

Supplemental Figures

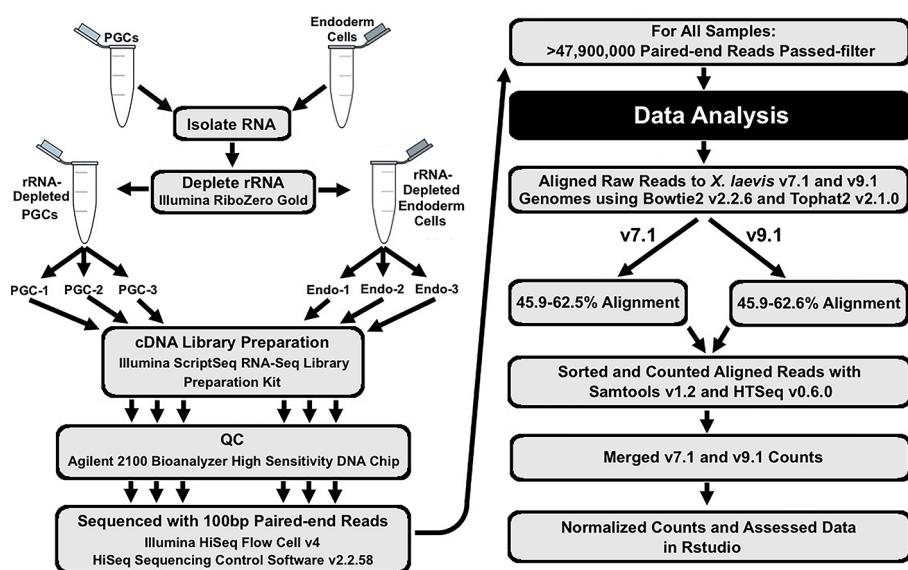


Figure S1. Methods flow chart for RNA-seq analysis of PGC versus endoderm cell transcripts in *X. laevis* embryos. 1,2,3 denote PGC and Endo sample sets.

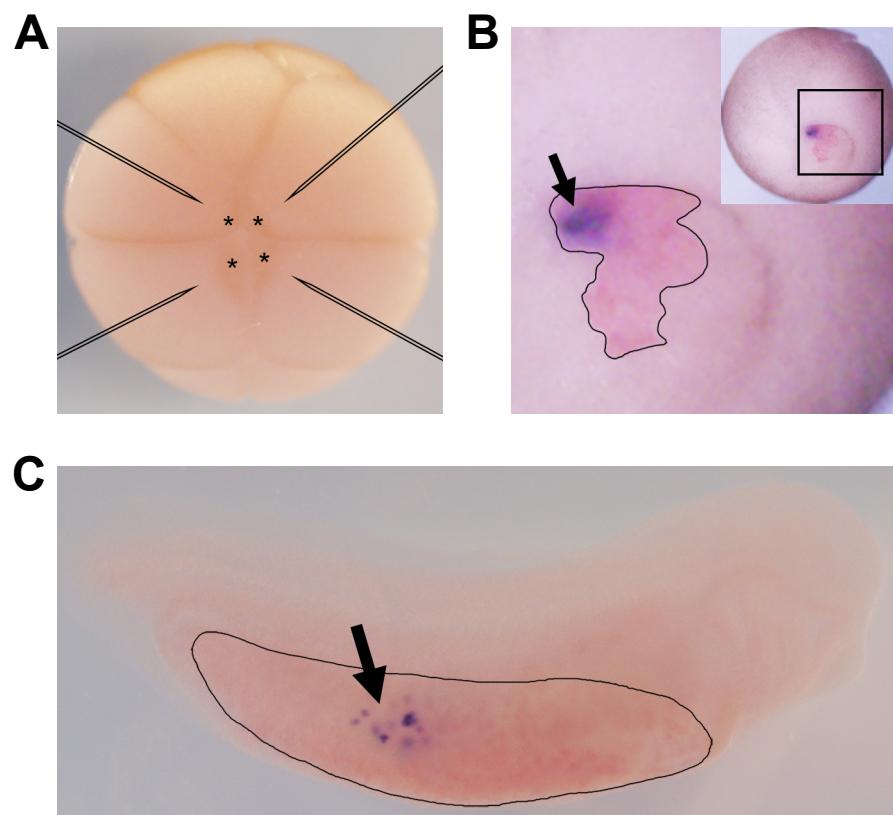


Figure S2. PGC-directed Injections. A) Live 16-32-cell embryo, vegetal pole up. Germ plasm is detected as dark regions in vegetal blastomeres (*). Typical injection sites are shown. B&C) Lineage tracer (red, outlined in black) showing gastrula (st. 11.5, B) and tailbud (st. 33-34, C) progeny injected as in A). *xpat (pgat)* WISH (purple, arrows) indicates PGCs. Note: quality of targeting of PGCs.

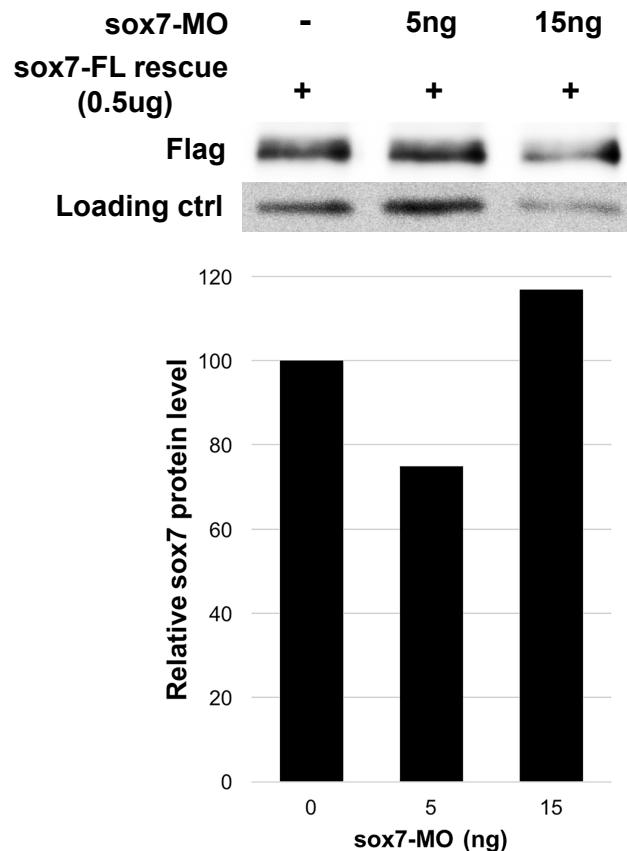


Figure S3. sox7-MO does not inhibit sox7-FL rescue protein expression. Expression of full length, flag-tagged sox7 rescue construct (sox7-FL rescue) in the presence of sox7-MO (concentrations are indicated). Western blot (top); histogram of the western (bottom).

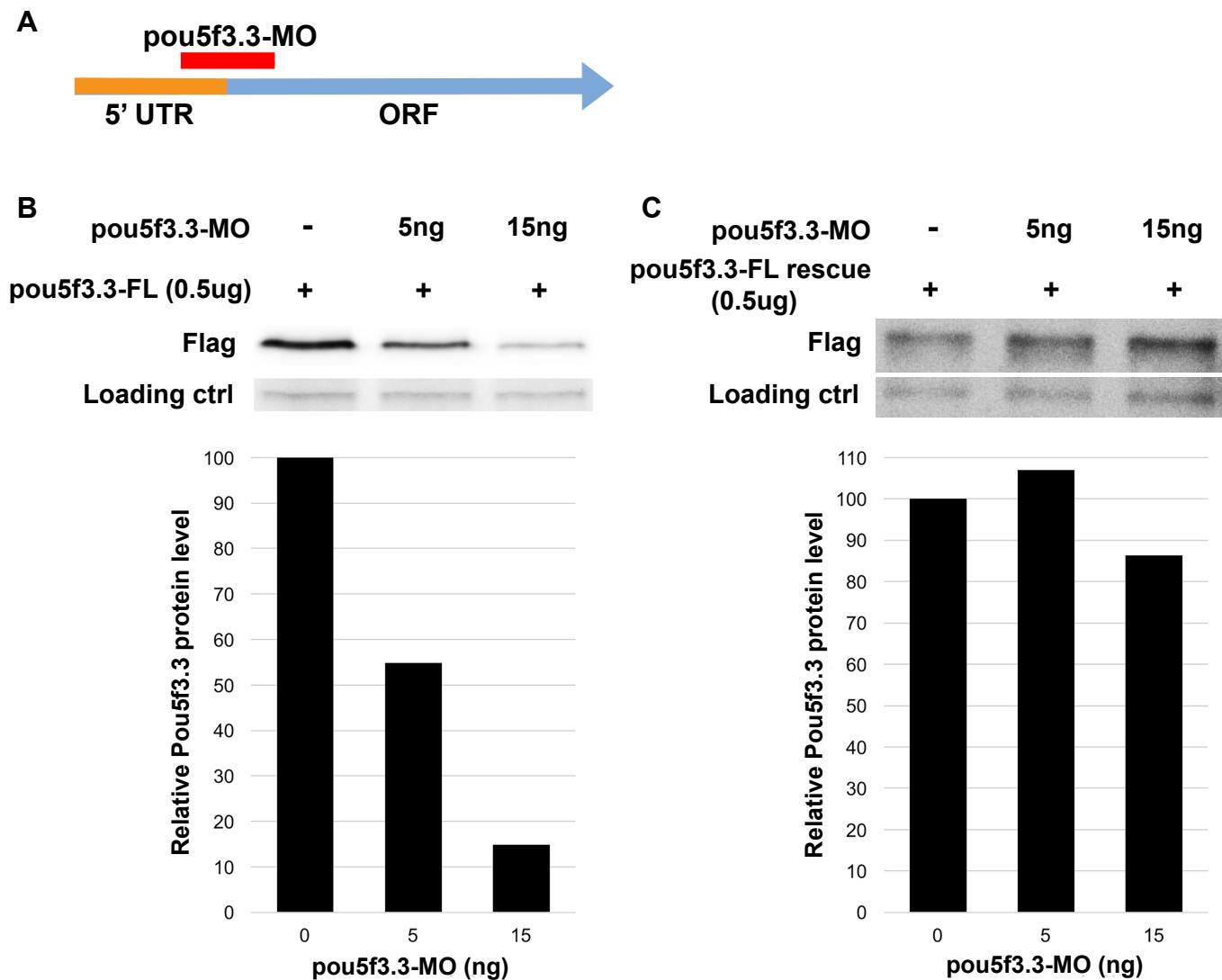


Figure S4. *pou5f3.3-MO* inhibits *pou5f3.3-FL* and not *pou5f3.3-FL* rescue protein expression. Schematic of the *pou5f3.3* (*oct60*) morpholino (*pou5f3.3-MO*) targeted region (A). Expression of full length, flag-tagged *pou5f3.3* (*pou5f3.3-FL*) (B) and *pou5f3.3-FL* rescue (C) in the presence of *pou5f3.3-MO* (concentrations are indicated). Respective histograms are shown.

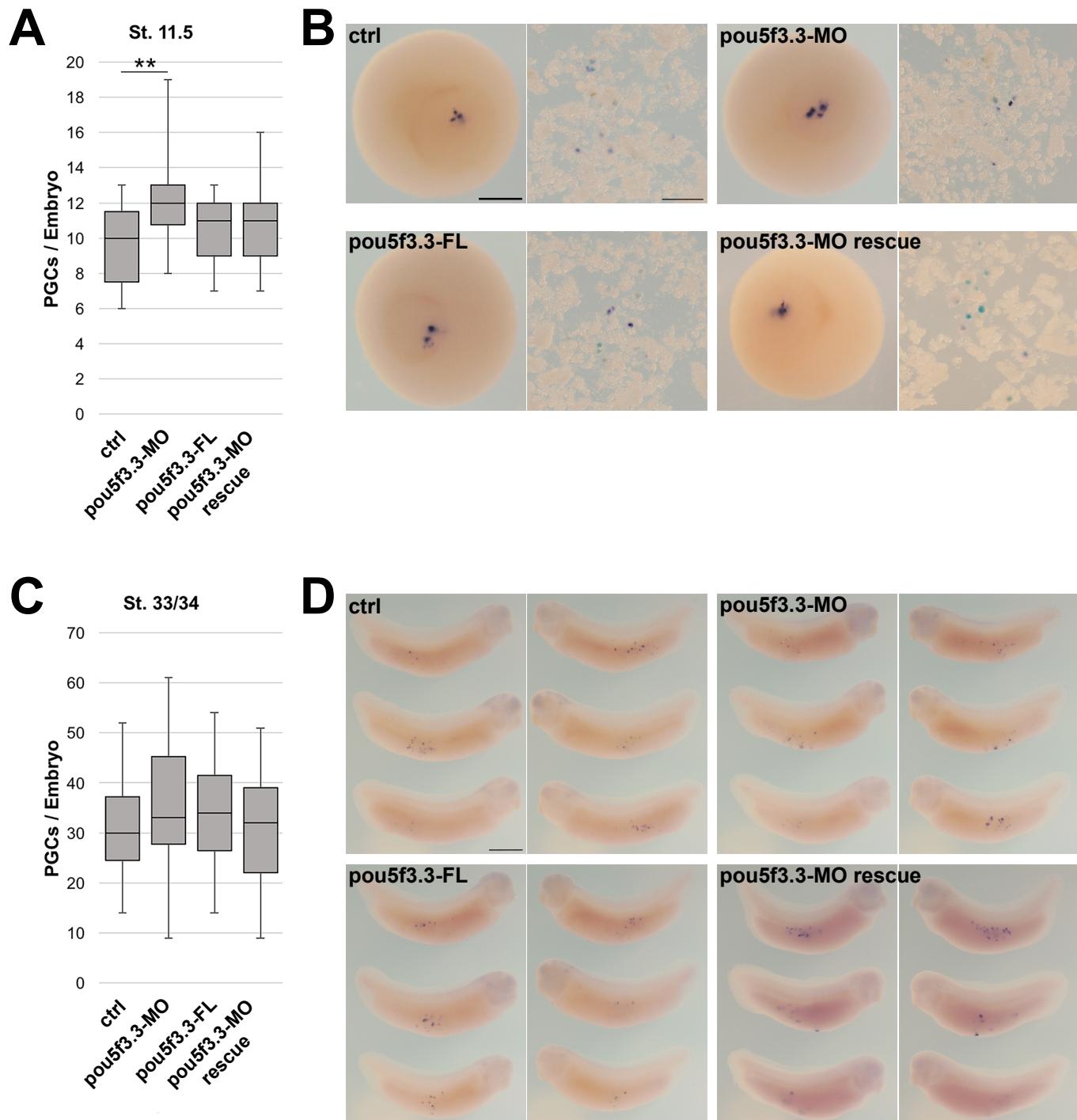


Figure S5. PGC number increased after PGC-directed *pou5f3.3* (*oct60*) knockdown in early development. The number of PGCs per embryo was determined in control, *pou5f3.3*-MO, *pou5f3.3*-FL, and *pou5f3.3*-MO rescue embryos at gastrula (st. 11.5) (A&B) and tailbud (st. 33-34) (C&D) stages. Representative images are shown (B&D). ctrl n=23, *pou5f3.3*-MO n=24, *pou5f3.3*-FL n=25, *pou5f3.3*-MO rescue n=24. ** statistically significant compared to uninjected control ($p<0.005$). *pgat* (*xpat*) expression by WISH was used to identify PGCs. Analysis shown is representative of 5 out of 8 independent experiments. ctrl = control; MO = morpholino; FL = full length, flag-tagged. Scale bar = 500um (B), or 1mm (D).

A

Negative Control (Sea urchin Wnt8):

CCCCACCGCTCCGAAGTCGAATCGCAACAAGTGTCTGGTCAGCTCTCAACTTTGTTAGCCTCACAGCAGGAGA
TAGTAATTGATACACATCTCTGTAACACTATCTCGCATCTTATCCTAACCCCTATCGTGGTGGAGACGATACATCACC
ATTTATTTCTATTGGACTGAACGTGAAATATTGGGGATTAGTTAAGTTTCGAGACGCATTTAAAGTTTGATT
TCTTTATTGGAGTGTACATCATGGATGTCTTACGAAATTGTTCGTCATCTTCTGCTCGTCGTCCTCCTCGT
CAACGTGCCATCTTCAAGCAATCATATGGTCACCAGCAAGCAACGTTCTCTAGCGGGATCCAAGAGGCACAT
CACGTATATCGACAGCGTGTCTGCGGGTATTCAAATGCTGTAAGGGAGTGTCAAGTCCAGTTCAAATGGGATAA
GTGGAATTGTCTGAAGTCTCGTCTTACAAACACTCAAGCTAATGTGAGATGTCTATTCAAGCCATCGCGTC
TGCAGGGGTATGTATTCACTTAAGAACACTGCACTCAGGGGGCTTTGATGACTGCGGATGTGATAAGAGACGA
AACGGAAATAGTGGTGGTGGAGGGCTGGACTGGGGCGCTGCAGTGACAATGTACGATTGGTGAACGAATGGC
GAGCGATATCATGGACGACGCAGAGAGTTCACAGGGCGCCATCTCCGTTATGACTCTACATAACAACGAGGCTGG
CAGAAAGGCTGTCAAACAGACCCCTAACGAAACCTGTAAGTGTATGGCGTCTCCGGCTCTGCTCTACAGAC
CTGCTGGAACCACGTCGAAACTCCGTGTCATGGGACGAGATCAAGCGCAAGTACTCCAGGCCGTACCG
TCGACTTCGTTGGAAACTCATCGATGGCAACAGCGCTGAAGATCGCTCCCTCAAGCCGTAGTGAGCGCAT
CGCACAAACCGTCGCGACCTCGTTCTGGACCAGTCCCCCGACTACTGCCGTGCCAACCTCACCATCGGTGCA
CCGGGACTGCGGGCAGGGAGTGCATGGTGCAGAGAGGATGTCACCGAGGGTATCATCGTCATCATTGACCAAG
GATTGACGTCGCCTTCATCGTCCGATGGGTGAAGCAGAGCTGTAGCAGACTGTGAGGAGCTGCGGGATGGT
GATTGCGTAAGACTCAAGTCATTATTACGTCCAGCTGCAACTGCAACTTCGTTGGTGTGAGGTGAAGTGCAC
ACCTGTCGGCGAACTGTGACCAGCGGACGTGCCAGCCGGTGGATAGAGGCTGTAGGTCCTTAAATATCTTG
ACTCTGAGGAAGATGAACCTTTAATGTCCACTAAGTTGGATGCTTGTCTATGACTACATTAAGACTGTG
ACAGAACACCAGCTTGTATAAATACAGTCTCTCTCTTTAAAAGGACAGGAAGGAAAAAGCGGCC

Positive Control (XI Wnt8):

TGTCATTGCTGAGGATACTGTTCAAGCATTACCCGGATAGCAGCCTCATCATGCAAAACACCACCTTGTTCAT
CCTTGCACACTCTCTGATCTGCCATTCTCACTGCCTCAGCATGGTCAGTCATAACTTCTGATGACAGGAC
CCAAGGCATATCTGACATACTCAGCGAGTGTGCCGGTGGCAGAATGGAATTGAGGAGTGAAATATCAGT
TTGCGTGGGAAAGATGGAATTGCCCTGAAAGTACCCCTGCAGCTGCTACCCACAATGGACTTCGAAGTGCAACCA
GAGAAACCTCCTTGTGCATGCCATTAGCTCAGCGGGAGTTATGTATAACTGACGAGAAACTGCAGCATGGGG
ACTTGATAACTGTGGATGTGATGACTCCAGAAATGCCGCATCGTGGCGAGGGCTGGGTATGGGGCGGCTGC
AGTGATAATGCAGAATTGGTGAGCGGATCTGAAACTATTCTGCGATGGCTTGGAGACGGGACAAGATGCCAGA
GCCCTAATGAACCTGCATAACAATGAAGCAGGAAGACTGCAGTAAAGAGACAATGAAAAGGACTTGCAGTGC
CATGGAATATCTGGAAGTTGCAGCATAAAACTGCTGGCTTCAGCTGGCGAGTTGGATATTGGCAATCACT
TAAAGATCAAGCACGACCAAGCGCTAAAGCTTGAGATGGACAAGAGGAAATGAGGTGGTAACAGTGTGACA
ACAGAGGAGCCATCGCTGATGCCCTCAGTTCTGTCAGGGCTGAACCTATTTCTGAAGACTCTCCCAGTAA
TTGCTAAAAAACATCAGTTGGCTCCAGGGGACAGAGGGACGAGAGTGCCTGCAAAGTGGCAAGAACTGTC
CCAGTGGAGAGAAGAAAGCTGCAAGAGGCTTGACAGACTGCAGCCTCCAGTGGAAAGAGAAAAGACGGAG
ATTATCAGTAGCTGCAACTGCAAATTCACTGGTCTGCACAGTCAAATGCGAGCAATGCAAACAGGTAGTGTATAA
AGCATTCTGCCAGGGAGGAAACGGGATTCCAACATGCTGAATACAAAAGGAAGAACAGAGGCCACCGGAGA
TGACGGCATTCCAGAAATGTCCTCGACCTCAACTCGGCCAGTGGATTGTTGCCTTCAGGATTCTCCTTAA
TTTATGGACTAAAGTAACGTATTAAAGTATAAGCTATAAGGTGGTCTGTTGAAATATTAATATATGCACTT
ATGAATATTCAATTAAACCCGTGTAGAGATTCTACACCGCAGAGTATTCCATGAAACAGAAACATGCTCCGTT
CGAAGGATATGTTGATTGATGAACAATATTAATTAAATGGTCTTTTCTGAATTCAAACGATTGGTC
TGTCTGAGATATGGGAGATATATATAAAATGGAATTGCAGACATTCTGCATTGCATCCATTGAGTT
TTCTGTGCTCATGTTTATGAACATCATCAGTATTAAATAAAAATATTTACAAAAAAAAAAAAAAAAAAAA
AAAA

vegt Conserved Region (Positive Control for vegt):

GTGCCTCCCTGGAAGACCAAGATCTATGGTCCCAGTTCCACCAGGAGGGGACAGAGATGATCATACCAAATCTG
GAAGGAGGATGTTCTCAGTGTAAAGATCCGTCCTCGGTCTCATCCTTATGCCAAGTACATGCTGCTGGTGG
CTTGTTCCCTGGACAACCTTAGGTATAAGTGGAAATAAGAACCGAGTGGGAAGCAGCAGGTAAGCAGAACCTCA
CCCACCCCTGAGGACGTATGCCACCCAGATTCACCTGCTCTGGTCCCCACTGGATGAAGGATCCGATCTGCTT
TCAAAAGCTCAAACCTACCAACAAACACATTGGATCAACAAGGCCATTATCTTGCAATTGCATCGCTACAAGC
CCAGGTTCCATGTTAGTCAGTCTGATGACATGTACAATTCTCATGGGGATTGGTACAAGTGTAGCTTCCCAGA
GACAGAGTTACTTCAGTGAUTGCCTACCAAGAATGAAAAGATTACTAAACTGAAAATTAAATCACAACCCATTGCTA

Maternal VegT:

ATATTGAGCATTACCTGCAGGGGCCATGTGATAGCCTAGCTACAGTCGTGGA **ATG**AGAAACTGCTGTCGGG
AATGCGGCCTCTCGGGCATCTGGAACAGAGGCTTCTTA~~CT~~ACTGTGCATCA **GAT**TAAAGTCTTCCCCAG
ACATGGACAGTGTCTCCAGCCAAGACTCCCTCACCTGCCAACACTGTTG

Zygotic VegT:

TGGATTAGTTAGGAAC**ATG**CATTCTCTGCCGGATGTAAAGTCTCCCCAGACATGGACAGTGTCTCCAGCCAAG
ACTCCCTTACCTGCCAACACTGTTG

B

Samples aligned to:	PGC1 (CPM*)	PGC2 (CPM*)	PGC3 (CPM*)	Endo1 (CPM*)	Endo2 (CPM*)	Endo3 (CPM*)
Negative Control	0	0	0	0	0	0
Positive Control	63	102	76	92	48	86
vegt Conserved Region	364	362	357	11	11	5
Maternal vegt	9	6	5	0	0	0
Zygotic vegt	0	0	0	0	0	0

*CPM (Before normalization)

Figure S6. Maternal vegt is expressed in PGCs. Raw reads from PGC and Endo samples were aligned to A) Sea urchin *wnt8* (negative alignment control), *Xenopus laevis wnt8* (positive alignment control), *vegt* region conserved between maternal and zygotic *vegt* isoforms (*vegt* Conserved Region; positive *vegt* control), maternal *vegt* (5' 131bp region unique to maternal *vegt*, black, plus 70bp of the conserved region, green), and zygotic *vegt* (5' 32bp region unique to zygotic *vegt*, black, plus 70bp of the conserved region, green), B) then counted. Transcription start site is indicated for maternal and zygotic *vegt*.

Supplemental Tables

Table S1. All annotated transcripts identified using v7.1 and v9.1 *Xenopus laevis* scaffold sets. Tab 1) PGC-enriched (red), Endo-enriched (blue), and not differentially expressed (white) transcripts are indicated. Tab 2) Transcripts enriched at least 3-fold in Endo versus PGCs. Tab 3) Transcripts enriched at least 3-fold in PGCs versus Endo. Tabs 4-6) Total transcription factors (TFs), homeodomain TFs, and TF co-factors enriched in PGCs. FC = fold change; PGC = primordial germ cell sample; Endo = endoderm cell sample; CPM = counts per million; LR = likelihood ratio statistics; FDR = false discovery rate. v7.1 transcripts are highlighted in yellow.

[Click here to Download Table S1](#)

Table S2. GO Processes of PGC-enriched transcripts. Top 300 PGC-enriched transcripts were subject to GeneGo analysis. The 50 most well-represented GO processes are categorized based on the greatest number of enriched transcripts identified.

GeneGo Process	p-value	FDR	# of genes
Cellular process	6.13E-09	1.04E-06	286
Single-organism process	1.59E-12	4.85E-10	276
Single-organism cellular process	3.33E-13	1.27E-10	261
Biological regulation	3.10E-07	3.47E-05	242
Regulation of metabolic process	2.42E-07	2.91E-05	161
Cellular component organization	5.01E-09	9.18E-07	142
Cellular component organization or biogenesis	2.66E-08	3.93E-06	142
Organelle organization	1.01E-15	6.60E-13	115
Positive regulation of metabolic process	4.22E-07	4.61E-05	101
Single-organism organelle organization	6.56E-13	2.31E-10	86
Cell cycle	1.32E-25	6.04E-22	83
Cell cycle process	6.53E-22	9.97E-19	70
Mitotic cell cycle	9.81E-23	2.25E-19	61
Mitotic cell cycle process	2.14E-21	2.46E-18	57
Single organism reproductive process	6.35E-07	6.19E-05	52
Regulation of cell cycle	6.76E-10	1.55E-07	49
Regulation of organelle organization	4.93E-07	5.13E-05	44
Nuclear division	1.32E-15	7.57E-13	39
Organelle fission	7.62E-15	3.17E-12	39
Regulation of cell cycle process	2.11E-10	5.38E-08	35
Mitotic cell cycle phase transition	6.44E-17	5.90E-14	34
Cell cycle phase transition	8.59E-17	6.56E-14	34
Cell division	1.52E-12	4.85E-10	34
Microtubule-based process	3.60E-10	8.68E-08	33
Regulation of mitotic cell cycle	3.97E-09	7.64E-07	31
Mitotic nuclear division	3.33E-12	9.54E-10	29
Microtubule cytoskeleton organization	1.44E-09	3.14E-07	26
Regulation of cell division	4.01E-09	7.64E-07	24
G2/M transition of mitotic cell cycle	2.20E-15	1.01E-12	23
Cell cycle G2/M phase transition	2.20E-15	1.01E-12	23
Regulation of cell cycle phase transition	2.76E-08	3.95E-06	22
Regulation of mitotic cell cycle phase transition	4.81E-08	6.11E-06	21
Negative regulation of organelle organization	5.71E-07	5.81E-05	21
Regulation of nuclear division	1.97E-10	5.32E-08	20
Chromosome segregation	5.83E-09	1.03E-06	20
Meiotic cell cycle	1.87E-08	2.95E-06	19
Cell cycle checkpoint	2.20E-08	3.36E-06	19
Negative regulation of cell cycle process	8.43E-07	8.04E-05	18
Regulation of mitotic nuclear division	3.49E-09	7.26E-07	17
G1/S transition of mitotic cell cycle	4.35E-08	5.69E-06	17
Cell cycle G1/S phase transition	4.35E-08	5.69E-06	17
Meiotic nuclear division	4.10E-08	5.69E-06	16
Meiotic cell cycle process	1.11E-07	1.38E-05	16
Regulation of cyclin-dependent protein kinase activity	4.49E-07	4.78E-05	14
Regulation of cyclin-dependent protein serine/threonine kinase activity	1.19E-08	1.94E-06	13
Regulation of microtubule cytoskeleton organization	1.01E-06	9.46E-05	13
Female meiotic division	2.90E-07	3.40E-05	8
Regulation of centrosome cycle	6.30E-07	6.19E-05	8
Regulation of transcription involved in G1/S transition of mitotic cell cycle	3.09E-07	3.47E-05	7
Chaperone-mediated autophagy	1.12E-06	1.03E-04	4

Table S3. *Xenopus* versus human PGC-enriched transcripts. 80% [1489/1865] of transcripts enriched in *Xenopus* PGCs are also expressed in human gonadal PGCs (Irie et al., 2015, gray), hPGC-like cells (hPGCLCs) enriched at least 2-fold vs. pre-induced cells (Irie et al., 2015, green), or both (orange). *Xenopus* PGC-unique transcripts (white). FC = fold change; PGC = primordial germ cell sample; Endo = endoderm cell sample; CPM = counts per million; LR = likelihood ratio statistics; FDR = false discovery rate. The left-most tab shows the 90% of transcripts that are conserved among the 50 most highly expressed *Xenopus* PGC-enriched transcripts.

[Click here to Download Table S3](#)

Table S4. Maternal, vegetally-enriched RNAs zygotically expressed. PGC-enriched (red), Endo-enriched (blue), and not differentially expressed (white) transcripts are indicated. FC = fold change; PGC = primordial germ cell sample; Endo = endoderm cell sample; CPM = counts per million; LR = likelihood ratio statistics; FDR = false discovery rate. v7.1 transcripts are highlighted in yellow.

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Table S5. *Xenopus* st. VI oocyte vegetally-enriched transcripts versus human oocyte transcripts. 60% [119/198] of transcripts enriched in the vegetal pole of stage VI *Xenopus laevis* oocytes (Owens et al., 2017) that are also expressed in mature human oocytes (Yan et al., 2013).

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