

Description of Additional Supplementary Files

File name: Supplementary Data 1

Description: Genetic interactions scores (qGI) from genome-wide CRISPR knockout screens in HAP1 *NAA30*-KO, *NAA35*-KO, and *NAA38*-KO cells.

File name: Supplementary Data 2

Description: Gene ontology (GO) biological process (BP) enrichment analysis of negative and positive genetic interactions of *NAA35*, *NAA30*, and *NAA38*.

File name: Supplementary Data 3

Description: N-terminal acetylome analysis of HAP1 WT, *NAA30*-KO, *NAA35*-KO, and *NAA38*-KO cells.

File name: Supplementary Data 4

Description: Label-free quantitative (LFQ) shotgun proteomic analysis of HAP1 WT, *NAA30*-KO, *NAA35*-KO, and *NAA38*-KO cells.

File name: Supplementary Data 5

Description: Knockdown of *UBR4* rescues *NAA30*-KO induced changes in protein abundance. Tandem mass tag (TMT) analysis of HAP1 WT and *NAA30*-KO cells treated with siCtrl or si*UBR1/UBR2/UBR4* (triple knockdown).

File name: Supplementary Data 6

Description: List of proteins co-immunoprecipitated with Myc-tagged *Drosophila* Naa30A from embryonic protein extracts.

File name: Supplementary Data 7

Description: List of all primers and peptides used in this study.

File name: Supplementary Data 8

Description: Key Resource Table (Antibodies, cell lines, fly strains, yeast strains, plasmids, siRNA, etc.).

File name: Supplementary Movie 1

Description: *Drosophila* Naa30A deletion males show significantly slower motility (climbing).

File name: Supplementary Movie 2

Description: Moving sperm from control (y1/Y) males.

File name: Supplementary Movie 3

Description: Moving sperm from Naa30A deletion (y1, Naa30 Δ 74/Y) males.