

Figure S1. Validation of siRNAs used in targeted screening. (A-J) 1nM siRNA transfected in HEK293 of each non-targeting (NTC) control and knockdown of ZNF598, RACK1, PELO, HBS1L, ABCE1, XRN1, NEMF, LTN1, VCP, and ANKZF1

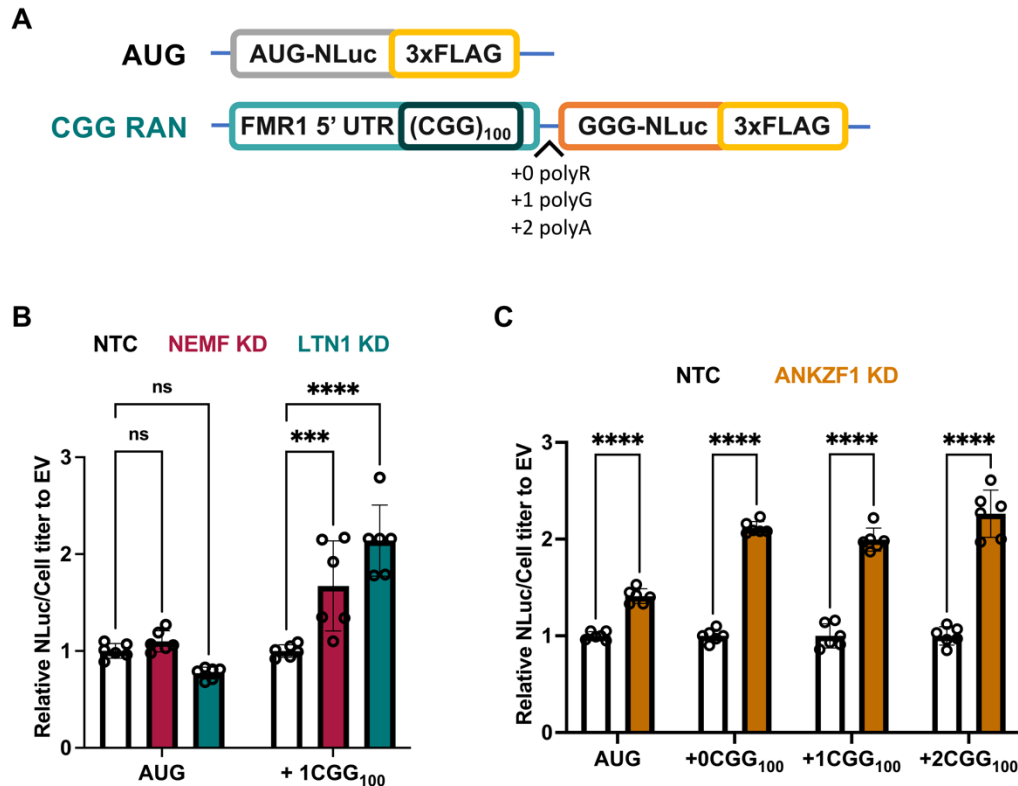


Figure S2.

Depletion of NEMF, LTN1, and ANKZF1 enhances CGG RAN translation. (A) Schematic AUG-driven and CGG RAN at different reading frame reporters. (B-C) Luciferase assays of RAN translation after NEMF, LTN1, or ANKZF1 depletion. All graphs show mean with error bars \pm SD. Each N is shown as an open circle ($n=6$ /group). Asterisks above each bar are comparisons of expression between NTC and gene(s) knockdown. ns = not significant; *** $P \leq 0.001$; **** $P \leq 0.0001$, as determined with one-way ANOVA with Sidak's multiple comparison test.

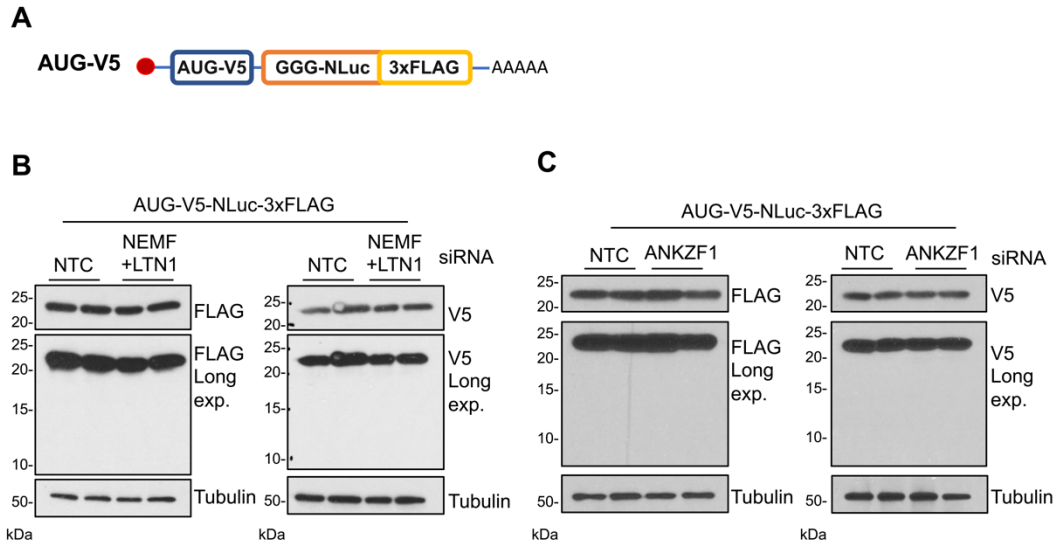


Figure S3. No detection of stall products from constructs lacking GC repeats. **(A)** Schematic of AUG-V5-NLuc-3xFLAG. **(B-C)** Knockdown of NEMF + LTN1, and ANKZF1 in HEK293 with AUG-V5-NLuc-3xFLAG RNA transfection.

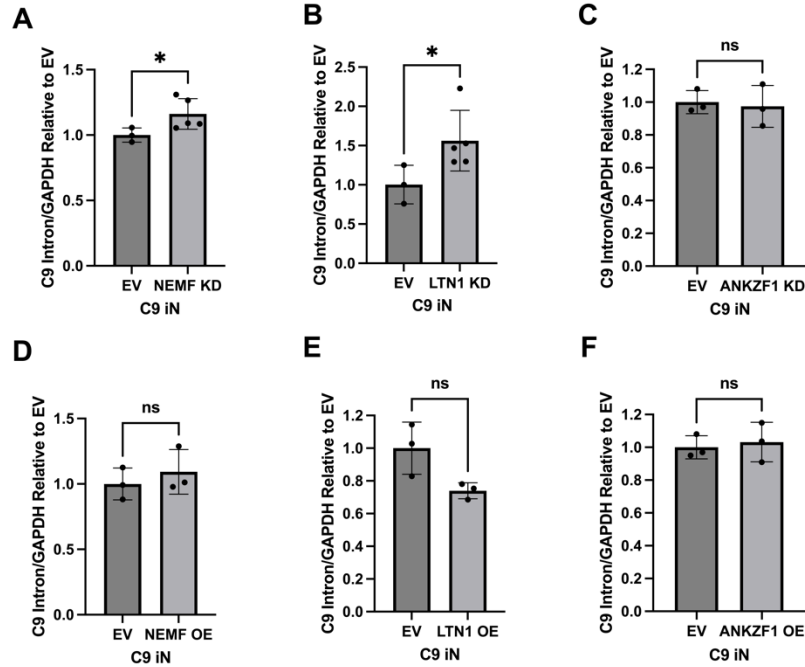


Figure S4. Effect of NEMF, LTN1, and ANKZF1 knockdown and overexpression on C9 transcripts. (A-F) Quantification of RNA abundance targeting the C9 intronic region normalized to GAPDH. C9 iN was treated with lentiviruses of empty vector, NEMF, LTN1, or ANKZF1 knockdown or overexpression. Leftover lysates from GP MSD were collected for RNA extraction and qRT-PCR analysis. Data represent means with error bars \pm SD of $n = 3-5$, ns = not significant; $*P \leq 0.05$ by Student's t-test.

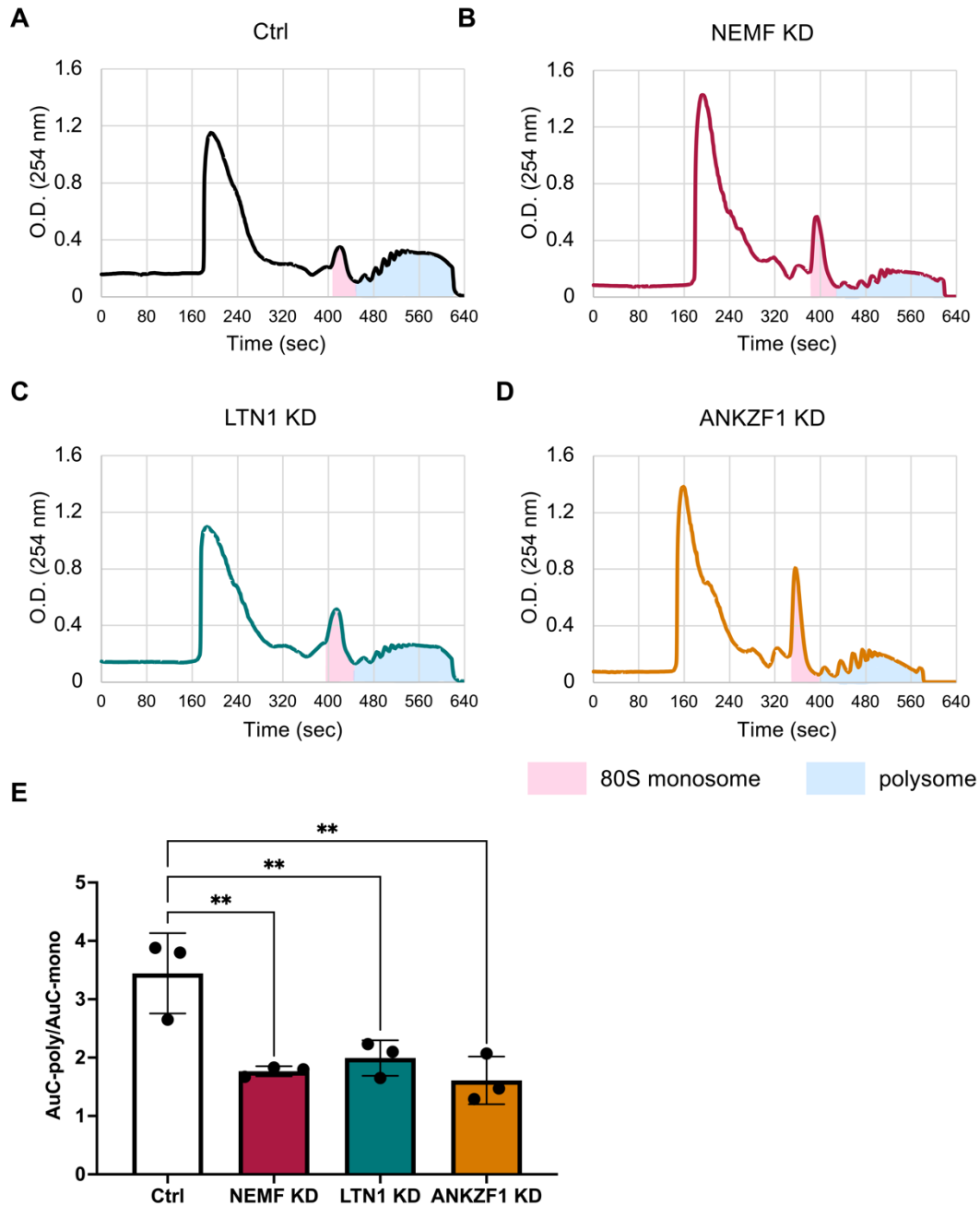


Figure S5. Knockdown of NEMF, LTN1, and ANKZF1 inhibit global translation. **(A-D)** Representative polysome-fractionation profiles of HEK293 lysates transduced with lentivirus of Ctrl KD, NEMF KD, LTN1 KD, or ANKZF1 KD. **(E)** The areas-under-the-curve (AuC) for monosomes and polysomes are shaded pink and blue, respectively. Global translation activity is calculated by normalizing AuC-polysome/AuC-mono. Data represent means with error bars \pm SD of $n = 3$, $**P \leq 0.001$ as determined with one-way ANOVA with Sidak's multiple comparison test.