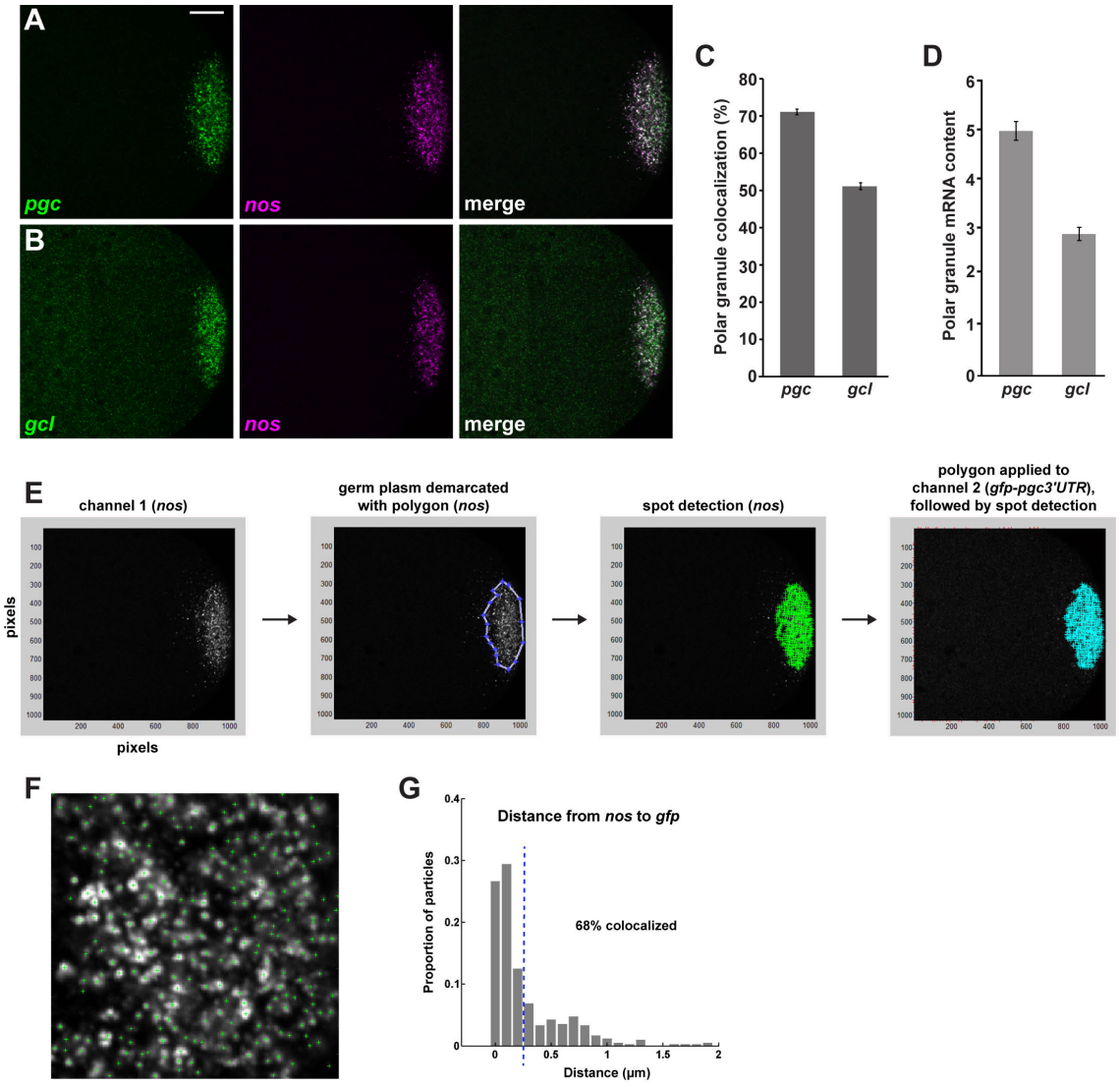
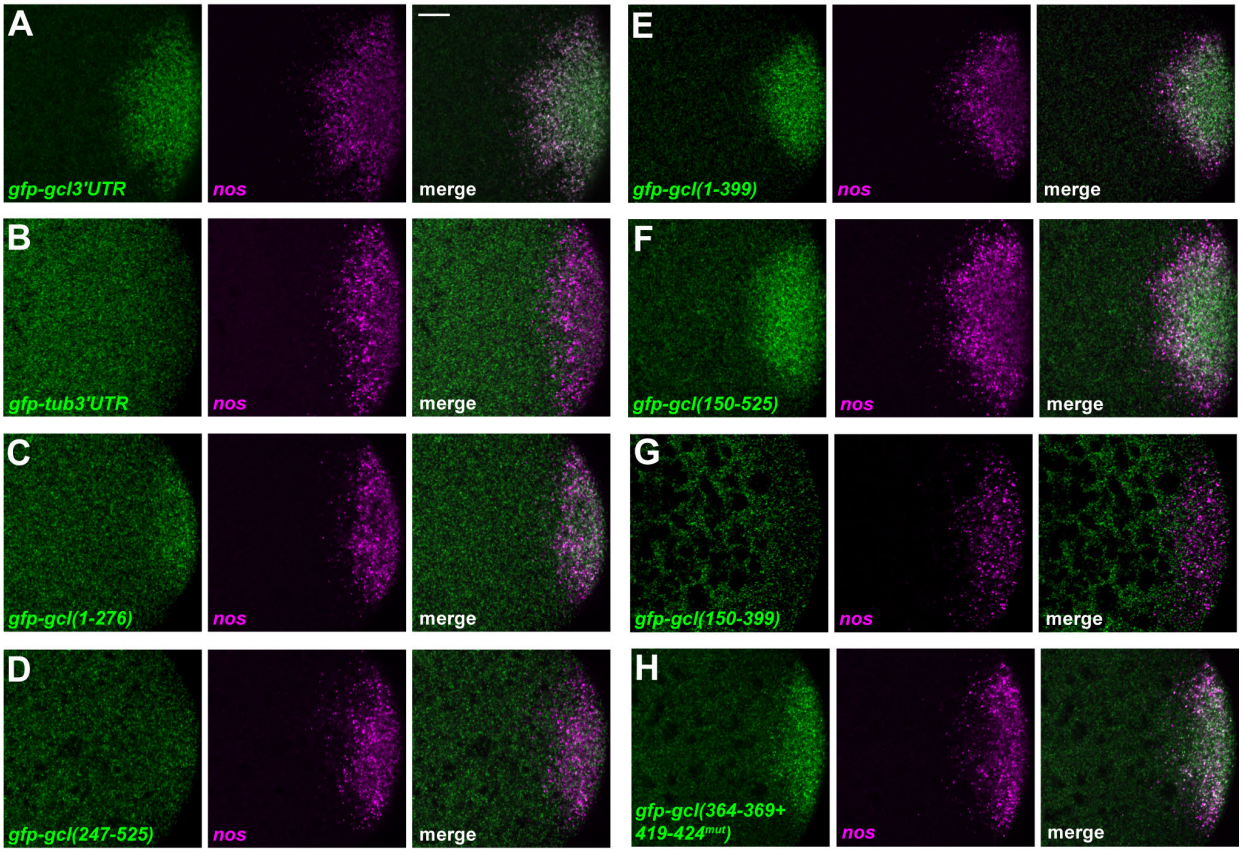
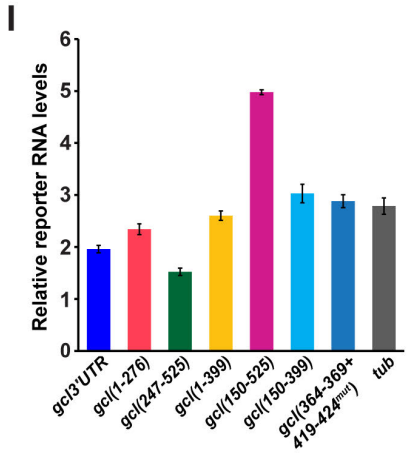


Figure S1, Related to Figure 1. Colocalization of endogenous *pgc* and *gcl* with *nos*, and germ plasm enrichment. (A, B) Confocal sections of the posterior region of 0-1 hr old wild-type embryos (anterior to the left, dorsal up). Embryos were probed simultaneously for *nos* mRNA (magenta) and *pgc* or *gcl* mRNAs (green). Scale bar = 15 µm. (C) Nearest-neighbor quantification of colocalization between *nos* and *pgc* or *gcl* (n = 30 embryos). (D) Quantification of mRNA content (average number of transcripts) in polar granules shows accumulation of *pgc* (5.0) and *gcl* (2.9) that is similar to the accumulation of *gfp-pgc3'UTR* and *gfp-gcl3'UTR* RNAs (n = 10 embryos each). Values shown are mean ± S.E.M. (E) Workflow of colocalization analysis for a representative image (see Materials and Methods). (F) Enlargement showing identification of particles by the spotDetector algorithm (Aguet et al., 2013). (G) Histogram from nearest-neighbor analysis showing the proportion of *nos* particles (spots detected) for which a *pgc* particle was detected at a particular distance. Particles within a distance of 300 nm (left of blue dashed line) were considered to be colocalized (68% in this example). Colocalization values shown in Figures 1-5, 7 represent the average for 20-30 embryos.





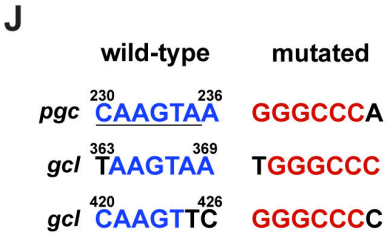
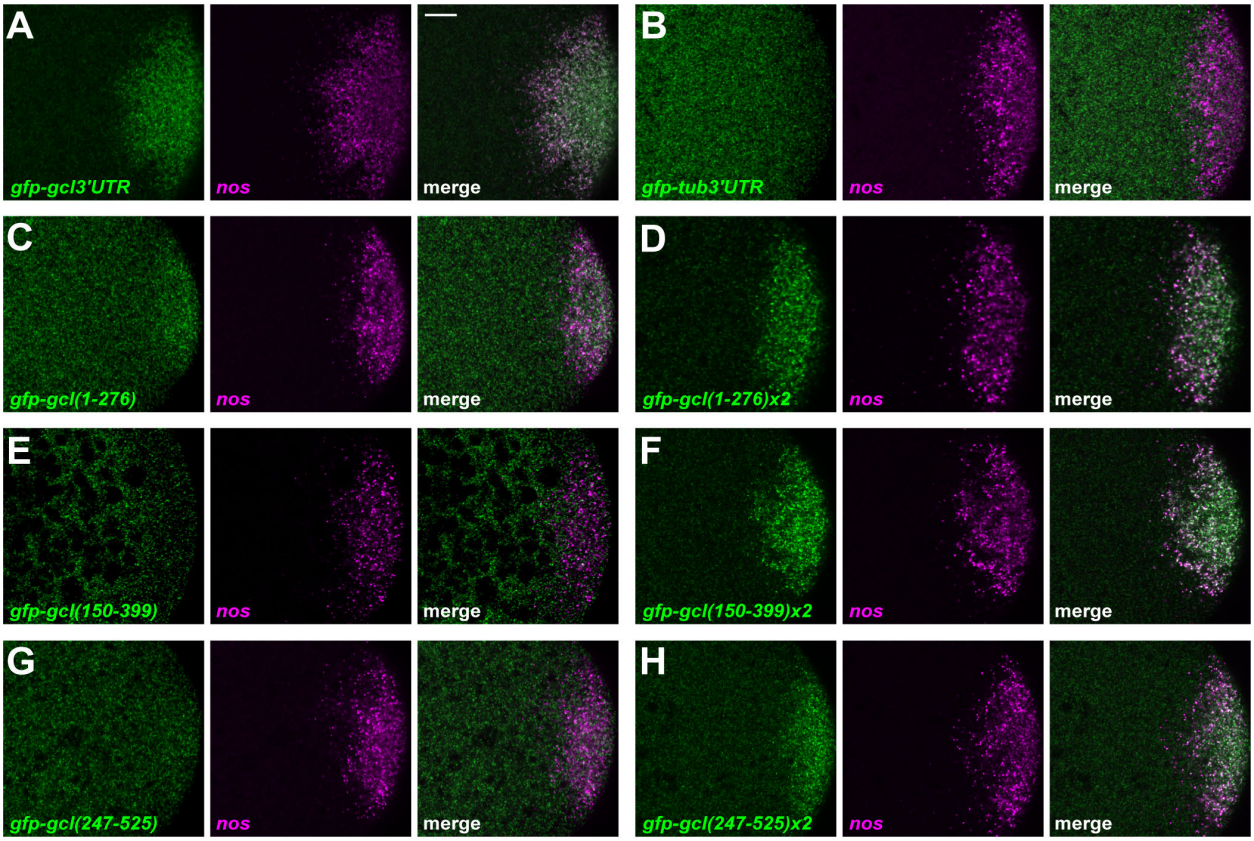


Figure S2, Related to Figure 2. *gfp-gcl3'UTR* reporter RNA levels do not correlate with localization efficacy. (A-H) Individual color channels are displayed along with the merged channels for the images shown in Fig. 2B. Scale bar = 10 µm. (I) RT-qPCR quantification of reporter RNA levels in early embryos normalized to *rpl7* and represented as fold difference from endogenous *gcl* expression. Values shown are mean ± s.d. (J) The zipcode located at nt 230-235 of the *pgc* 3'UTR (underlined) and the two closely matching regions in the *gcl* 3'UTR. Nucleotides matching the *pgc* motif (and adjacent 5' nucleotide) are shown in blue and were mutated to the sequences shown in red.



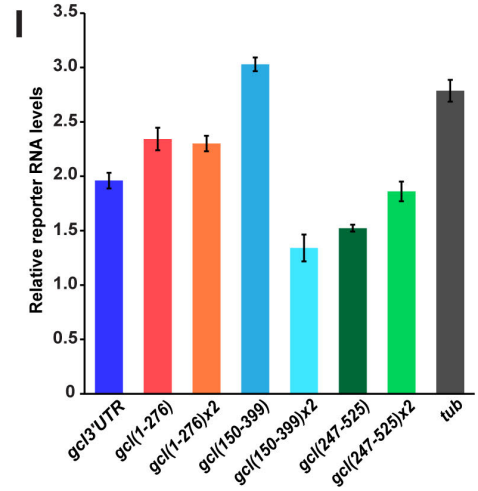


Figure S3, Related to Figure 3. *gfp-gcl3'UTR* reporter RNA levels do not correlate with localization efficacy. (A-H) Individual color channels are displayed along with the merged channels for the images shown in Fig. 3B. Scale bar = 10 µm. (I) RT-qPCR quantification of reporter mRNA levels in early embryos normalized to *rpl7* and represented as fold difference from endogenous *gcl* expression. Values shown are mean ± s.d.

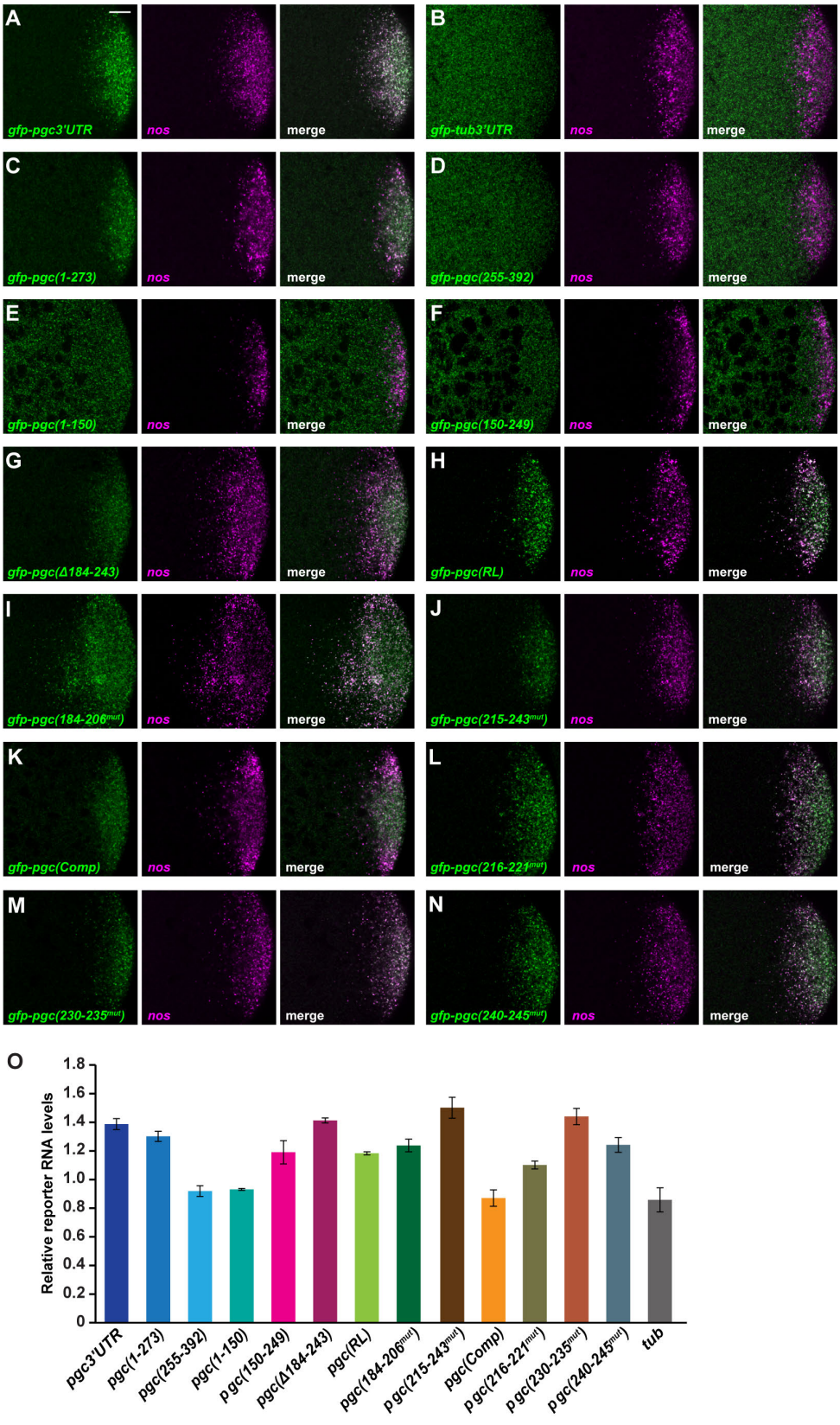


Figure S4, Related to Figure 4 and Figure 5. *gfp-pgc3'UTR* reporter RNA levels do not correlate with localization efficacy. (A-O) Individual color channels are displayed along with the merged channels for the images shown in Fig. 4B; Fig 5B. Scale bar = 10 µm. (P) RT-qPCR quantification of reporter mRNA levels in 0-1 hr old embryos internally normalized to *rpl7* and represented as fold difference from endogenous *pgc* expression. Values shown are mean ± s.d.

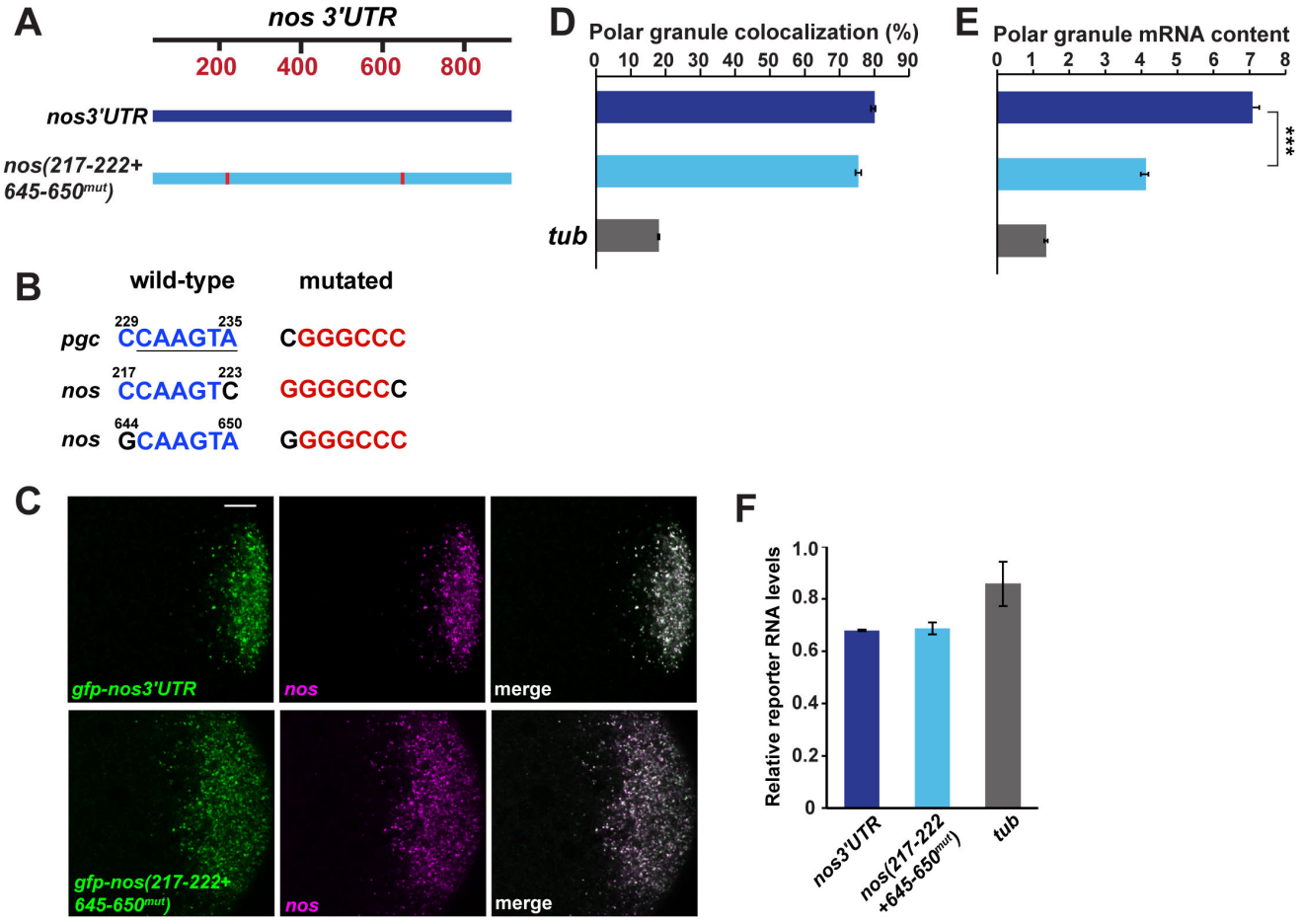


Figure S5, Related to Figure 5. *nos* 3'UTR sequences related to the *pgc* CAAGUA motif regulate *nos* homotypic cluster growth. (A) Schematic of the wild-type *nos* 3'UTR and *nos* 3'UTR with mutations shown in (B) indicated in red boxes. (B) The clustering element motif located at nt 230-235 of the *pgc* 3'UTR (underlined) and the two closely matching regions in the *nos* 3'UTR. Nucleotides matching the *pgc* motif (and adjacent 5' nucleotide) are shown in blue and were mutated to the sequences shown in red. (C) Confocal sections of the posterior region of 0-1 hr old transgenic embryos (anterior to the left, dorsal up). Embryos were probed simultaneously for *nos* mRNA (magenta) using probes restricted to the *nos* coding sequences and reporter mRNA (green). Scale bar = 10 µm. (D) Nearest-neighbor quantification of colocalization between *nos* and reporter transcripts (n = 30 embryos each). (E) Quantification of the average number of reporter mRNAs per polar granule (n = 12 embryos). Values shown in (D,E) are mean ± s.e.m., *** $p<0.001$ as determined by a two tailed t-test. (F) RT-qPCR quantification of reporter mRNA levels in 0-1 hr old embryos normalized to *rpl7* and represented as fold difference from endogenous *nos*. Values shown are mean ± s.d.

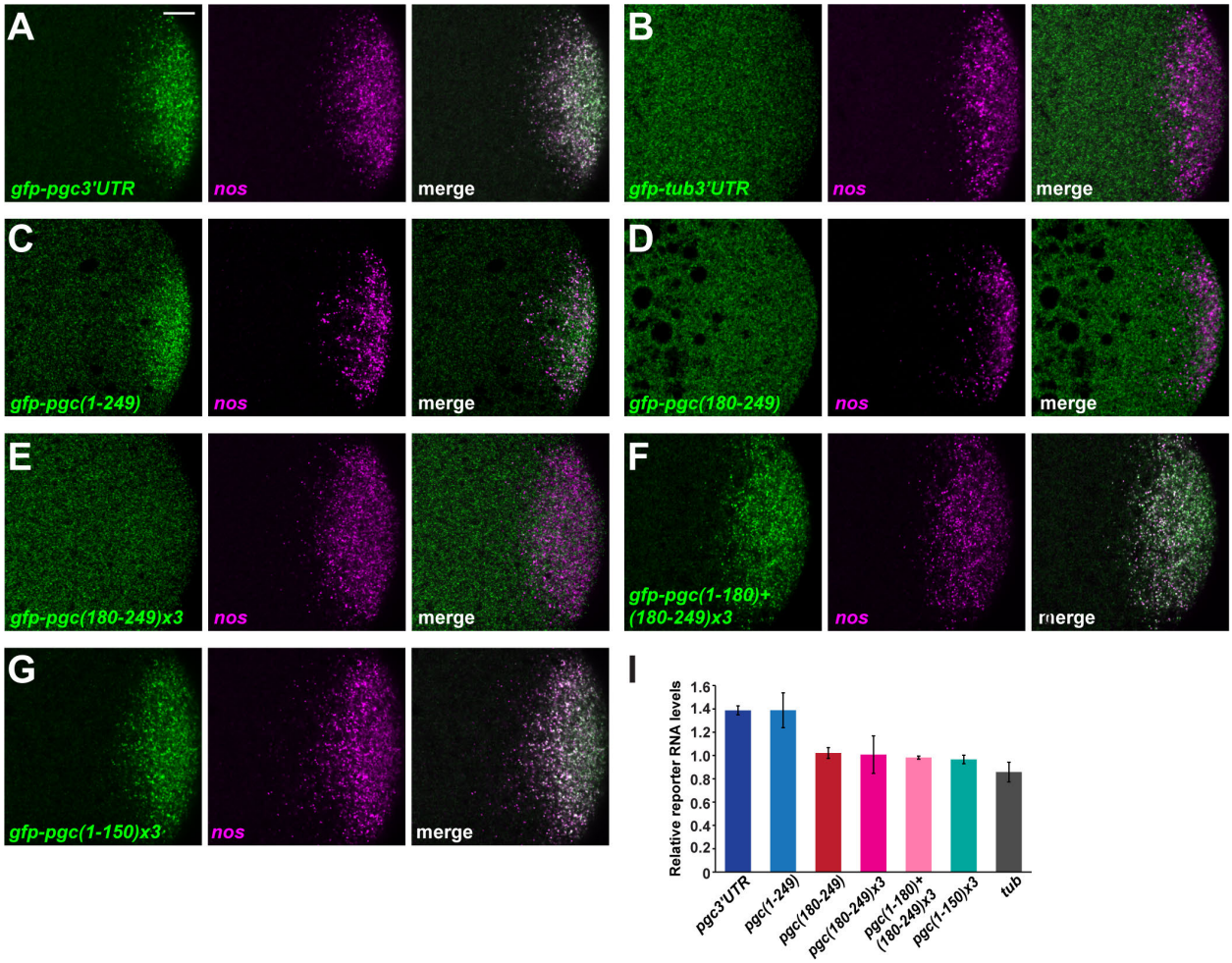


Figure S6, Related to Figure 7. *gfp-pgc3'UTR* reporter RNA levels do not correlate with localization efficacy. (A-G) Individual color channels are displayed along with the merged channels for the images shown in Fig. 5B. Scale bar = 10 µm. (H) RT-qPCR quantification of reporter RNA levels in early embryos internally normalized to *rpl7* and represented as fold difference from endogenous *pgc*. Values shown are mean ± s.d.