

**Figure S1** Co-localization of cytoskeleton markers at cytokinetic rings within the abnormal anterior cells of *step* RNAi embryos. Anillin staining (Turquoise) is shown as in Fig. 1F but additionally with co-stained Peanut (Red).



**Figure S2** *step* mRNA localization is indistinguishable between forming PGCs and the surrounding syncytial soma. Confocal imaging of fluorescent *in situ* hybridization of *step* mRNA in the posterior of an early syncytial embryo (nuclear cycle 10). The phase contrast image, right, shows the position of forming PGCs at the posterior pole. For the *in situ* image, left, note the indistinguishable *step* mRNA signal around the embryo periphery (for both the posterior pole where PGCs are forming and the surrounding soma). A greater interior signal is also present.



**Figure S3** PGC numbers at early blastoderm cellularization for various control genotypes. (A) Supplement to Fig. 3B. Each *rho1* heterozygote produced significantly fewer PGCs than control (p<.001). (B) Supplement to Fig. 4B. (C) Supplement to Fig. 4B.



**Figure S4** step loss suppresses the loss of PGC numbers in *rho1* heterozygotes at nuclear cycles 11 and 12. Supplement to Fig. 3B. Each point represents one embryo and the numbers of embryos with zero PGCs are indicated in brackets.



**Figure S5** Enhancement of the effects of Step over-expression on PGC numbers with two additional *gcl* perturbations. Supplement to Fig. 4B. In each case, the reduction of PGC counts was significant (p<.001).

## Files S1-S2

## Available for download as .avi files at www.genetics.org/lookup/suppl/doi:10.1534/genetics.115.176867/-/DC1

**File S1** Live phase contrast imaging of a control (mcherry RNAi) embryo showing the normal posterior formation of PGCs (right) accompanied by minimal changes elsewhere around the embryo. Images were acquired once every 30 seconds, and the movie is shown at 8 frames/sec (240 times real-time).

**File S2** Live phase contrast imaging of a *step* RNAi embryo showing the normal posterior formation of PGCs (right) accompanied by abnormal anterior cell formation (left). Images were acquired once every 30 seconds, and the movie is shown at 8 frames/sec (240 times real-time).