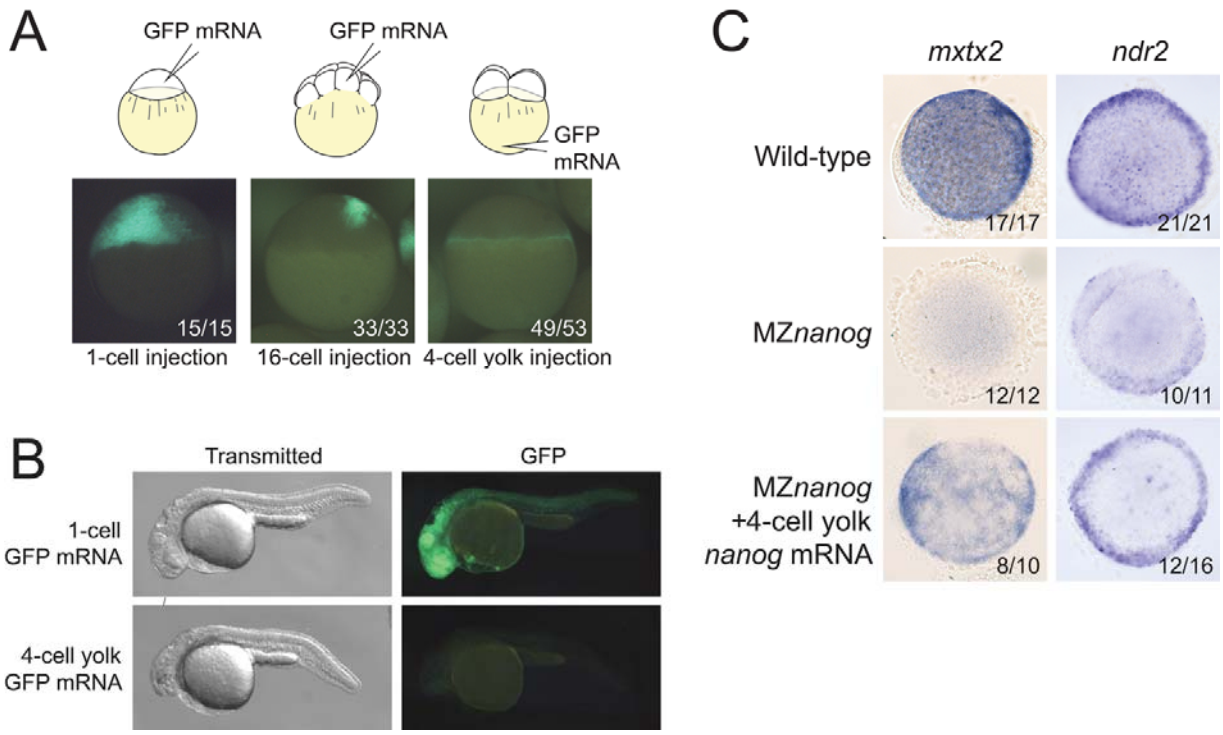

Fig. S1. Predominant expression in the YSL after 4-cell yolk mRNA injection. A. MZ*nanog* embryos were injected with 33 pg GFP mRNA and 25 pg *nanog* mRNA at 1-cell stage, 4-cell stage in vegetal yolk, or in 1 of the cells at 16-cell stage. At sphere (4 hpf) stage, embryos were imaged and scored for spatial GFP expression. **B.** Representative rescued embryos (as indicated) were imaged at 24 hpf for GFP expression in embryonic tissues. **C.** In situ hybridization for *mxtx2* and *ndr2* expression at 4.5 hpf (dome / 30% stage) in wild-type, MZ*nanog,* and MZ*nanog* embryos injected with *nanog* mRNA into the vegetal yolk at the 4-cell stage.

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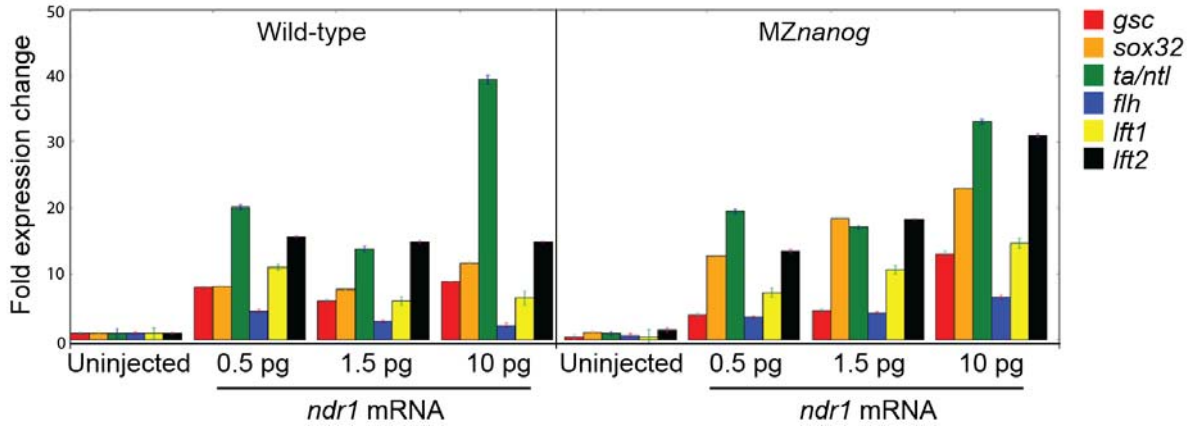

Fig. S2. MZ*nanog* embryos respond to Nodal signaling by expressing mesendodermal marker genes. Wild-type or MZnanog embryos were injected at the 1-cell stage with indicated concentrations of *ndr1* mRNA, then collected at 4 hpf. Fold expression change for the indicated genes was determined using RT-qPCR, normalized to wild-type uninjected expression. Error bars show standard deviation for three technical replicates (10 embryos per replicate).

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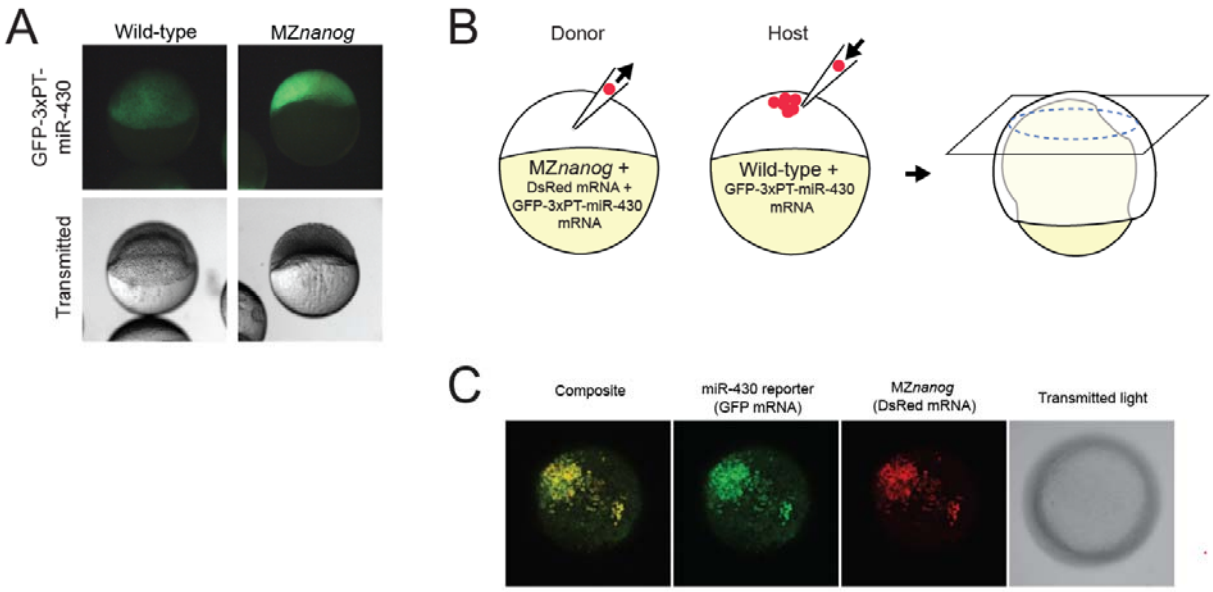

Fig. S3. A cell autonomous requirement for *nanog* for miR-430 activity. A. Wild-type and MZ*nanog* embryos were injected at the 1-cell stage with 100 pg GFP-3xPT-miR-430 mRNA, which contains three targets sites for miR-430 in the 3' UTR. Images were taken at 6.5 hpf, and representative images are shown for each genotype (wildtype n=28; MZ*nanog* n=11). **B.** A diagram of transplantation of MZ*nanog* cells (injected with DsRed mRNA and GFP miR-430 reporter mRNA) into wild-type host embryos (injected only with GFP miR-430 reporter mRNA). Transplants were performed at 3-4 hpf, and embryos were imaged at 8 hpf. Donor cell contribution to the host embryo is detected by DsRed expression. **C.** A representative transplant host embryo imaged at the animal pole (from 3 independent experiments, n=33). GFP miR-430 reporter expression is repressed in wild-type host cells but detected in transplanted MZ*nanog* cells (co-expressing DsRed).

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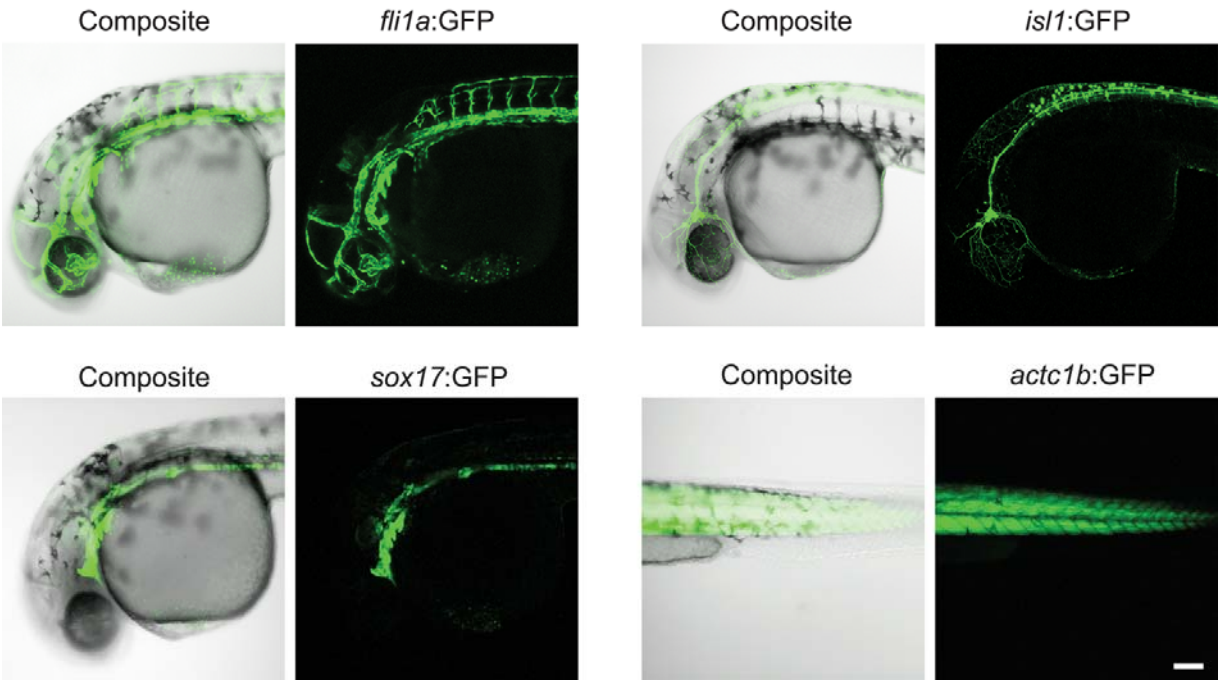

Fig. S4. Transgene expression in wild-type embryos. For comparison with transplanted cells in **Figure 6D**, representative wild-type transgene expression patterns are shown at 30 hpf, imaged and processed as in **Figure 6D**. Scale bar indicates 100 microns.

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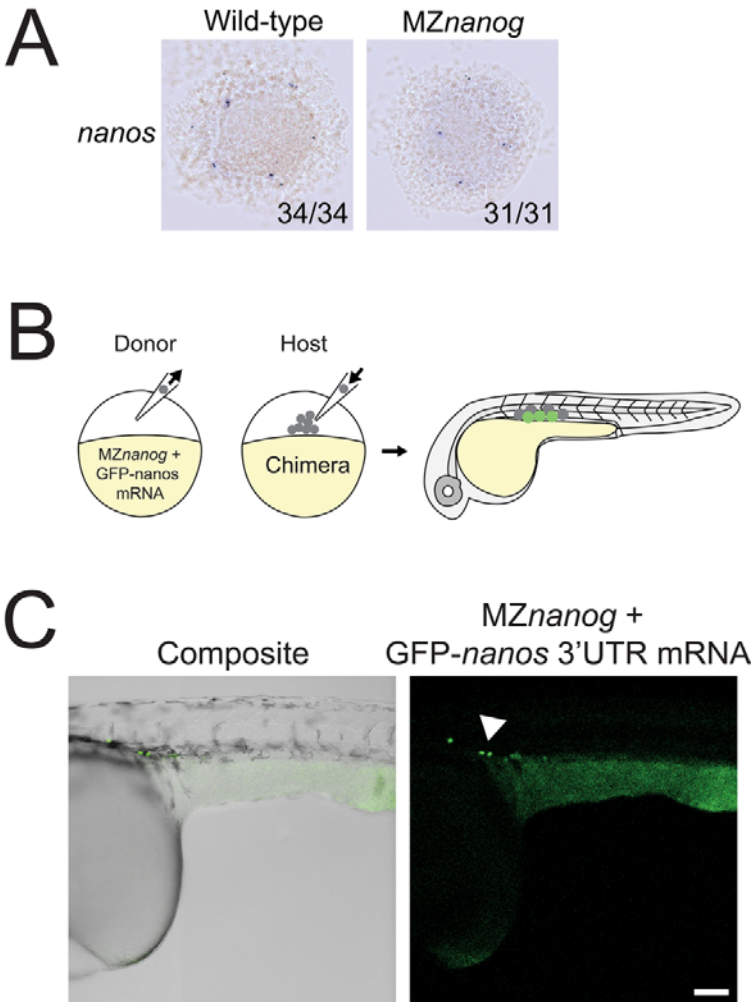

Fig. S5. MZ*nanog* germ cells are specified and migrate correctly when transplanted into wild-type host embryos. A. In situ hybridization for *nanos* expression at sphere stage in wild-type and MZ*nanog* embryos. **B.** A diagram of transplantation of cells from MZ*nanog* embryos injected with 50 pg GFP-*nanos*1 3'UTR mRNA ("GFP-nanos", Köprunner et al., 2001) into a wild-type host embryo, with green cells in the host embryo indicating expression of the mRNA in transplanted cells at 30 hpf. **C.** Approximately 20 cells were transplanted from donor injected embryos into uninjected wild-type host embryos. At 30 hpf, embryos were anaesthetized, mounted, imaged, and processed as in **Figure 6D** (n=6). Germ cells are indicated with an arrowhead. Scale bar indicates 100 microns.

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Table S1. RNAseq expression of early zygotic genes reduced >2-fold in MZ*nanog* embryos. ENSEMBL ID, gene name, chromosome location, and expression level (FPKM) in MZ*nanog* and wild-type embryos at sphere stage for 79 early zygotic genes.

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Table S2. DNA sequences for RT-qPCR primers, GESTALT barcode and amplification primers, vectors and the *nanog* mutant allele.

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