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Supplemental information

Nucleoporins are degraded via upregulation of ESCRT-III/Vps4 complex in *Drosophila* models of C9-ALS/FTD

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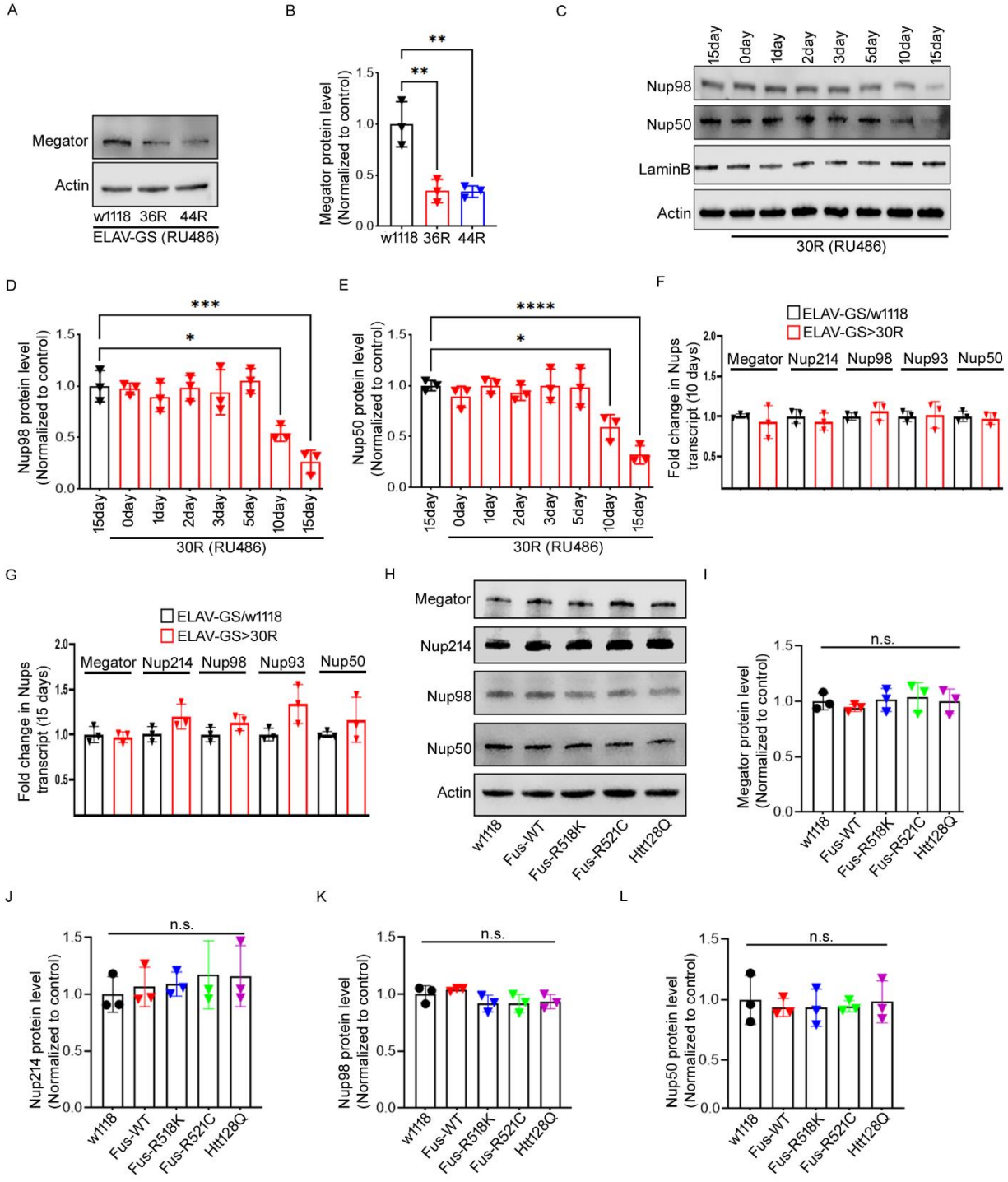
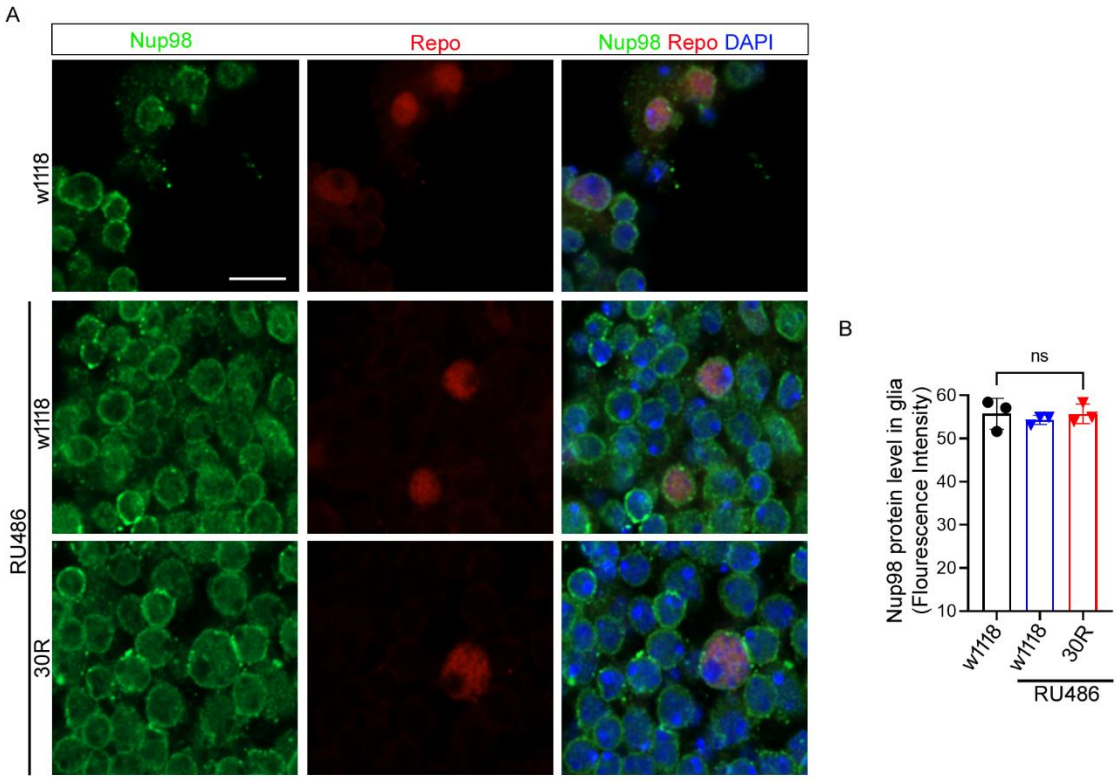
Figure S1


A
Megator
Actin
w1118 36R 44R
ELAV-GS (RU486)
B
Megator protein level (Normalized to control)
w1118 36R 44R
C
15day 0day 1day 2day 3day 5day 10day 15day
Nup98
Nup50
LaminB
Actin
30R (RU486)
D
Nup98 protein level (Normalized to control)
15day 0day 1day 2day 3day 5day 10day 15day
30R (RU486)
E
Nup50 protein level (Normalized to control)
15day 0day 1day 2day 3day 5day 10day 15day
30R (RU486)
F
ELAV-GS/w1118
ELAV-GS>30R
Megator Nup214 Nup98 Nup93 Nup50
Fold change in Nups transcript (10 days)
G
ELAV-GS/w1118
ELAV-GS>30R
Megator Nup214 Nup98 Nup93 Nup50
Fold change in Nups transcript (15 days)
H
Megator
Nup214
Nup98
Nup50
Actin
w1118 Fus-WT Fus-R518K Fus-R521C Htt128Q
I
n.s.
Megator protein level (Normalized to control)
w1118 Fus-WT Fus-R518K Fus-R521C Htt128Q
J
n.s.
Nup214 protein level (Normalized to control)
w1118 Fus-WT Fus-R518K Fus-R521C Htt128Q
K
n.s.
Nup98 protein level (Normalized to control)
w1118 Fus-WT Fus-R518K Fus-R521C Htt128Q
L
n.s.
Nup50 protein level (Normalized to control)
w1118 Fus-WT Fus-R518K Fus-R521C Htt128Q

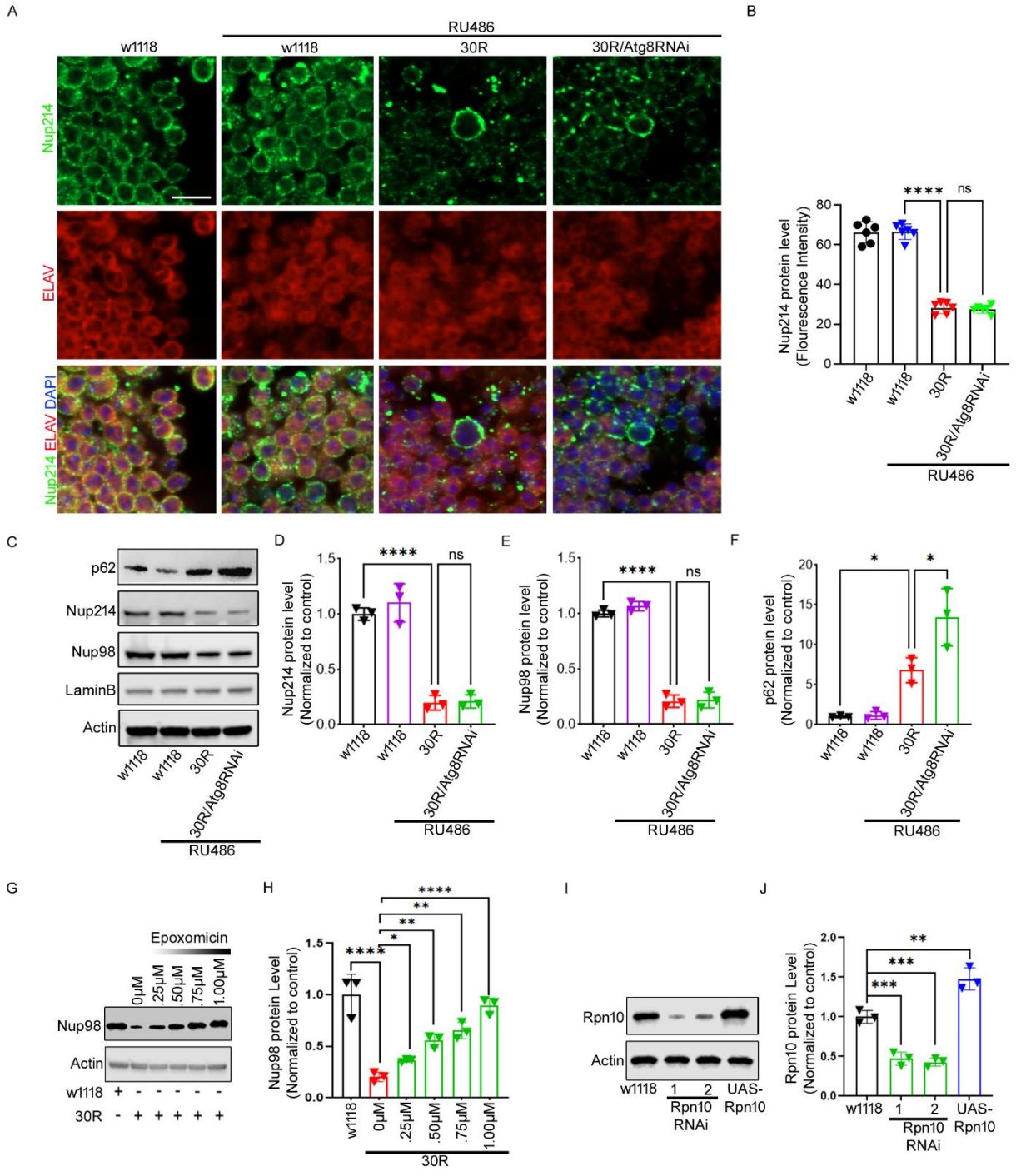
Supplementary Figure 1. Nucleoporins are reduced in multiple C9-ALS fly models but not in Fus-ALS or Huntington's disease fly models, Related to Figure 1. (A) Immunoblot of the nucleoporin Megator and Beta-actin from adult brain of 15-day-old flies. Genotypes are Ctrl (*ELAV-GS/w1118)*, 36R (*ELAV-GS>(GGGGCC)36R),* and 44R (*ELAV-GS>(GGGGCC)44*. **(B)** Quantification of Megator from Western blot in A. (n = 3; Tukey's multiple comparisons test **P < 0.01). **(C)** Western blot of Nup98, Nup50, LaminB and Beta-actin of adult brains of 15-days-old *ELAV-GS/w1118* or *ELAV-GS>30R* flies at indicated days after eclosion. Indicated flies were raised on food containing RU486 until collection. **(D and E)** Quantification of Nup98 and Nup50 from western blot in C. One-way ANOVA, p<0.0001, followed by Sidak's multiple comparisons, (n = 3; *P < 0.05, ***P < 0.001, ****P < 0.0001). Data are reported as mean ± SEM. **(F and G)** Quantitative RT-PCR of nucleoporins transcript of ELAV-GS/w1118 and ELAV-GS>30R (brain of 10 and 15 days old flies). **(H)** Immunoblot of Megator, Nup214, Nup98, Nup50, and Beta-actin of adult brain of 15-days-old *ELAV-GS/w1118*, *ELAV-GS>Fus-WT*, *ELAV-GS>Fus-R518K*, *ELAV-GS>Fus-R521C*, and *ELAV-GS>HttP128Q* flies. **(I-L)** Quantification of Megator, Nup214, Nup98, and Nup50 of immunoblot in H. One-way ANOVA, followed by Sidak's multiple comparisons, (n = 3; n.s.= nonsignificant). Data are reported as mean ± SEM.

Figure S2



Supplementary Figure 2. Nup98 is not altered in G4C2 expressing glial cells in adult brain, Related to Figure 1. (A) 15-days-old *Drosophila* adult brain expressing (G4C2)30 under the control of the inducible, pan-glia Repo-Gene Switch induced with 100 µM RU486. Representative Images showing Nup98 (green) with glia marker Repo (red) and DAPI (blue) in mentioned genotypes (scale bar: 10 µm and n=10 brain per genotype). **(B)** Quantification of Nup98 immunofluorescence in glial cells in A. Data are reported as mean ± SEM, (One-way ANOVA, followed by Sidak's multiple comparisons, n.s.= nonsignificant, 30>n cells per genotype).

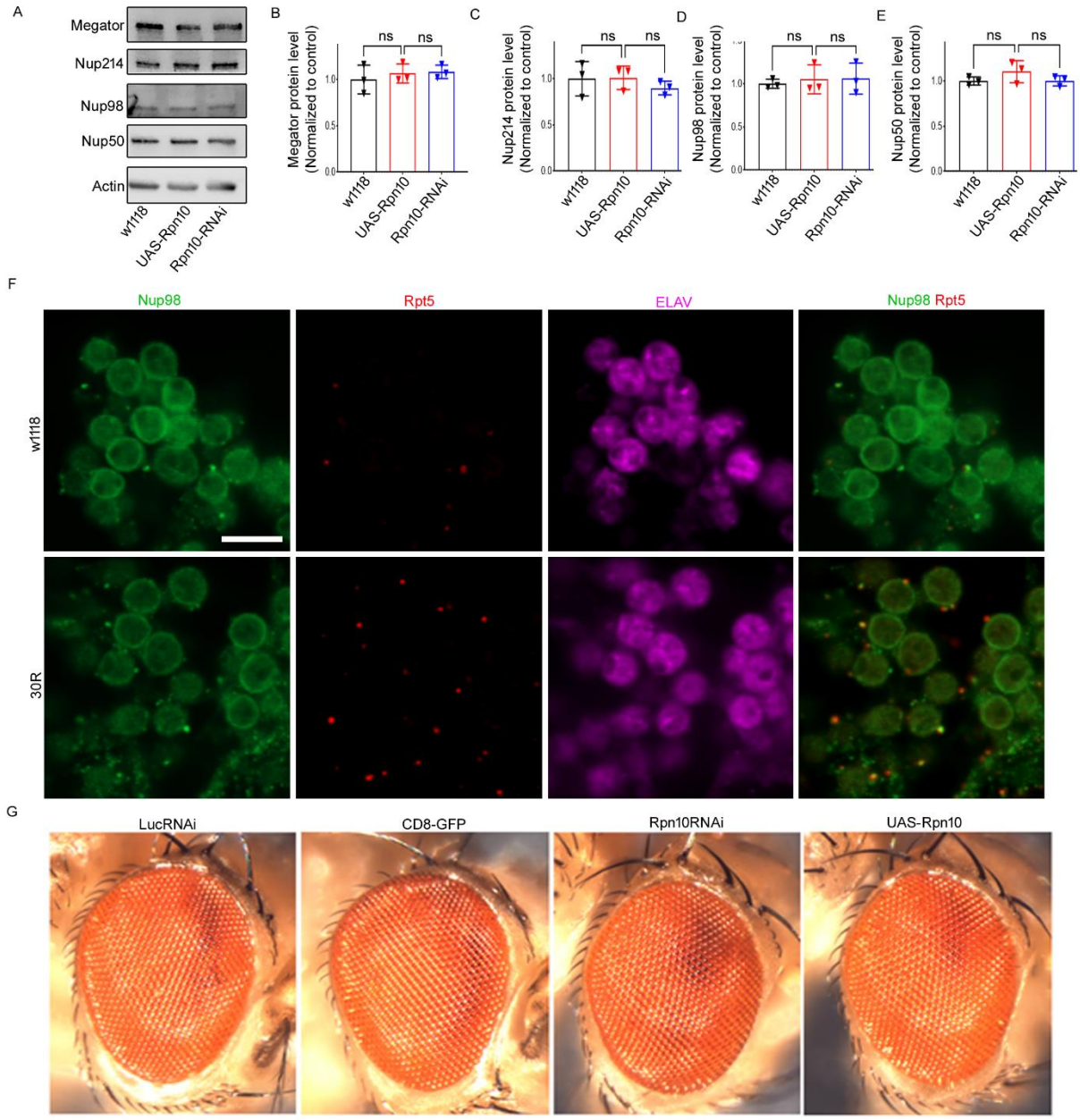
Figure S3



Supplementary Figure 3. Inhibition of autophagy does not alter nucleoporins in 30R expressing adult brain, Related to Figure 2. (A) Confocal images show Nup214 (green) with neuronal marker ELAV (red) and DAPI (blue)

in above mentioned genotypes of 15-days-old fly brain (scale bar denotes 5 μm and n=15 brain per genotype). **(B)** Quantification of Nup214 immunofluorescence in A, data are reported as mean ± SEM (One-way ANOVA, followed by Tukey's multiple comparisons test; ****P < 0.0001, n.s.= nonsignificant, 50>n cells per genotype). **(C)** Immunoblot of Ref(2)p, Nup214, Nup98, LaminB and Beta-actin of adult brain of 15-days-old fly in below mentioned genotypes. **(D-F)** Quantification of Nup214, Nup98, and Ref(2)p from western blots in C. One-way ANOVA, p<0.0001, followed by Sidak's multiple comparisons, (n = 3; *P < 0.05, ****P < 0.0001, n.s= nonsignificant). Error bars indicate ± SEM. **(G)** Immunoblot of Nup98 from adult brains of 15-day-old flies in control or 30R-expressing flies fed different concentrations of the proteasomal pathway inhibitor epoxomicin. **(H)** Quantification of Nup98 levels in immunoblots shown in G. One-way ANOVA, followed by Sidak's multiple comparisons, (n = 3; n.s.= nonsignificant). Data are reported as mean ± SEM. **(I and J)** The Knock down (KD) and overexpression efficiency of the Rpn10 transgenic lines are analyzed by Western blot and normalized to actin. One-way ANOVA, p<0.0001, followed by Sidak's multiple comparisons, (n = 3; ***P < 0.001, ****P < 0.0001). Error bars indicate ± SEM.

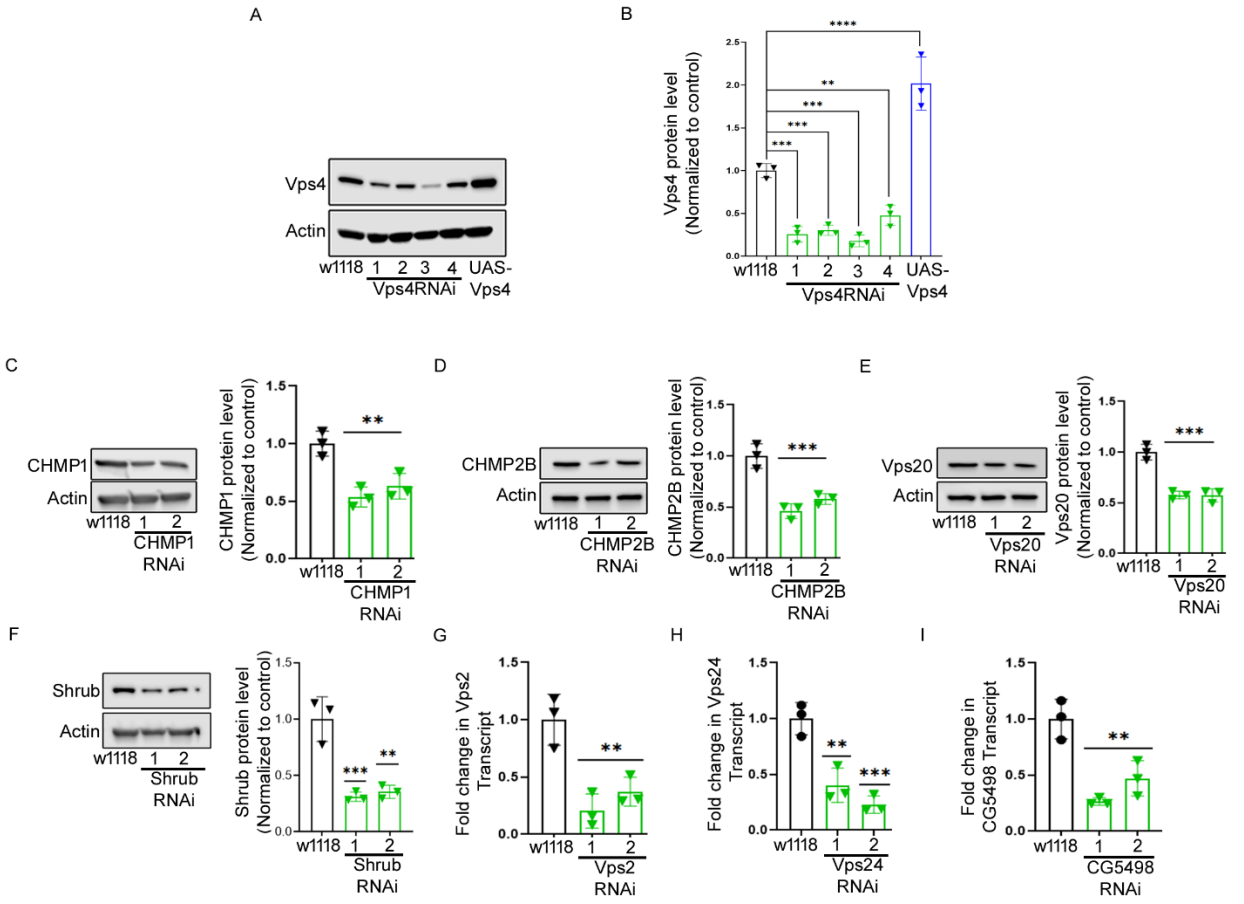
Figure S4



Supplementary Figure 4. Proteasome co-localizes with Nup98 in G4C2 expressing neurons, Related to Figure 2. (A) Western blot of Megator, Nup214, Nup98, Nup50, and Beta-actin of adult brain of 15-days-old fly from *ELAV-GS/w1118*, *ELAV-GS>Rpn10RNAi* and *ELAV-GS>Rpn10RNAi* genotypes. **(B-E)** Quantification of Megator, Nup214, Nup98 and Nup50 from western blot in G. One-way ANOVA, $p<0.0001$, followed by Sidak's multiple comparisons, ($n = 3$; ***$P < 0.001$, ****$P < 0.0001$). Error bars indicate ± SEM. **(F)** Representative images showing Nup98 (green),

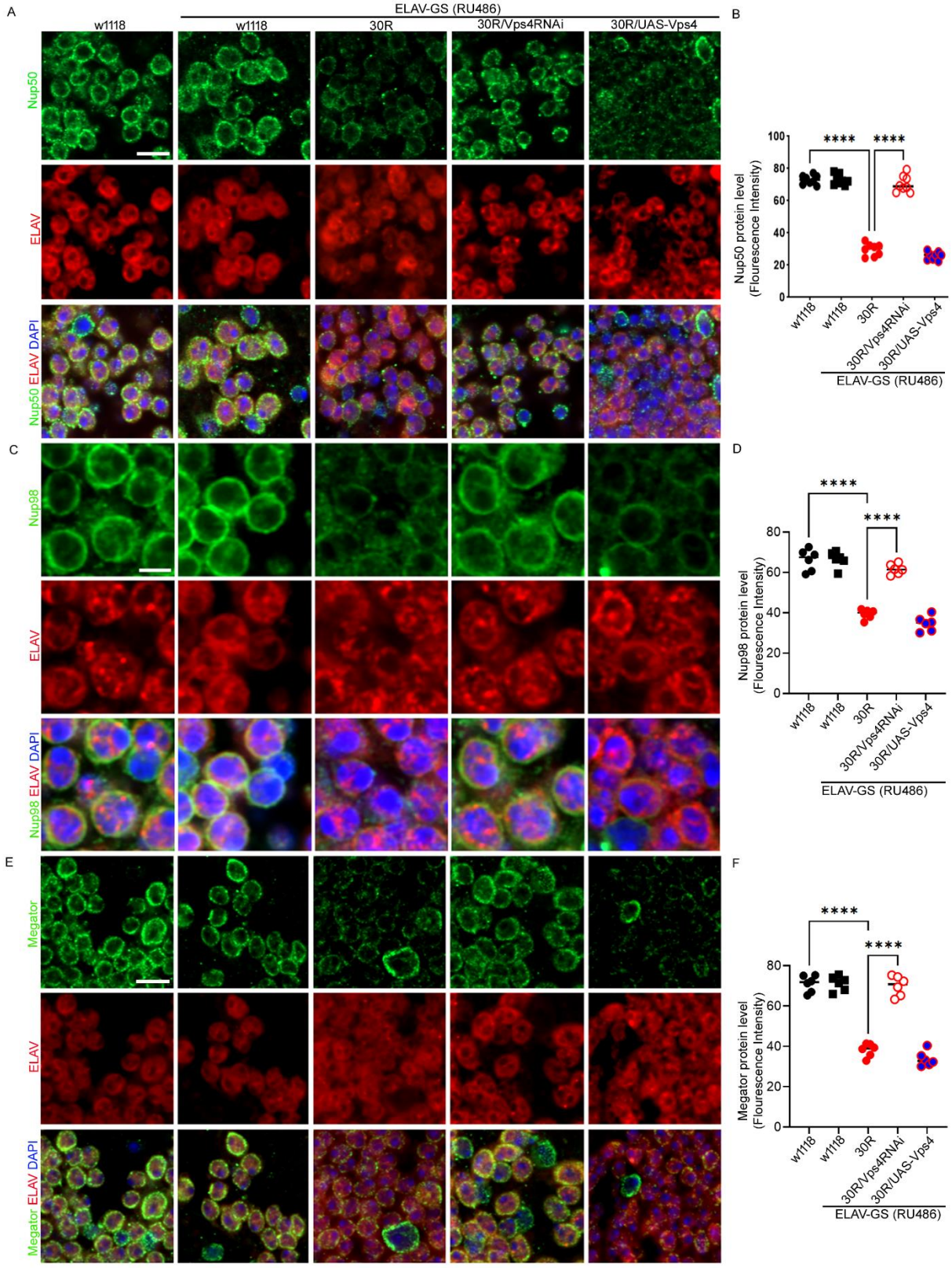
Rpt5 (red) and ELAV (magenta) staining in control and 30R expressing neurons (scale bar denotes 5 μm and n=15 brain per genotype). **(G)** External eye images in several genetic background driven with *GMR-GAL4* (*LucRNAi, CD8-GFP, Rpn10RNAi, and UAS-Rpn10*).

Figure S5



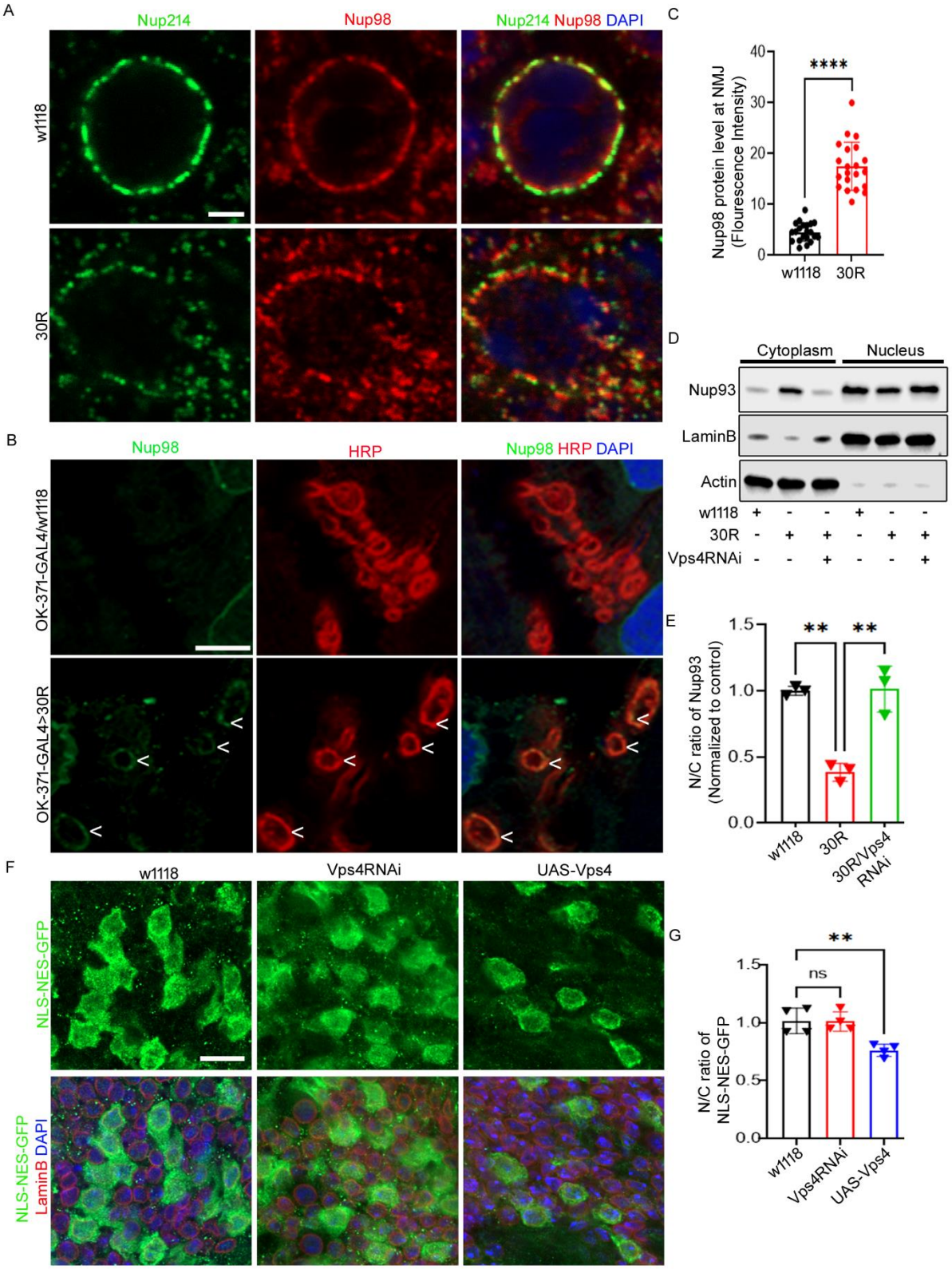
Supplementary Figure 5. Validation of RNAi and overexpression lines of Vps4 and ESCRT-III, Related to Figure 3 and 5. (A) The KD and overexpression efficiency of the Vps4 transgenic lines are analyzed by western blot and normalized to actin (Vps4RNAi lines#1-4 lines, respectively). **(B)** Quantification of Vps4 of immunoblot A. One-way ANOVA, followed by Dunnett's multiple comparisons test, (n = 3; *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001). **(C-F)** The KD efficiency of the CHMP1, CHMP2B, Vps20 and Shrub transgenic lines is analyzed by western blot and normalized to actin. One-way ANOVA, followed by Dunnett's multiple comparisons test, (n = 3; *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001). **(G-I)** The KD efficiency of the RNAi lines of Vps2, Vps24 and CG5498 is analyzed by quantitative polymerase chain reaction (qPCR) and normalized to actin. One-way ANOVA, followed by Dunnett's multiple comparisons test, (n = 3; *P < 0.05, **P < 0.01).

Figure S6


A
w1118
ELAV-GS (RU486)
w1118
30R
30R/Vps4RNAi
30R/UAS-Vps4
Nup50
ELAV
Nup50 ELAV DAPI
B
Nup50 protein level (Flourescence Intensity)
****
****
w1118
w1118
30R
30R/Vps4RNAi
30R/UAS-Vps4
ELAV-GS (RU486)
C
Nup98
ELAV
Nup98 ELAV DAPI
D
Nup98 protein level (Flourescence Intensity)
****
****
w1118
w1118
30R
30R/Vps4RNAi
30R/UAS-Vps4
ELAV-GS (RU486)
E
Megator
ELAV
Megator ELAV DAPI
F
Megator protein level (Flourescence Intensity)
****
****
w1118
w1118
30R
30R/Vps4RNAi
30R/UAS-Vps4
ELAV-GS (RU486)

Supplementary Figure 6. Depletion of Vps4 restores Nup50, Nup98 and Megator in G4C2 expressing adult brain, Related to Figure 3. (A, C and E) Immunofluorescence staining of adult brain showing Nup50, Nup98 and Megator (green) with neuronal marker ELAV (red) and DAPI (nucleus) in following genotypes (w1118, ELAV-GS/+ (RU486), ELAV-GS>30R (RU486), ELAV-GS>30R/Vps4RNAi (RU486) and ELAV-GS>30R/Vps4) (scale bar in Nup50 and Megator denotes 5 μm, while 2μm in Nup98 and n=15 brain per genotype). **(B, D and F)** Quantification of Nup50, Nup98 and Megator immunofluorescence of A, C and E. Data are reported as mean ± SEM. One-way ANOVA, followed by Sidak's multiple comparisons, (60>n cells per genotype; ****P < 0.0001). Error bars indicate ± SEM.

Figure S7


A
Nup214
Nup98
Nup214 Nup98 DAPI
w1118
30R
B
Nup98
HRP
Nup98 HRP DAPI
OK-371-GAL4/w1118
OK-371-GAL4>30R
C
Nup98 protein level at NMJ (Flourescence Intensity)
****
w1118
30R
D
Cytoplasm
Nucleus
Nup93
LaminB
Actin
w1118
30R
Vps4RNAi
E
N/C ratio of Nup93 (Normalized to control)
**
**
w1118
30R
30R/Vps4 RNAi
F
w1118
Vps4RNAi
UAS-Vps4
NLS-NES-GFP
NLS-NES-GFP LaminB DAPI
G
N/C ratio of NLS-NES-GFP
**
ns
w1118
Vps4RNAi
UAS-Vps4

Supplementary Figure 7. Localization of nucleoporins are disrupted in G4C2 expressing neurons and upregulation of Vps4 disrupts nucleocytoplasmic transport, Related to Figure 7. (A) Expansion microscopy (ExM) images showing Nup214 (green), Nup98 (red) and DAPI (blue) of adult brain in mentioned genotypes from 10 days old fly (scale bar: 10μm and n=15 brains per genotype). **(B)** Immunostaining showing Nup98 and HRP (NMJ) with DAPI in larva in *vGLUT-GAL4/w1118* and *vGLUT-GAL4>30R* genotypes (scale bar: 10μm and n=15 brain per genotype). **(C)** Quantification of Nup98 immunofluorescence in B, data are reported as mean ± SEM (20>n boutons per genotype; Unpaired t test, ****P < 0.0001). **(D)** Western blot for Nup93, LaminB and Beta-actin from cytoplasmic and nuclear fractionated samples from adult brain of 10-days old fly in above mentioned genotypes (all genotypes are raised on RU486 containing food, except w1118). Beta-actin and LaminB were used for cytoplasmic and nuclear fraction control. **(E)** Quantification of nucleocytoplasmic ratio of Nup93 of cytoplasmic and nuclear fraction in D. Beta-actin was used to normalize cytoplasmic fraction, whereas LaminB for nuclear fraction (n = 3; Tukey's multiple comparisons test, **P < 0.01). **(F)** Images showing localization of NLS-NES-GFP in motor neuron in *vGlut-GAL4:NLS-NES-GFP/+*, *vGlut-GAL4:NLS-NES-GFP/Vps4RNAi* and *vGlut-GAL4:NLS-NES-GFP/UAS-Vps4*. LaminB (red) staining showing nuclear membrane with DAPI (blue) (scale bar denotes 10 μm and n=10 brain per genotype). **(G)** Quantification of the nucleocytoplasmic ratio (N/C ratio) of NLS-NES-GFP of motor neuron in F. One-way ANOVA, followed by Tukey's multiple comparisons test, (40>n cells per genotype; **P < 0.01, n.s.= nonsignificant). Error bars indicate ± SEM.