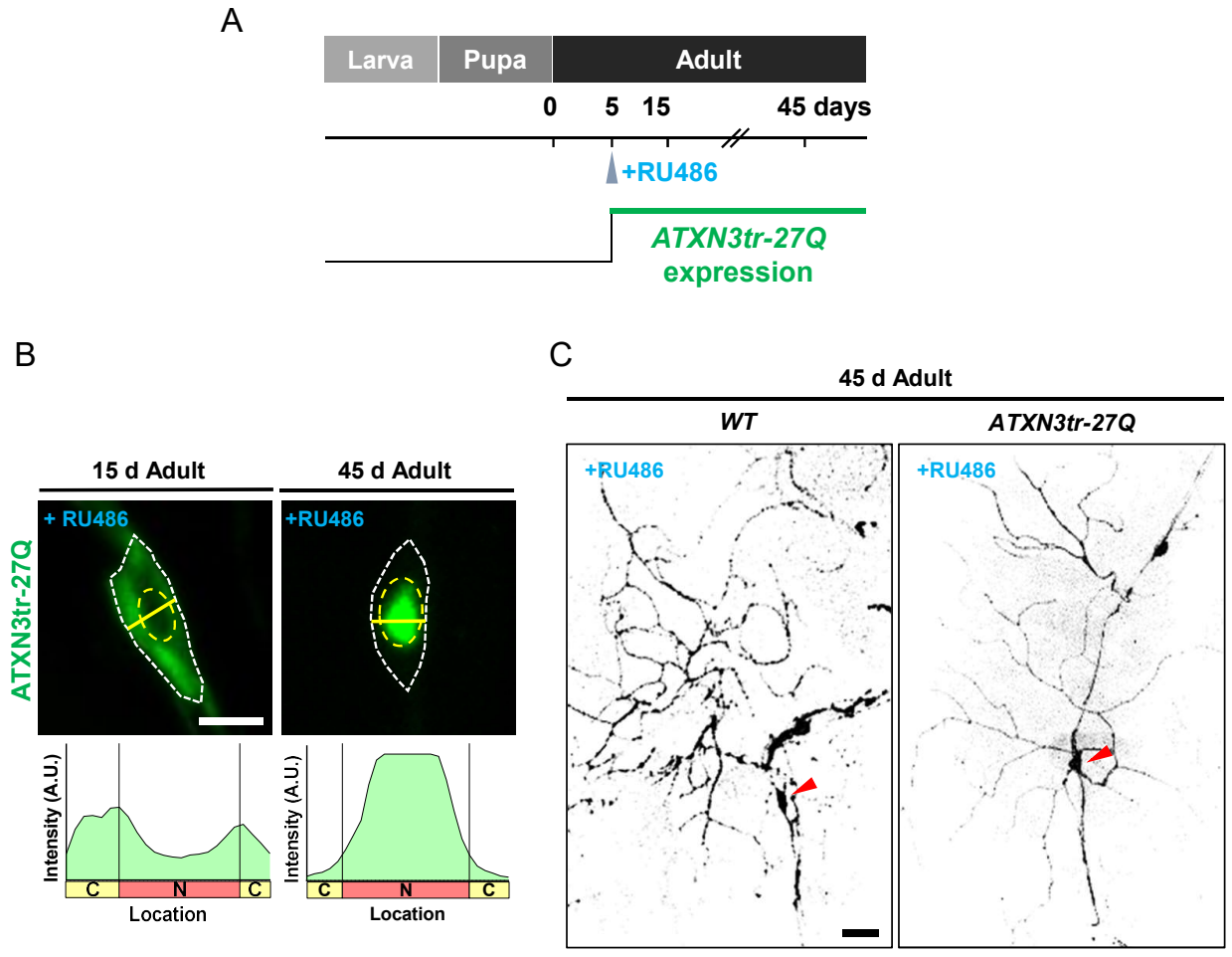
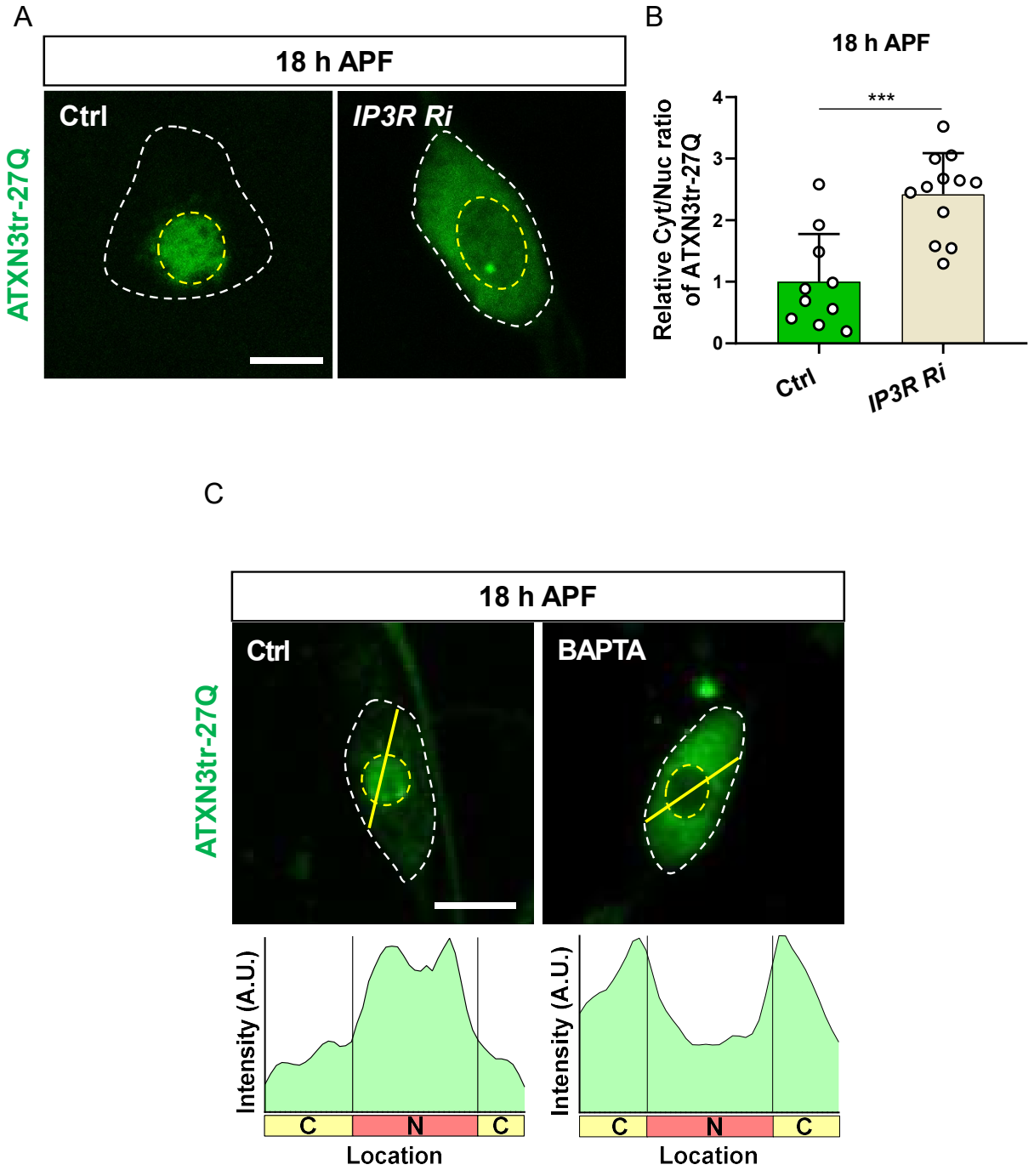


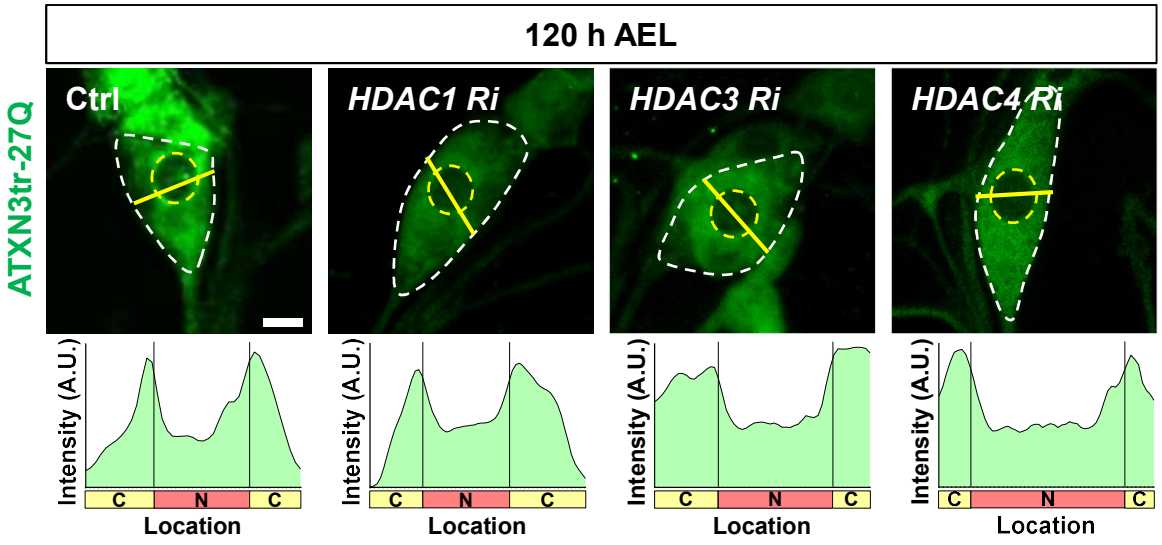

**Supplementary Fig. S1. ATXN3tr-78Q shows a strong tendency to accumulate within the nucleus of *Drosophila* sensory neurons during development.** Subcellular localization of overexpressed HA-ATXN3tr-78Q proteins in C4da neurons during development stages (120 h AEL, 18 h APF, 1 d adult, and 20 d adult) [*+/+;ppk$^{1a}$-GAL4>UAS-CD4-tdGFP/UAS-HA-ATXN3tr-78Q*]. Outer and inner dashed lines indicate the borders of cell bodies and nuclei, respectively. Scale bar = 5 μm. The intensity profile of fluorescent signals representing ATXN3tr-78Q proteins across cell bodies along yellow lines are presented at the bottom.



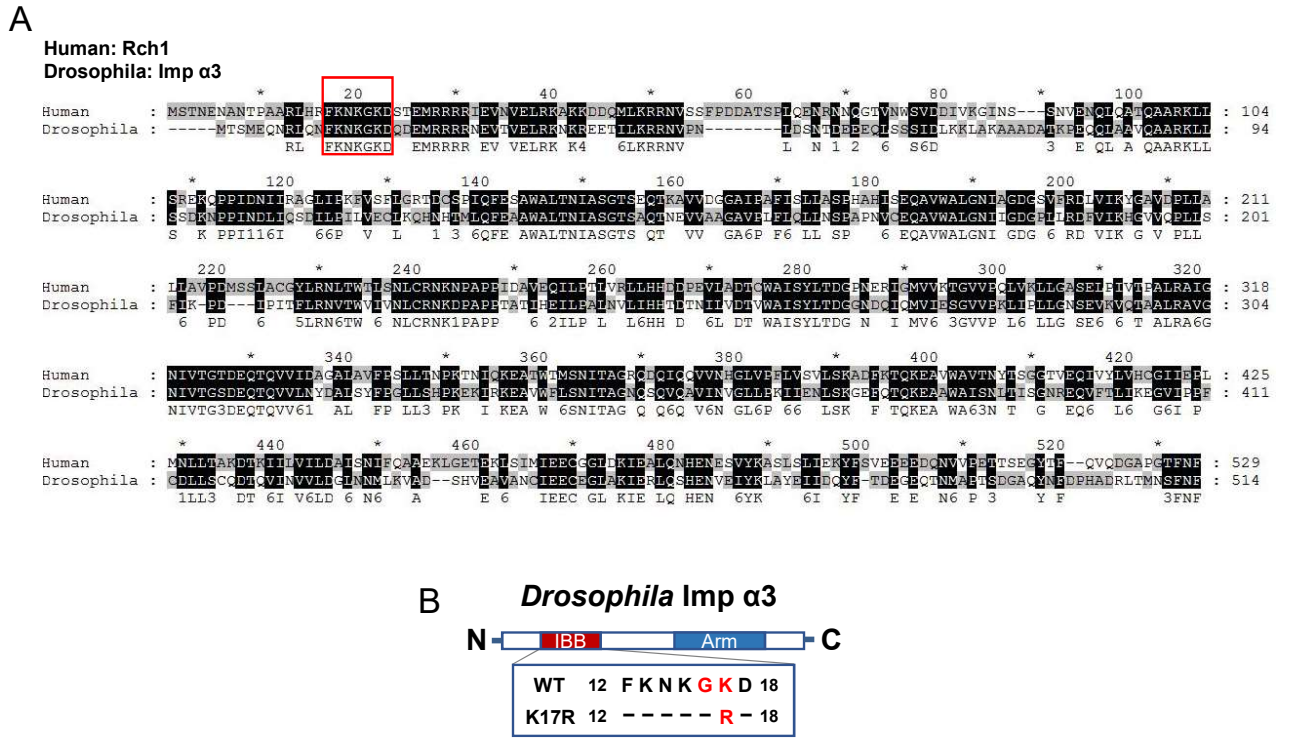

**Supplementary Fig. S2. Adult-specific expression of ATXN3tr-27Q leads to aging-dependent nuclear accumulation in neurons accompanied by dendrite defects.** (A) Experimental scheme for adult-specific expression of HA-ATXN3tr-27Q in C4da neurons. To activate gene switch-mediated transcription of GAL4 (*GS-ppk$^{1a}$-GAL4*), adult flies were fed food with RU486 from 5 days. (B) Subcellular localization of overexpressed HA-ATXN3tr-27Q proteins in C4da neurons at 15 days adult and 45 days adult [*+/+;GS-ppk$^{1a}$-GAL4>ppk-CD4-tdtom/UAS-HA-ATXN3tr-27Q*]. Outer and inner dashed lines indicate the borders of cell bodies and nuclei, respectively. Scale bar = 5 μm. The intensity profile of fluorescent signals representing ATXN3tr-27Q proteins across cell bodies along yellow lines are presented at the bottom. (C) Representative images of dendrites of C4da neurons of *WT* or expressing *HA-ATXN3tr-27Q* [*WT, +/+;GS-ppk$^{1a}$-GAL4>ppk-CD4-tdtom/+, ATXN3tr-27Q, +/+;GS-ppk$^{1a}$-GAL4>ppk-CD4-tdtom/UAS-HA-ATXN3tr-27Q*]. Red-colored arrowheads indicate cell bodies of C4da neurons. Scale bar = 100 μm.



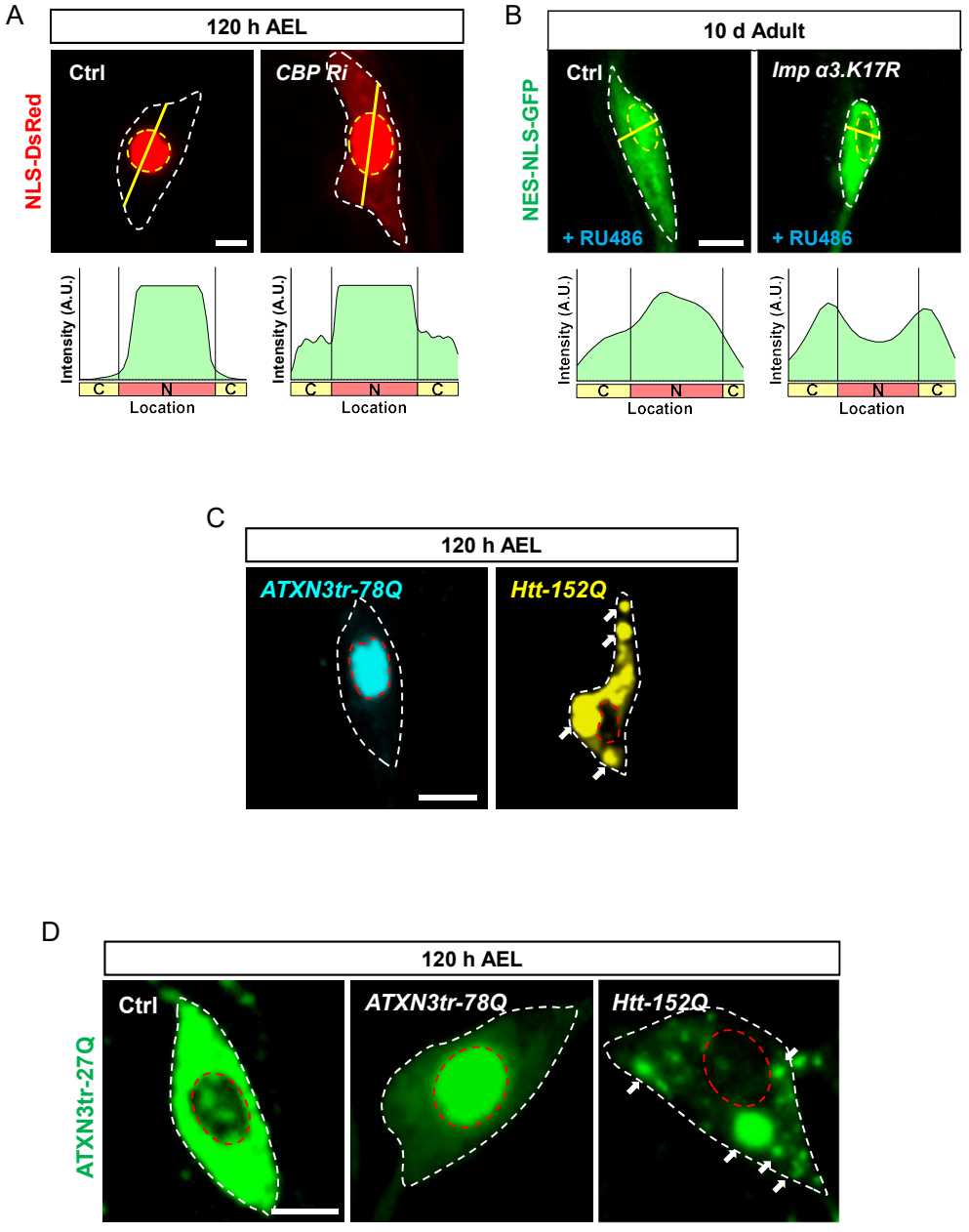

**Supplementary Fig. S3. Genetic and chemical manipulation of intracellular calcium level prevents nuclear accumulation of ATXN3tr-27Q in neurons during pupal stage.** (A) Subcellular localization of overexpressed HA-ATXN3tr-27Q proteins in C4da neurons of Ctrl or expressing *IP3R Ri* at 18 h APF [Ctrl, *+/+;ppk$^{1a}$-GAL4/UAS-HA-ATXN3tr-27Q, IP3R Ri, UAS-IP3R RNAi/+;ppk$^{1a}$-GAL4/UAS-HA-ATXN3tr-27Q*]. Outer and inner dashed lines indicate the borders of cell bodies and nuclei, respectively. Scale bar = 5 μm. (B) Quantification of Cyt/Nuc ratio of HA-ATXN3tr-27Q proteins in C4da neurons of Ctrl or expressing *IP3R Ri* 18 h APF. Values are presented as mean ± SD. ***$P$ = 0.0002 by two-tailed $t$-test; n = 10 for Ctrl, n = 12 for *IP3R Ri*. (C) Subcellular localization of overexpressed HA-ATXN3tr-27Q proteins in C4da neurons of ctrl or feeding with BAPTA at 18 h APF [*+/+;ppk$^{1a}$-GAL4/UAS-HA-ATXN3tr-27Q*]. Outer and inner dashed lines indicate the borders of cell bodies and nuclei, respectively. Scale bar = 5 μm. The intensity profile of fluorescent signals representing ATXN3tr-27Q proteins across cell bodies along yellow lines are presented at the bottom.




**Supplementary Fig. S4. CBP-associated histone acetylation is not essential for NCT of ATXN3tr-27Q in neurons.** Subcellular localization of overexpressed ATXN3tr-27Q proteins in pan-da neurons of Ctrl or expressing a subset of *HDACs Ri* at 120 h AEL [*Ctrl, 109(2)80-GAL4/+;UAS-HA-ATXN3tr-27Q/+, HDAC1 RNAi, 109(2)80-GAL4/+;UAS-HA-ATXN3tr-27Q/UAS-HDAC1 RNAi, HDAC3 RNAi, 109(2)80-GAL4/+;UAS-HA-ATXN3tr-27Q/UAS-HDAC3 RNAi, HDAC4 RNAi, 109(2)80-GAL4/+;UAS-HA-ATXN3tr-27Q/UAS-HDAC4 RNAi*]. Outer and inner dashed lines indicate the borders of cell bodies and nuclei, respectively. Scale bar = 5 μm. The intensity profile of fluorescent signals representing ATXN3tr-27Q proteins across cell bodies along yellow lines are presented at the bottom.




**Supplementary Fig. S5. *Drosophila* Imp α3 has a consensus sequence conserved from human Rch1 for CBP-dependent acetylation.** (A) Sequences alignment between human Rch1 and *Drosophila* Imp α3 using GeneDoc. Amino acids on a black background are identical. Those on a gray background are similar for side chain hydrophobicity. Red rectangle indicates "FKNKGK" sequence, acetylation site. (B) Schematic representation of the domain structure of *Drosophila* Imp α3. The WT protein sequence (amino acids 12-18) and the GK motif, known to be acetylated by CBP (Bannister et al., 2000), is shown in red. The positions and sequence of the point mutation (K17R) introduced into Imp α3 is shown below the WT protein sequence. A dash indicates no change. IBB, Importin β binding domain; ARM, Armadillo repeats.




**Supplementary Fig. S6. CBP-mediated acetylation of *Drosophila* Imp α3 is involved in NCT of additional NLS-containing proteins in neurons.** (A) Subcellular localization of overexpressed NLS-DsRed proteins in C4da neurons at 120 h AEL [Ctrl, *UAS-RedStinger/+;ppk$^{1a}$-GAL4/+, CBP Ri, UAS-RedStinger/UAS-CBP RNAi;ppk$^{1a}$-GAL4/+*]. Outer and inner dashed lines indicate the borders of cell bodies and nuclei, respectively. Scale bar = 5 μm. The intensity profile of fluorescent signals representing NLS-DsRed proteins across cell bodies along yellow lines are presented at the bottom. (B) Subcellular localization of overexpressed NES-NLS-GFP proteins in C4da neurons at adult 10 days [Ctrl, *+/+;ppk$^{1a}$-GAL4/UAS-NES-NLS-GFP, Imp α3.K17R, UAS-2xFlag-Imp α3.K17R/+;ppk$^{1a}$-GAL4/UAS-NES-NLS-GFP*]. Outer and inner dashed lines indicate the borders of cell bodies and nuclei, respectively. Scale bar = 5 μm. The intensity profile of fluorescent signals representing NES-NLS-GFP proteins across cell bodies along yellow lines are presented at the bottom. (C) Subcellular localization of overexpressed HA-ATXN3tr-78Q and Htt-152Q-eGFP proteins in pan-da neurons at 120 h AEL [*ATXN3tr-78Q, 109(2)80-GAL4/+;UAS-HA-ATXN3tr-78Q/+, Htt-152Q, 109(2)80-GAL4/+;UAS-Htt-152Q-eGFP/+*]. Outer and inner dashed lines indicate the borders of cell bodies and nuclei, respectively. White-colored arrows indicate cytosolic protein aggregates in the cell bodies of C4da neurons. Scale bar = 5 μm. (D) Subcellular localization of overexpressed ATXN3tr-27Q proteins in pan-da neurons of Ctrl or expressing *HA-ATXN3tr-78Q* or *Htt-152Q-eGFP* at 120 h AEL [*Ctrl, 109(2)80-GAL4/+;UAS-HA-ATXN3tr-27Q/+, ATXN3tr-78Q, 109(2)80-GAL4/+; UAS-HA-ATXN3tr-27Q/UAS-HA-ATXN3tr-78Q, Htt-152Q, 109(2)80-GAL4/+; UAS-HA-ATXN3tr-27Q/UAS-Htt-152Q-eGFP*]. Outer and inner dashed lines indicate the borders of cell bodies and nuclei, respectively. White-colored arrows indicate cytosolic protein aggregates in the cell bodies of C4da neurons. Scale bar = 5 μm.