Figure S1

5’…..agtactcg**ctg**agggtgaacaagaaaagac**ctg**a**taa**agat**taa**ccagaagaaaacaaggagggaaacaacc
gcagc**ctgtag**caagct**ctg**gaactcaggagtcgcgcgc**tag**gggccggggcc…...

Fig. S1. The sequence of 113 nucleotides upstream from the G_4C_2 repeats. The first two G_4C_2 repeats are shown and underlined. There are four CUGs upstream of the repeats. The CUG at -24 (red) with respect to the beginning of the repeat has a good Kozak consensus sequence, and is in the GA reading frame. The other three CUGs (-105 (green, in the GA reading frame), -83 (blue, in the GP reading frame), and -36 (pink, in the GA reading frame) that meet stop codons prior to the repeat: at -72 (in the GA reading frame), -2 (in the GP reading frame), and -33 respectively (in the GA reading frame). There are additional stop codons at -33 in the GA reading frame and -79 (brown) in the GR reading frame.

Figure S2

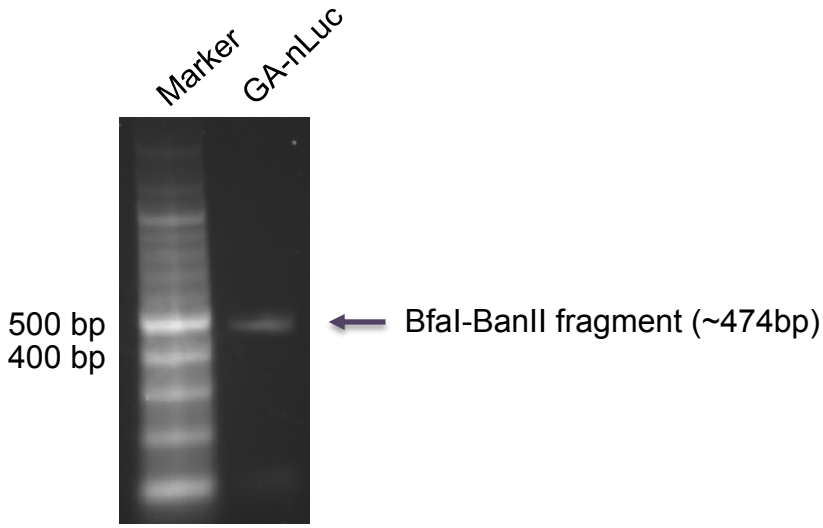

Fig. S2. Gel electrophoresis of 75 G_4C_2 repeats. A construct containing 75 repeats was digested with HindIII and NotI, and then further digested with BfaI and BanII. As predicted, a ~474 base pair size fragment corresponding to the repeats was detected.

Figure S3

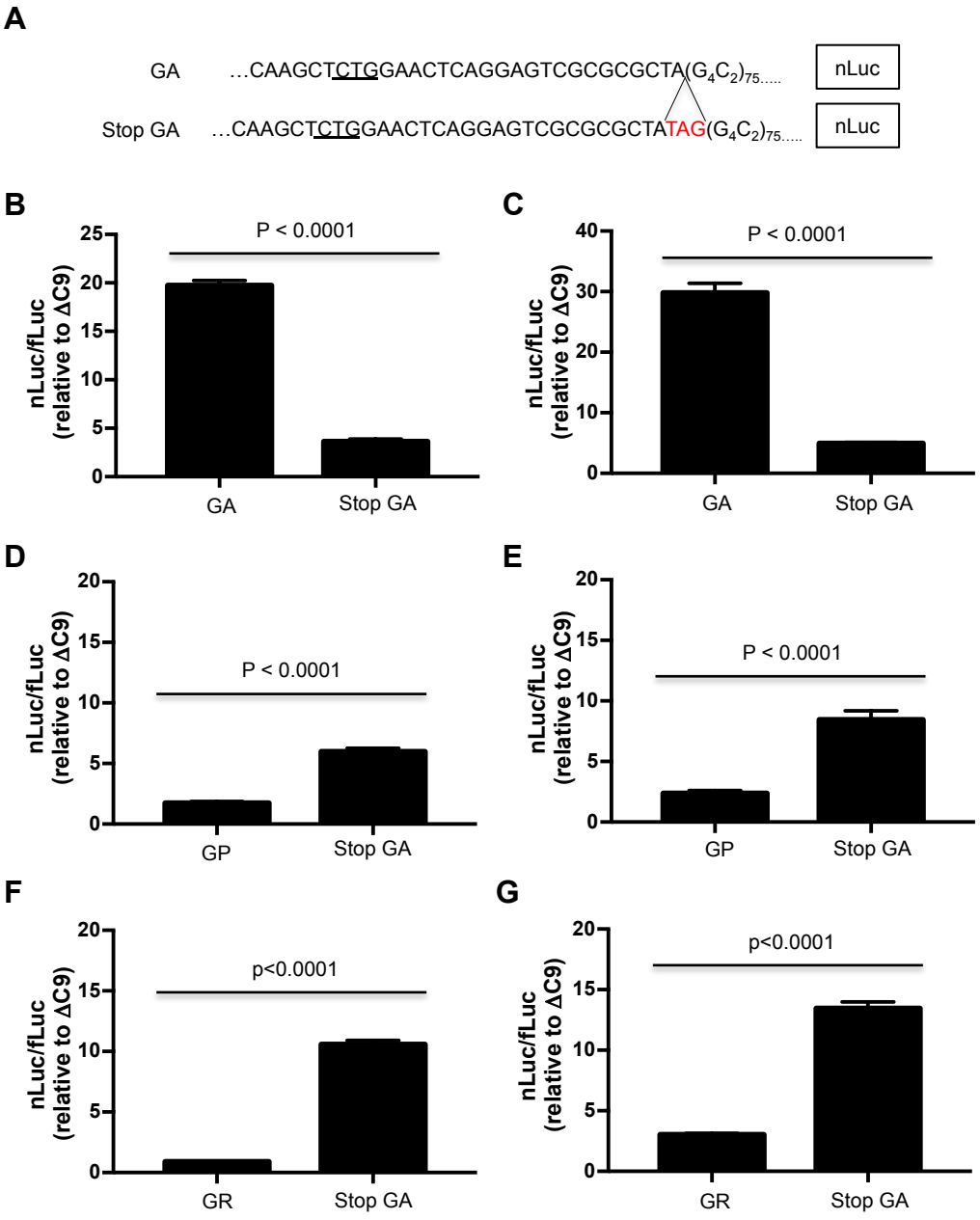

Fig. S3. Translation of GA, GP, and GR with -1 TAG insertional mutation. (A) Sequence upstream from the G_4C_2 repeats showing insertion of TAG (red) in GA reading frame. The CUG putative translation start codon is underlined. NSC34 (B, D, F) and HEK293 (C, E, G) cells were cotransfected with fLuc plasmid along with either C9 or a plasmid with -1 TAG mutation of C9 followed by nLuc in the reading frame of GA (B, C), GP (D, E), or GR (F, G). Cells were harvested after 48h for dual luciferase assays.

Figure S4

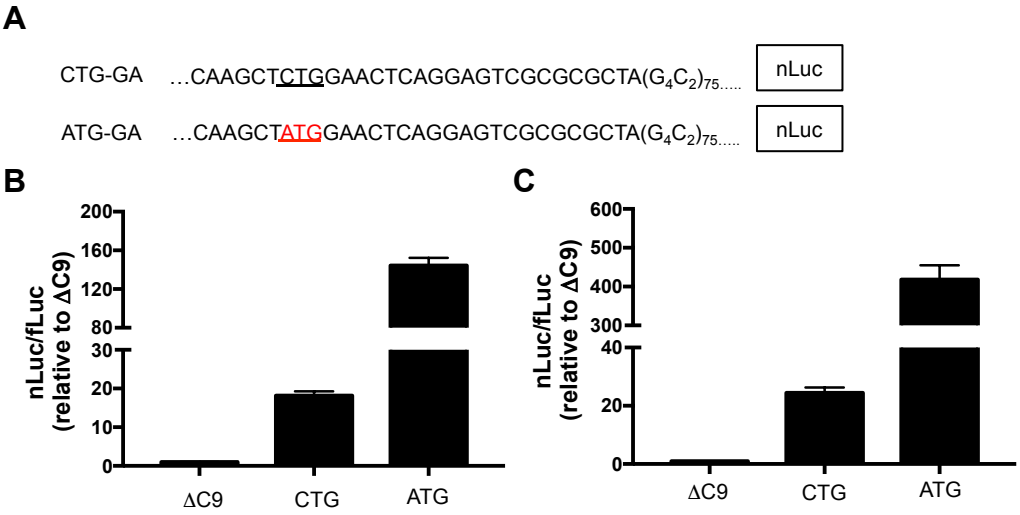

Fig. S4. Translation of GA with mutation of -24 CTG to ATG. (A) Nucleotide sequence upstream from the G_4C_2 repeats showing the CUG putative translation initiation codon (underlined) and with mutation of CTG to ATG (in red). NSC34 (B) and HEK293 (C) cells were cotransfected with fLuc along with either ΔC9, CTG-GA-nLuc, or ATG-GA-nLuc. Cells were harvested for dual luciferase assays.

Figure S5

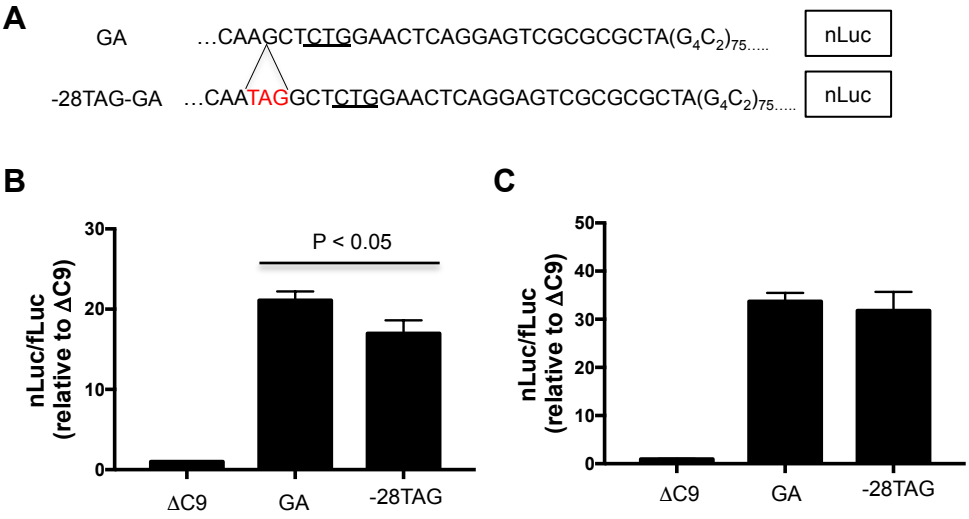

Fig. S5. Translation of GA with -28 TAG insertional mutation. (A) Sequence upstream from the G_4C_2 repeats showing insertion of TAG (red) in the GA reading frame upstream of the CUG putative initiation codon (underlined) in GA-nLuc. NSC34 (B) and HEK293 (C) cells were cotransfected with fLuc plasmid along with either C9 or a plasmid with a -28 insertional mutation of TAG in the reading frame of GA in the GA-nLuc plasmid. Cells were harvested after 48h for dual luciferase assays.

Figure S6

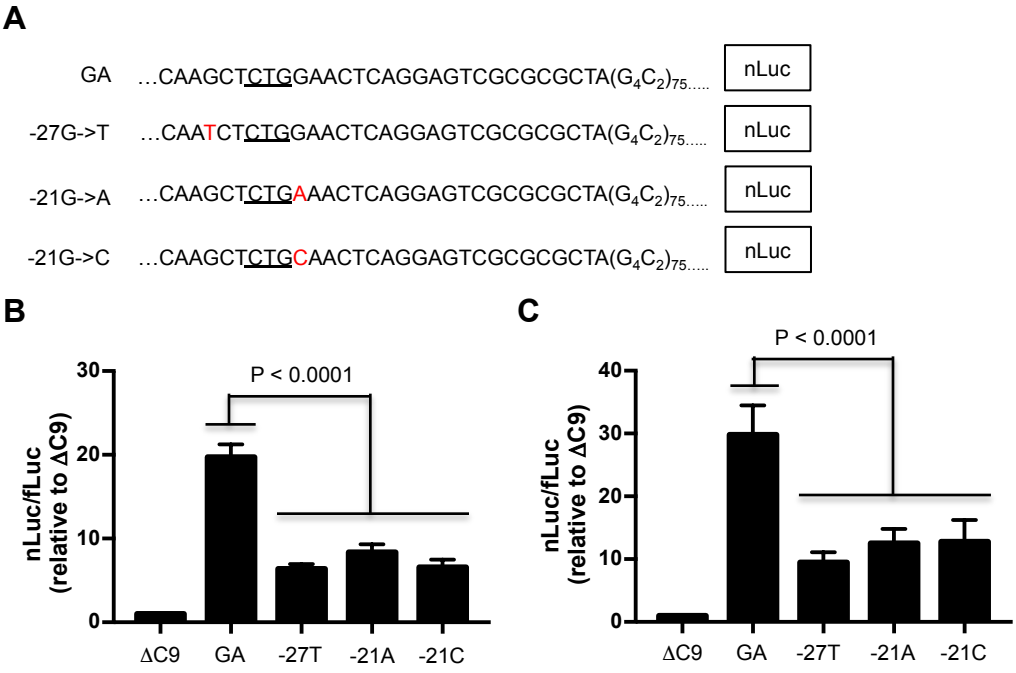

Fig. S6. Mutation in CUG KOZAK consensus significantly reduces translation of GA. (A) Nucleotide sequence upstream from the G_4C_2 repeats showing the CUG putative translation initiation codon (underlined) and mutations (in red) at -27 and -21. NSC34 (B) and HEK293 (C) cells were cotransfected with fLuc along with either ΔC9, GA-nLuc or GA-nLuc that had -27T, -21A or -21C mutations. Cells were harvested for dual luciferase assays.

Figure S7

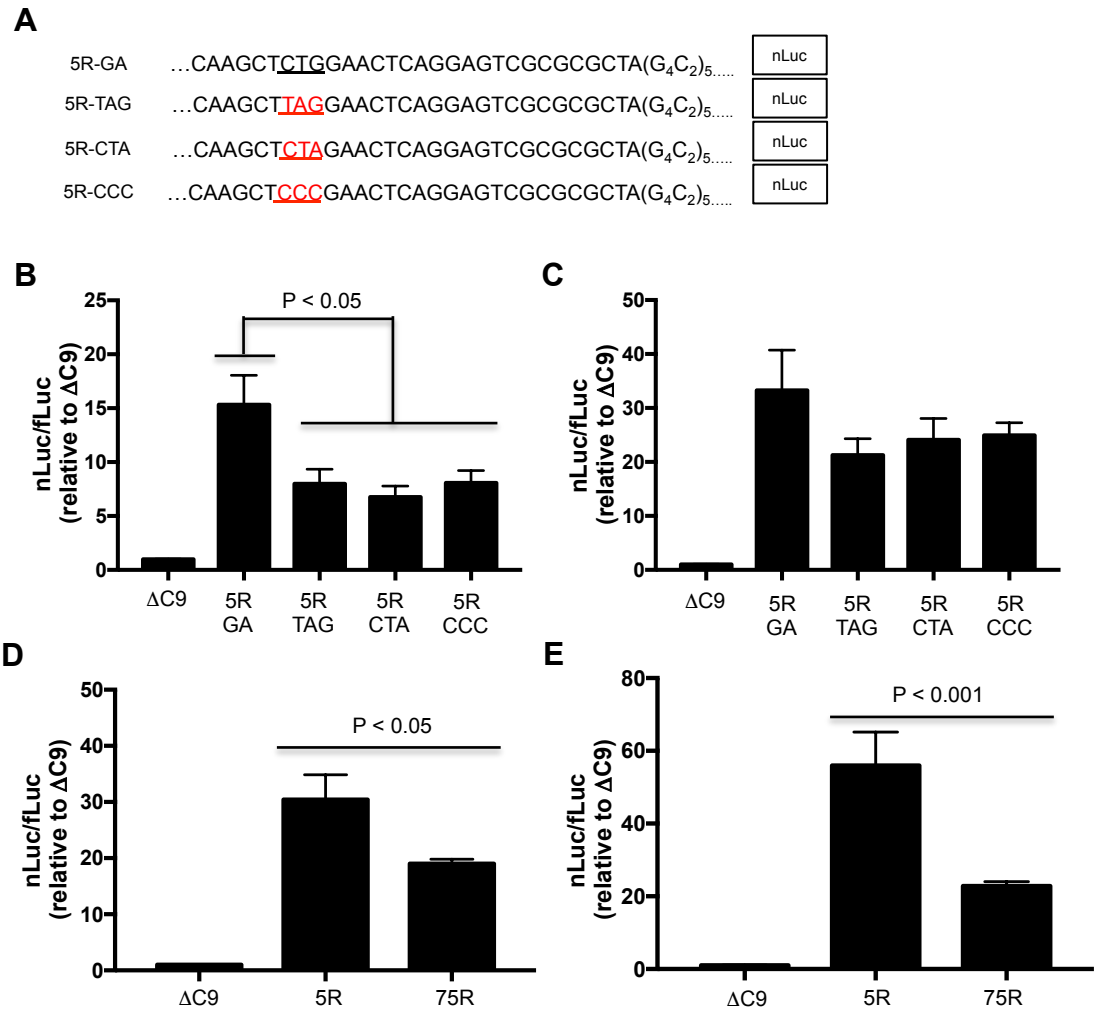

Fig. S7. Poly-GA translation initiation at a CUG -24 nucleotides upstream of 5 G_4C_2 repeats. (A) Nucleotide sequence upstream from 5 G_4C_2 repeats shows the CUG putative translation initiation codon (underlined) and mutation of CTG to TAG, CTA, or CCC (in red). NSC34 (B) and HEK293 (C) cells were cotransfected with fLuc along with either ΔC9, 5R-GA or 5R-GA that had TAG, CTA, or CCC mutations of the -24 CTG. Cells were harvested for dual luciferase assays. (D, E) NSC34 (D) and HEK293 (E) cells were cotransfected with fLuc along with either ΔC9, 5R-GA or 75R-GA. Cells were harvested for dual luciferase assays.

Figure S8

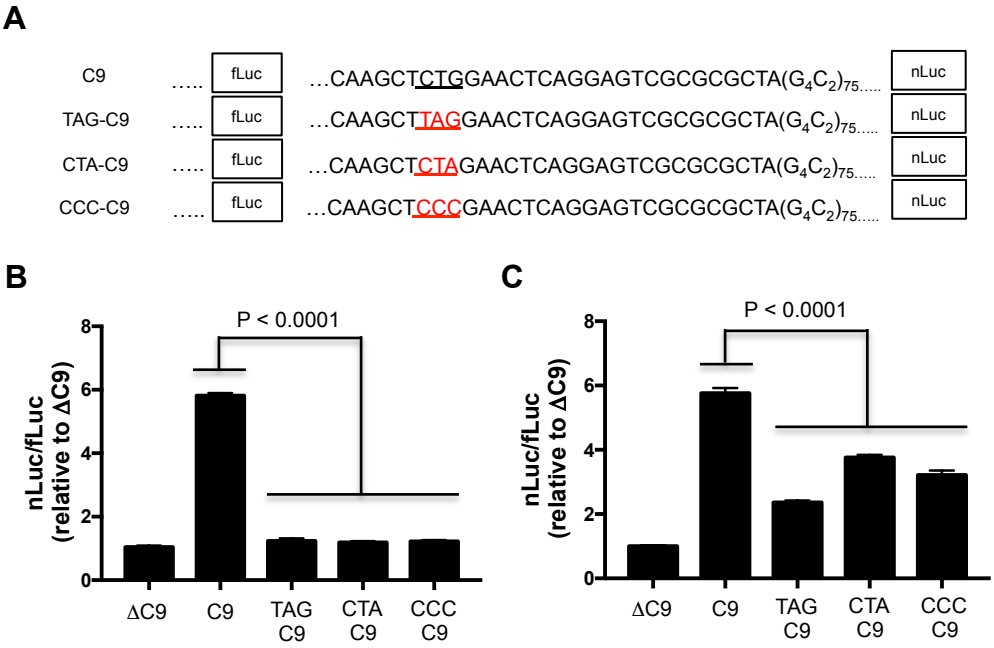

Fig. S8. Translation of GA with mutation of the -24 CTG to TAG, CTA, or CCC in the second cistron of C9 bicistronic construct. (A) Nucleotide sequence upstream from the G_4C_2 repeats shows the CUG putative translation initiation codon (underlined) and mutation of CTG to TAG, CTA, or CCC (in red). NSC34 (B) and HEK293 (C) cells were transfected with ΔC9, C9, TAG-C9, CTA-C9, or CCC-C9 bicistronic plasmid. Cells were harvested for dual luciferase assays.

Figure S9

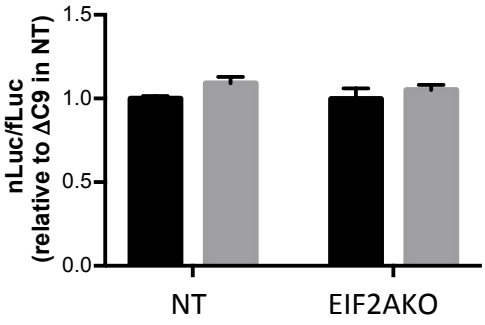

Fig. S9. Knockout of *EIF2A* does not affect levels of transfected bicistronic construct mRNA. HEK293 NT and *EIF2A*-KO cells were transfected with ΔC9 (black) or C9 (gray) bicistronic DNA constructs, and harvested 48hs later. The levels of bicistronic construct mRNA in the cell lysates were assessed by RT-PCR.

Figure S10

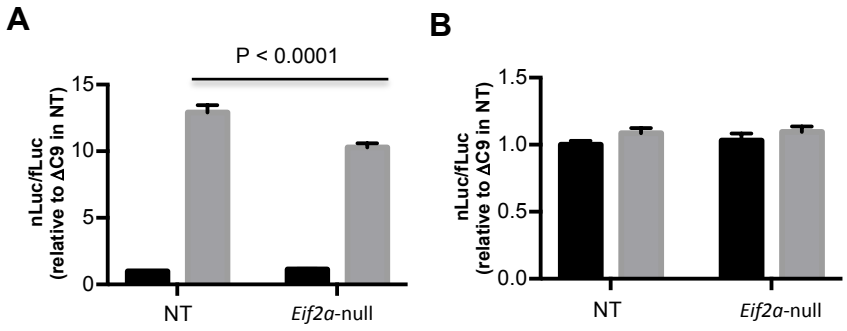

Fig. S10. Translation of GA in mouse keratinocytes utilizes eIF2A. (A) Non-targeted and *Eif2a*-null cells were transfected with either ΔC9 or C9 bicistronic plasmids. The cells were harvested after 48h for dual luciferase assays. (B) RT-PCR shows that non-targeted and *Eif2a*-null cells transfected with ΔC9 or C9 bicistronic plasmids had comparable amounts of RNA by RT-PCR.

Figure S11

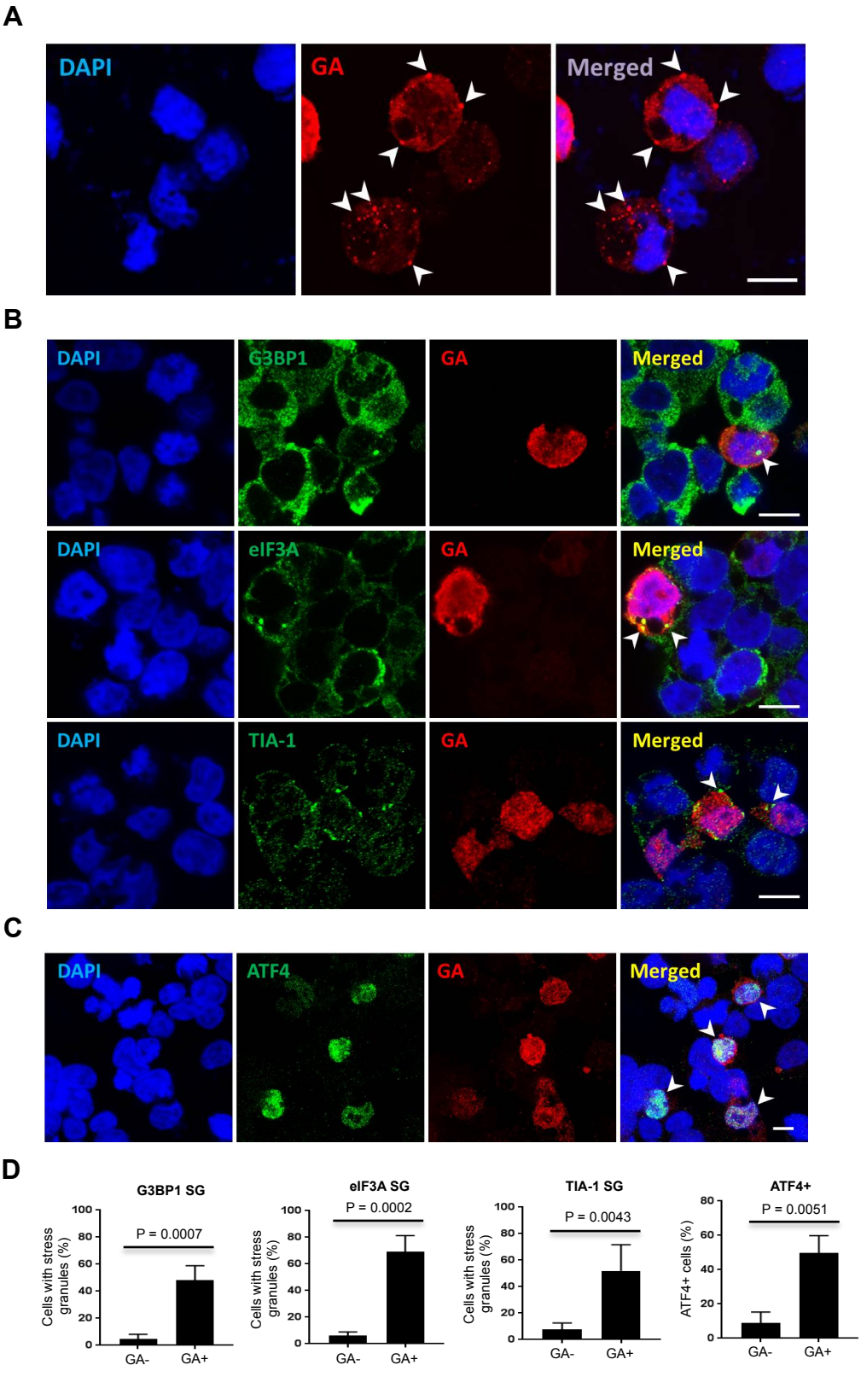

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Fig. S11. Upregulation of ATF4 and formation of stress granule in poly-GA expressing cells
(A) Immunofluorescent staining for poly-GA (red) in HEK293 cells transfected with GA-nluc plasmid. Cells transfected with GA-nluc plasmid had poly-GA protein aggregates in their cytoplasm (arrowheads). (B) Double immunostaining for stress granule markers (green) and poly-GA (red) in HEK293 cells transfected with GA-nluc plasmid. Stress granules containing G3BP1, eIF3A and TIA-1 were formed in poly-GA expressing cells (arrowheads). (C) Double immunostaining for ATF4 (green) and poly-GA (red) in HEK293 cells transfected with GA-nluc plasmid. Immunoreactivity for ATF4 was upregulated in the nuclei of poly-GA expressing cells (arrowheads). (**D**) The number of GA- vs. GA+ cells with stress granules (SG) containing G3BP1, eIF3A, and TIA-1 or with fluorescent staining of ATF4 were compared in 50 cells in 6 random fields. Scale bars, 10 μm.

Figure S12

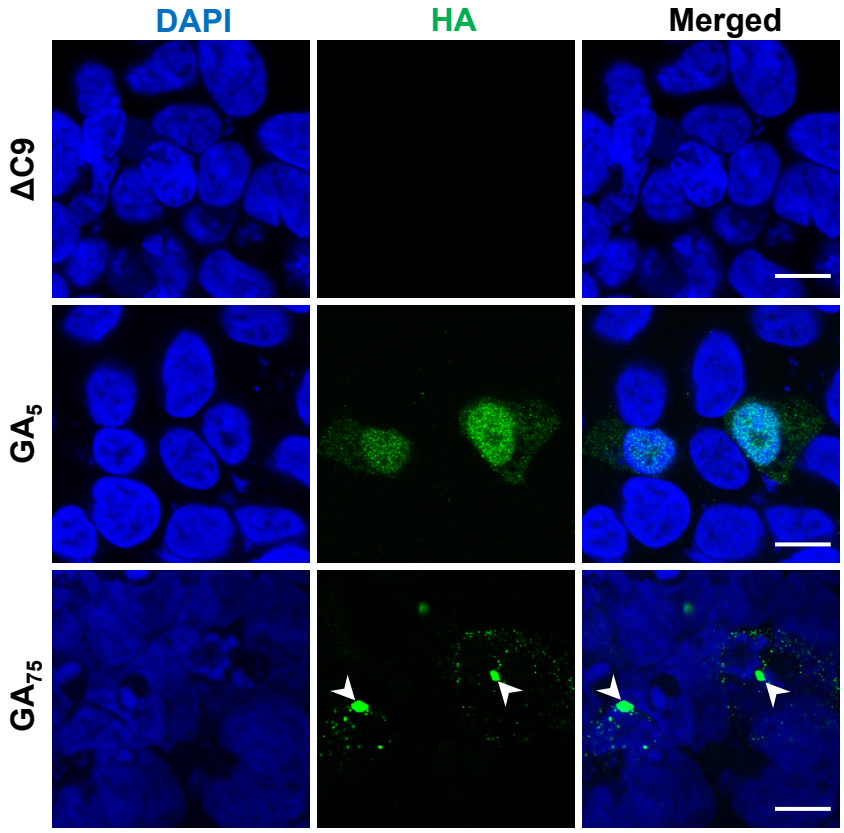

Fig. S12 Formation of cytoplasmic aggregates in cells transfected with GA_{75}-HA plasmids. Immunofluorescent staining for HA (green) in HEK293 cells transfected with ΔC9-HA, GA_5-HA, or GA_{75}-HA plasmids. HEK293 cells transfected with ΔC9-HA plasmids had no evidence of HA-tagged protein (upper panel). GA_5-HA had diffusely distributed HA throughout the cytoplasm (middle panel). GA_{75}-HA formed cytoplasmic aggregates (*arrowheads,* lower panel). Scale bar 10 μm.

Figure S13

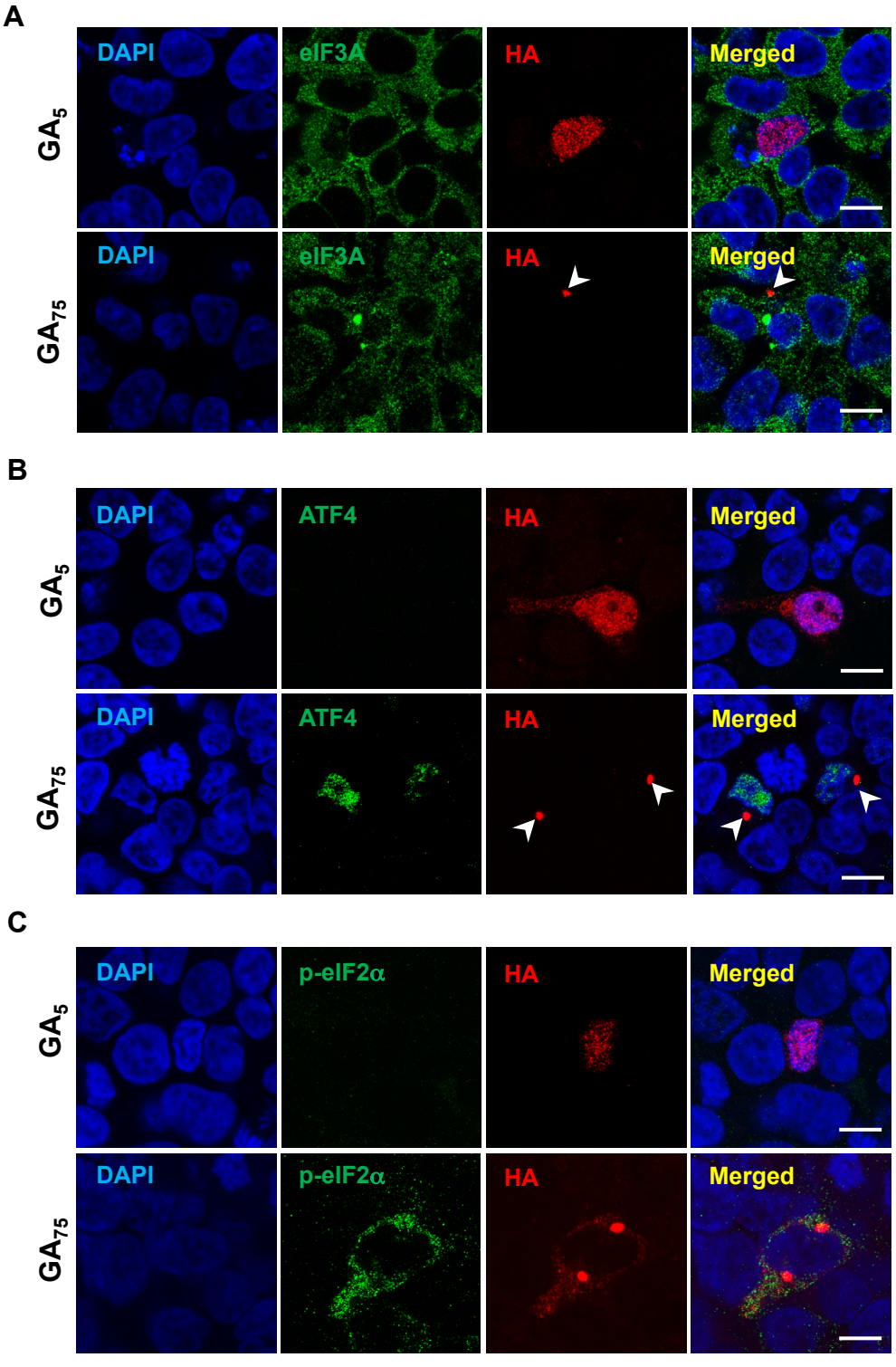

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Fig. S13 Induction of ER stress and formation of stress granules in cells transfected with GA_{75}, but not GA_5-HA plasmids. (**A**) Double immunostaining for eIF3A (green) and HA (red) in HEK293 cells transfected with GA_5-HA or GA_{75}-HA plasmids. Stress granules containing eIF3A was formed in the cytoplasm of GA_{75}-HA-expressing cells (*arrowhead*), but not in GA_5-HA. (**B**) Double immunostaining for ATF4 (green) and HA (red) in HEK293 cells transfected with GA_5-HA or GA_{75}-HA plasmids. ATF4 immunoreactivity was upregulated in nuclei of GA_{75}-HA-expessing cells (*arrowheads*). (**C**) Double immunostaining for p-eIF2α (green) and HA (red) in HEK293 cells transfected with GA_5-HA or GA_{75}-HA plasmids. Expression of phosphorylated eIF2α was increased in the cytoplasm of GA_{75}-HA expressing cells (*arrowheads*). (**D**) The number of GA_5-HA- vs. GA_{75}-HA-expressing with stress granules (SG) containing eIF3A or with fluorescent staining of ATF4 and p-eIF2α were compared in 50 cells in 6 random fields.Scale bars, 10 μm.