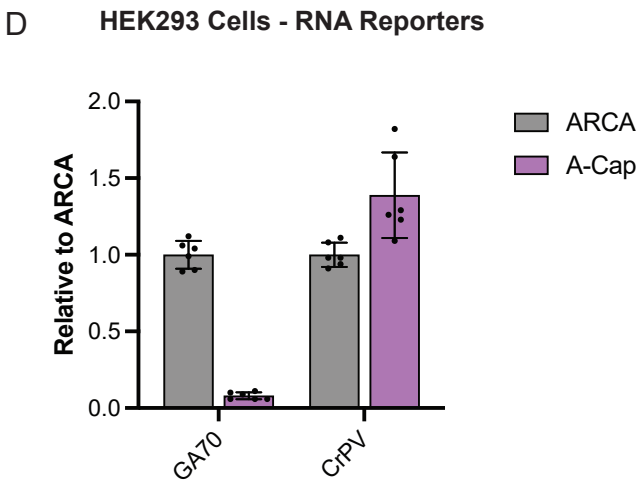
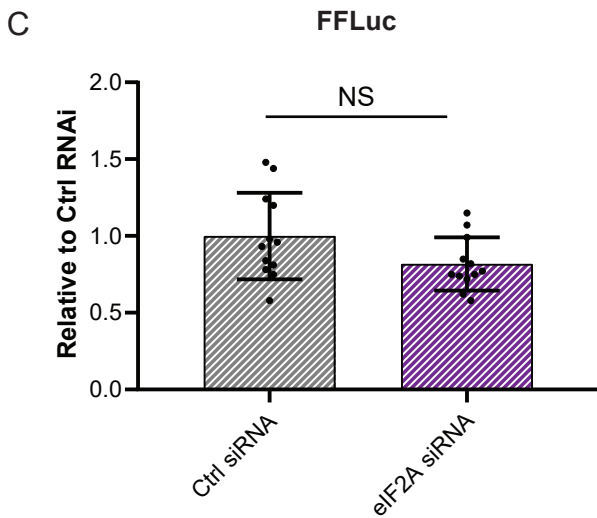
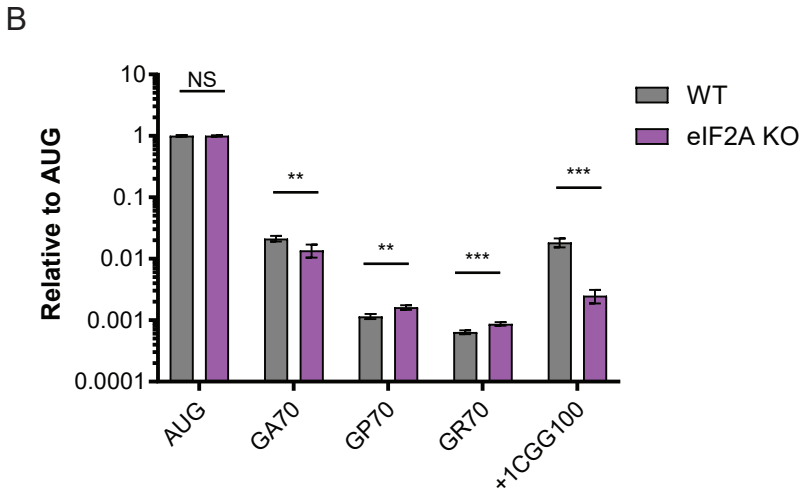
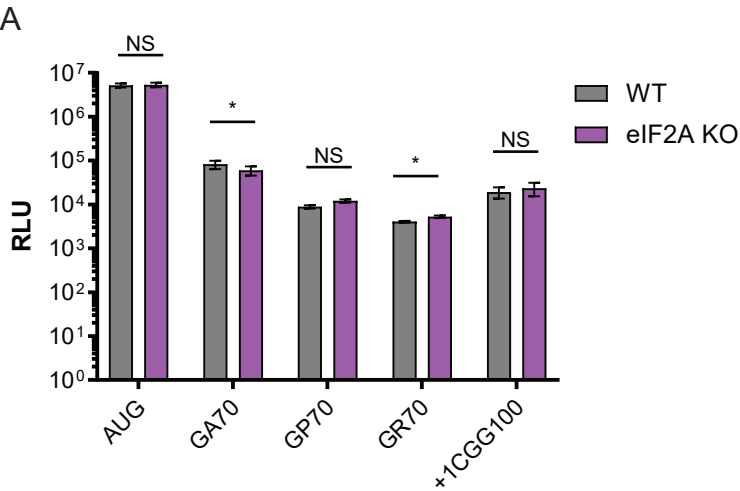


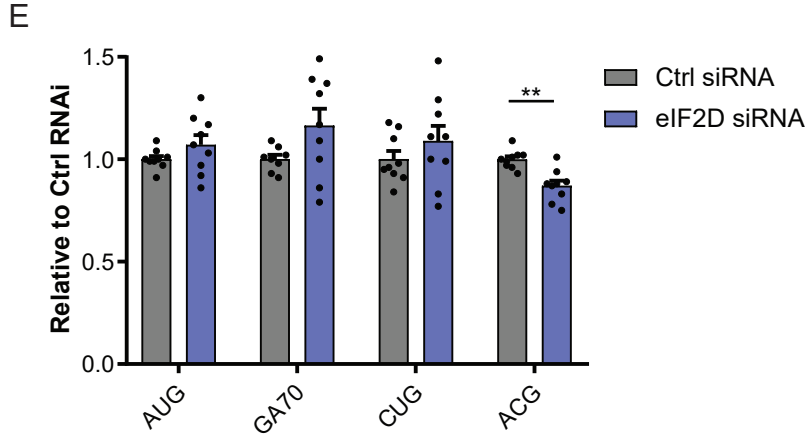
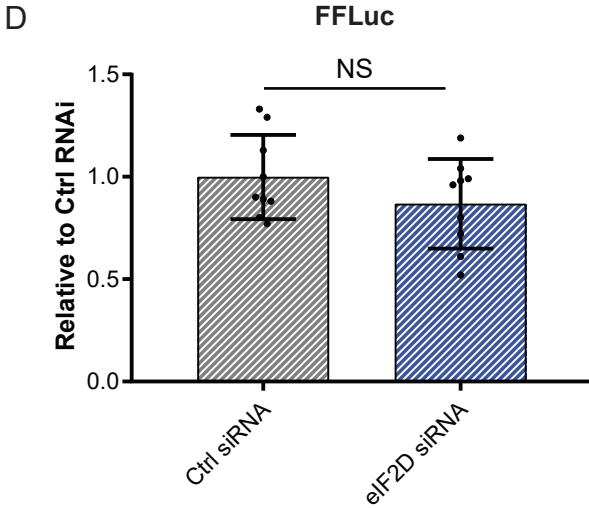
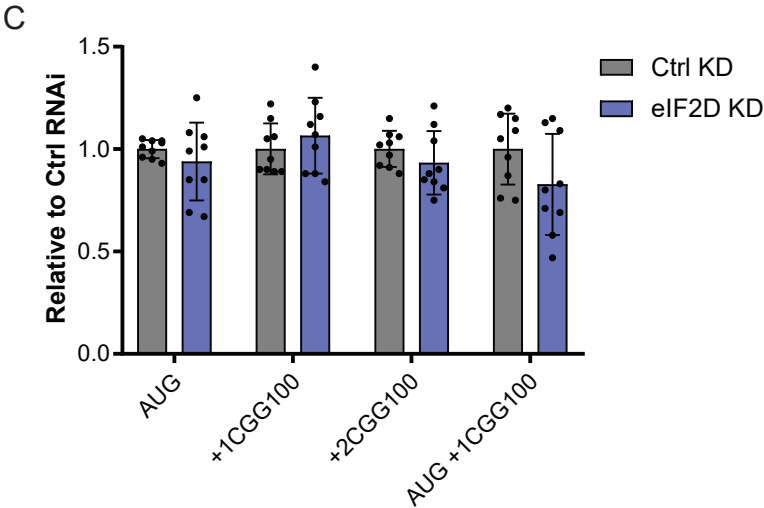
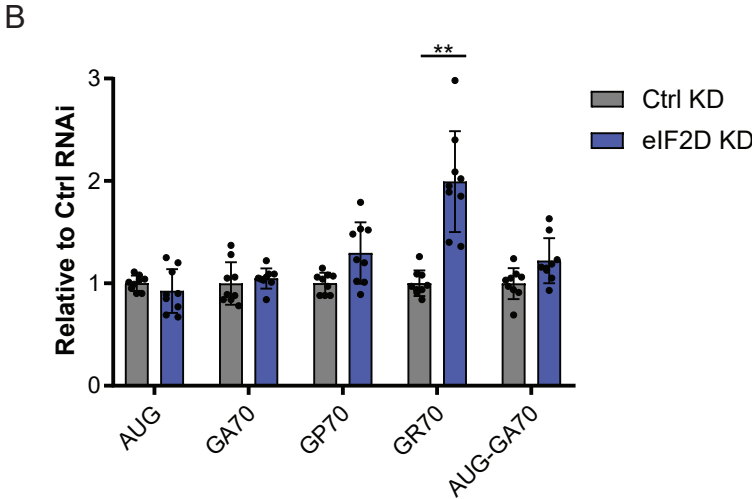
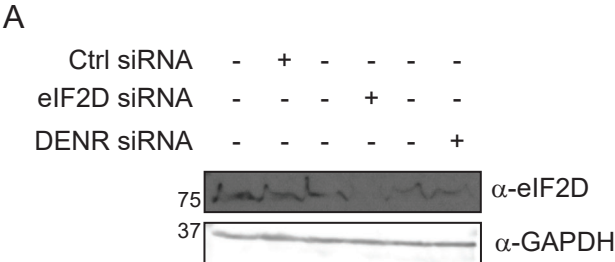
Supplemental Figure 1



**Figure S1: Effect of eIF2A depletion and 5' m7G cap on translation.**

(A) Raw RLU values for the indicated NLuc reporter mRNAs in WT and eIF2A KO lysates, n=12-42. Bars represent mean and error bars +/- SEM. Multiple paired Wilcoxon tests with Holm-Sidak correction for multiple comparisons. (B) RLU values for indicated reporter mRNAs normalized to AUG-NLuc controls in each trial, within WT and eIF2A KO lysates, n=12-42. (A-B) Graphs represent mean and error bars +/- SEM. \*p<0.05, \*\* p< 0.01, \*\*\* p< 0.001, multiple paired Wilcoxon tests with Holm-Sidak correction for multiple comparisons. (C) Expression of FFLuc reporter in wells co-transfected with AUG-NLuc, 24 h post transfection with non-targeting or eIF2A siRNAs. FFLuc levels are expressed relative to levels in cells transfected with the non-targeting siRNA, n=12. (D) NLuc expression of indicated A-capped reporter mRNAs expressed relative to corresponding ARCA reporter mRNAs, transfected into HEK293 cells 24 h post transfection with a non-targeting siRNA, n=6. (C-D) Graphs represent mean with error bars +/- standard deviation.

Supplemental Figure 2

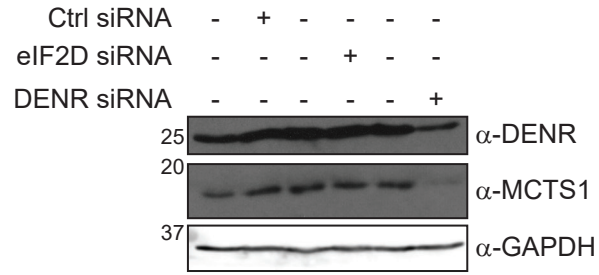


**Figure S2: Knockdown of eIF2D does not reduce RAN translation reporter expression**

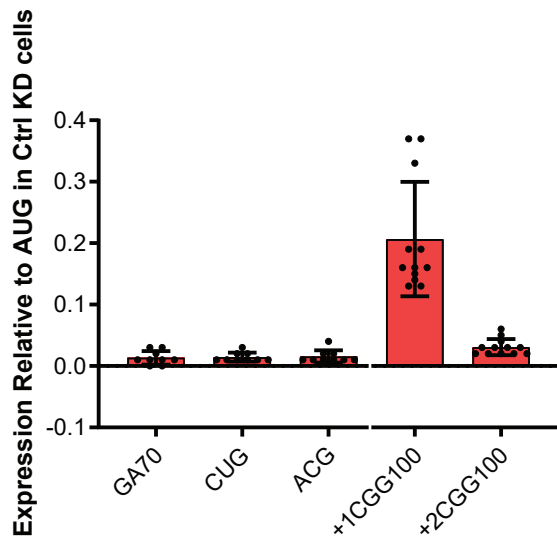
(A) Western blot showing efficiency of eIF2D KD in HEK293 cells 48 h post transfection with an eIF2D targeting siRNA. GAPDH was used as a loading control. (B-C) NLuc expression of indicated reporters expressed from DNA plasmids transfected into HEK293 cells 24 h post transfection with non-targeting or eIF2D siRNAs. NLuc levels are expressed relative to levels in cells transfected with the non-targeting siRNA, n=9. (D) Expression of FFLuc reporter in wells co-transfected with AUG-NLuc, 24 h post transfection with non-targeting or eIF2D siRNAs. FFLuc levels are expressed relative to levels in cells transfected with the non-targeting siRNA, n = 9. (E) NLuc expression of indicated reporters expressed from DNA plasmids transfected into HEK293 cells 24 h post transfection with non-targeting or eIF2D siRNAs. NLuc levels are expressed relative to levels in cells transfected with the non-targeting siRNA, n=9. All graphs represent mean with error bars +/- standard deviation, \*\* p< 0.01 (B-C, E) Two-way ANOVA with Sidak's multiple comparison test, (D) two-tailed unpaired t-test.

Supplemental Figure 3

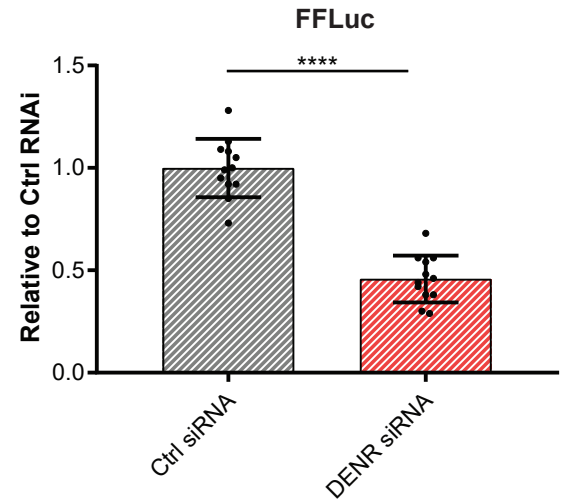
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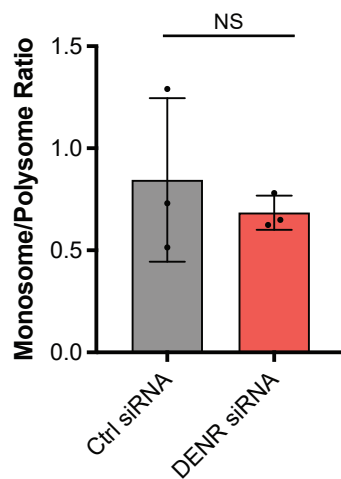
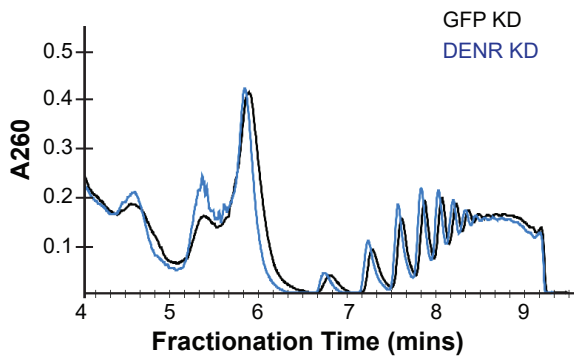
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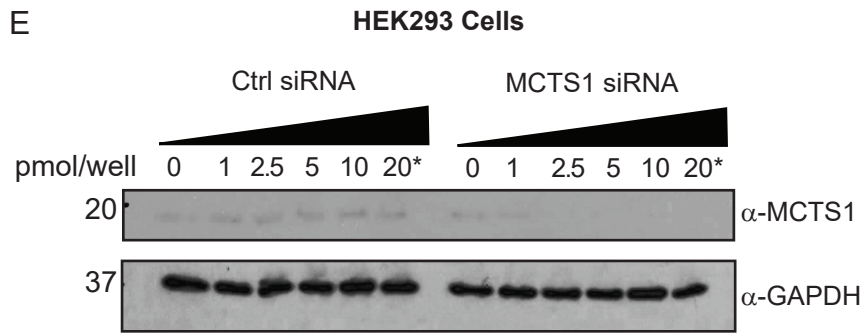
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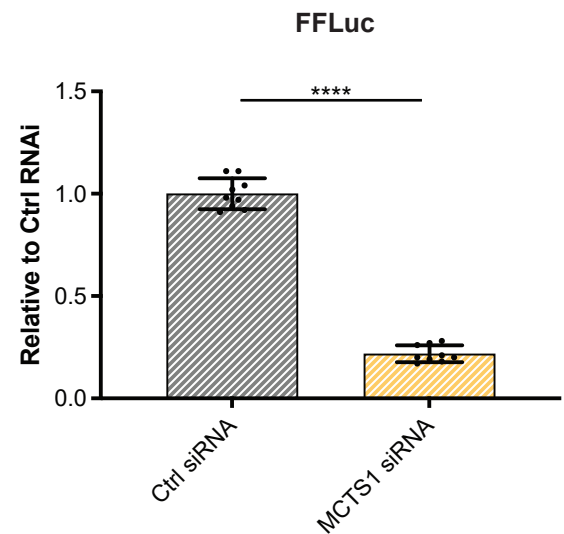
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E



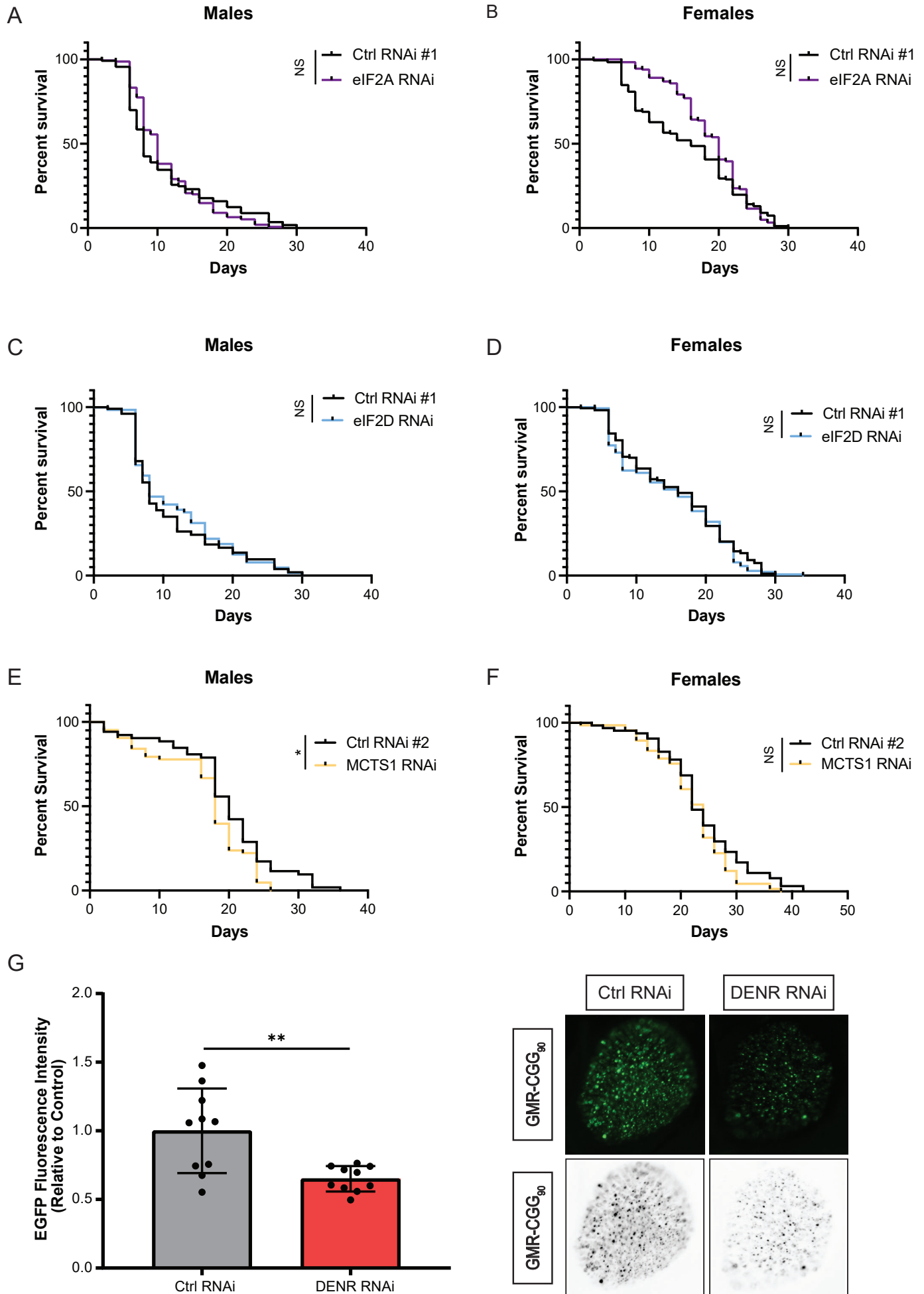
F



### **Figure S3: Effects of DENR and MCTS1 knockdown on canonical translation**

(A) Western blot showing efficiency of both DENR and MCTS1 KD in HEK293 cells 48 h post transfection with an DENR targeting siRNA. GAPDH was used as a loading control. (B) NLuc expression of non-AUG initiation reporters, relative to the AUG-NLuc control, in cells transfected with a non-targeting siRNA in DENR KD experiments, n=9-12. (B) Expression of FFLuc reporter in wells co-transfected with AUG-NLuc, 24 h post transfection with non-targeting or DENR siRNAs. FFLuc levels are expressed relative to levels in cells transfected with the non-targeting siRNA, n=12. (C) Representative polysome profiles and monosome/polysome ratios of HEK293 cells 24 h post transfection with non-targeting or DENR siRNAs, n=4. (D) Western blot showing efficiency of MCTS1 KD in HEK293 cells following increasing concentrations of MCTS1 siRNA. The starred lane indicates siRNA concentration used in subsequent experiments. GAPDH was used as a loading control. (E) Expression of FFLuc reporter in wells co-transfected with AUG-NLuc, 24 h post transfection with non-targeting or MCTS1 siRNAs. FFLuc levels are expressed relative to levels in cells transfected with the non-targeting siRNA, n=9. All graphs represent mean with error bars +/- standard deviation, \*\*\*\* p< 0.0001, two-tailed unpaired t-test.

Supplemental Figure 4



**Figure S4: Effects of eIF2A, eIF2D and MCTS1 knockdown on GGGGCC repeat-suppressed *Drosophila* survival**

(A-F) Survival curves of RU486-treated GGGGCCx28 male and female Tub5-GS-Gal4 flies, expressing ctrl or eIF2A, eIF2D, or MCTS1 targeting shRNAs. (A) Ctrl n = 113, eIF2A n = 155. (B) Ctrl n = 177, eIF2A n = 182. (C) Ctrl n = 103, eIF2D n = 63. (D) Ctrl n = 173, eIF2D n = 141. (E) Ctrl n = 52, MCTS1 n = 63. (F) Ctrl n = 64, MCTS1 n = 66. \*p < 0.05 by mantel-cox test. (G) Bar graph and representative images of EGFP fluorescence intensity within GMR-Gal4 fly eyes expressing 90 CGG repeats with an EGFP tag on the poly(G) RAN translation product, in the presence of either non-targeting or DENR targeting shRNAs, n=10. Graph represent mean with error bars +/- standard deviation, \*\* p < 0.01, Welch's two-tailed unpaired t-test.