## Supplemental MATERIALS AND METHODS

### Isolation of animal and vegetal pole samples (continued)

After surgical removal, ovaries were teased into small clumps and rinsed in Modified Barth's Solution. Oocytes were enzymatically released from ovarian tissue and from follicles with 0.2% collagenase (Type 1; Worthington Biochemical Corp.) in calcium free OR2 (Sive et al., 2000). Stage VI oocytes were selected and incubated overnight in OR2 with 1 mM $CaCl_2$ at 18°C. The following day, oocytes were transferred into P10EM solution (Elinson et al., 1993) and oocyte-matched vegetal and animal poles were cut (Cuykendall and Houston, 2010). Samples were immediately collected into Eppendorf tubes, frozen on dry ice, then stored at -80°C until RNA was extracted.

### RNA isolation

Total RNA was isolated using Trizol reagent (Invitrogen) as per the manufacturer's protocol with the following modifications: samples were homogenized before adding Trizol reagent; RNA precipitation was done overnight in isopropanol and the RNA pellets were rinsed three times with ice-cold ethanol then air-dried and reconstituted in nuclease free water. RNA concentration was determined using Nanodrop 2000c (Thermo Scientific). For each sample, total RNA was used to synthesize cDNA via the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems). All procedures for RNA isolation were done using filtered pipette tips. *X. laevis nanos* RNA is exclusively found within the germ plasm and associates with the vegetal cortex (Elinson et al., 1993). To assess both the purity and cortical quality of our samples, we determined the amount of *nanos* RNA in the vegetal and animal pole samples by semi-quantitative RT-PCR (Lai et al., 2011). Vegetal pole samples with the highest levels of *nanos* RNA and oocyte-matched animal pole samples with no detectable *nanos* RNA were selected for RNA-seq analysis.

**Small RNA analysis and novel non-coding RNA identification (continued)**

For small RNA analysis, Cutadapt was used to remove adapter sequences from read ends. Reads trimmed to < 20bp were discarded. Next, the reads were aligned to a *X. laevis* specific microRNA (miRNA) reference using TopHat v2. 23% alignment was achieved. Samtools was used to filter then count the aligned reads using the following command:

```
$ samtools view aligned_file.bam miR21 > miR21.sam | wc –l
```

Since *X. laevis* miRNAs are not well annotated, we identified miRNA homologues in *X. tropicalis*. We subsequently queried TargetScan for miRNA targets. Target mRNAs were identified based on highly conserved regions in homologues human genes. Thus *X. tropicalis* miRNAs that match with human mRNA target homologs of vegetally localized RNAs from our RNA-seq analysis were reported.

To identify novel long non-coding (lnc) RNAs, Trinity software was used for de novo assembly of the transcripts in each sample. These assemblies were analyzed by Coding-Potential Assessment Tools (CPAT) and Coding Potential Calculator (CPC), which determined that there were no viable transcripts. Further analysis of the potential function of the lncRNAs was done using advanced modeling, but nothing significant could be detected based on the current platforms. Further analyses of lncRNAs will have to await better annotation of the Xenopus genome.

**Gene name identification and GO analysis (continued)**

The total transcript set of all vegetal pole up-regulated genes (≥4 fold enrichment; total = 198) was submitted to GeneGO for enrichment and pathway analysis. Un-annotated sequences represented approximately half of the total RNAs enriched at either the animal (12/27) or vegetal

poles (213/411) (Fig. 1D) and these are listed in supplemental Table 3. Metacore Pathway Analysis (GeneGO v6.18, www.genego.com) was used as previously described (Egan et al., 2014). Pathway analysis was determined using the direct interaction and analyze network building algorithms.

**Whole-mount *in situ* hybridization (WISH) (continued)**

*X. laevis* embryos were obtained as described in Sive et al., 2000. WISH was performed as described in Agüero et al., 2012 with the exception of day 1, which was performed as described in Colozza and De Robertis, 2014. Plasmids containing full-length clones were purchased from GE Dharmacon (*spire1*, *atrx*, *wnk2*, *rras2*, *mov10*, *trank1*, *sybu*, *otx1*, *e2f1*, and *dand5*) and Transomic Technologies (*efnb1*, *slc18a2*), and provided by Dr. Jing Yang (*hook2*, *tob2*, and *sox7*). Inserts were verified by restriction digestion and sequencing. Inserts were PCR amplified using the following primer sets: T7/Sp6 (*efnb1*, *rras2*, *mov10*, *tob2*, *sybu*, *sox7*, *rnf38*, *spire1*, *hook2*, *e2f1*, *trank1*, *otx1*, and *slc18a2*) or T3/Sp6 (*wnk2*, *atrx*, and *dand5*). *Xpat* clone was synthesized as described in Lai et al., 2012. Antisense probes containing Digoxigenin-11-UTP were synthesized using the T7, SP6 or T3 RNA polymerase (NEB). Primer sequences: T7: 5'-taatacgactcactatag-3'; T3: 5'-aattaaccctcactaaag-3'; Sp6: 5'-atttaggtgacactatag-3'.

**Gain-of-function analysis of vegetally enriched genes of interest**

cDNA clones encoding full-length genes were obtained from GE-Dharmacon (*parn*, # MXL1736-202774978; *rras2*, # MXL1736-202773625; *wwtr1*, # MXL1736-202784975; *e2f1*, # MXL1736-202809814; *otx1*, # MXT1765-202789330; *spire1*, # MXL1736-202787536), transOMIC (*efnb1*, ID: TCL1007) or as a gift (pCS107-Xt*sox7*, from Dr. Jing Yang, University of Illinois at Urbana). Synthetic capped RNAs for microinjection were obtained by *in vitro* transcription using the mMESSAGE mMACHINE SP6, T7 or T3 Kit (Ambion). DNA templates were linearized or amplified by PCR, transcribed and 0.5 ng of each mRNA was injected in the vegetal pole of 1-cell

embryos as follows: pCS107-Xt*sox7* (SP6/NotI); pCMV-SPORT6-Xl*parn* (SP6/NotI), pCMV-SPORT6-Xl*rras2* (SP6/NotI); pExpress1-Xl*e2f1* (SP6/PCR: T7/SP6); pExpress1-Xl*otx1* (SP6/PCR: T7/SP6); pCR4-TOPO-Xl*efnb1* (T3/PCR: T3/T7); pCMV-SPORT6-Xl*spire1* (SP6/NotI). All clone identities were confirmed by sequencing. All results shown are representative of at least two independent experiments. All embryos were fixed for WISH at stage 32-35 (tailbud). Embryos were staged according to Nieuwkoop and Faber (1956). WISH was performed using the germ plasm marker *xpat* to see PGC differences of injected RNAs. Both sides of each embryo were used for counting PGC number.

**Loss-of-Function analysis of novel germline RNAs**

Morpholinos targeting the following RNAs were purchased from gene tools: *efnb1*-MO (5'-CCACCTGTGCGGATGGAAGGGCTCC), *sox7*-MO (5'-GTCATTATTCCAACTGACTTGCTGA), *otx1*-MO (5'-TAGGACATCATGCTCAAGGCTGGAT), and *wwtr1*-MO (5'-TGGTACAACAGCTACTTCCCAAGGC). To test MO efficiency full length *X. laevis efnb1* was PCR amplified from stage VI oocyte cDNA with the following forward and reverse primers: 5'-GGATAATACAAAGCTGGTTTCTGTG and 5'- CTTTGCTCCTGTGATTGGATTG, respectively, and subsequently a flag tag (FL) was added using the following primers: forward: 5'-CAGCATGAATTCGGATTTAGCAGCTGAGGGCAAG; reverse: 5'-ATATTCTCGAGTCACTTGTCGTCATCGTCTTTGTAGTCCTTGTAGTAAATGTTTGCAGG. The flag-tagged *efnb1* insert was then cloned into PCS2+ using EcoRI/XhoI to make *efnb1*-FL. Additionally, *otx1* and *wwtr1* (GE-Dharmacon) were cloned into flag(FL)-pCS2+ using BamHI/ClaI and ClaI/ClaI to generate *otx1*-FL and *wwtr1*-FL, respectively. Flag-tagged full length *sox7* (*sox7*-FL) was synthesized and purchased from Genewiz. *efnb1*-FL-rescue was generated from *efnb1*-FL, by introducing conservative mutations in the region that binds the morpholino (5'ATGGAgGGtCTtCGGCGTCTTCTC), rendering the morpholino ineffective.

Mutations were introduced using the Q5 Site-Directed Mutagenesis Kit (New England Biosciences, E0554) according to the manufacturer's protocol.

All morpholinos were tested for knockdown efficiency using the Wheat Germ Extract kit (Promega, REF: L4380) according to the manufacturer's protocol. Translation of *efnb1*-FL, *otx1*-FL, *wwtr1*-FL, and *sox7*-FL were detected by Western blot analysis for flag (Figs. S2 and S3). Primary antibody used: Monoclonal mouse ANTI-FLAG (Sigma, REF: F1804); secondary antibody used: Anti-Mouse IgG HRP Conjugate (Promega, REF: W402B).

Embryos were injected vegetally with *otx1*-MO (15ng), *wwtr1*-MO (15ng), *efnb1*-MO (16ng), *efnb1*-FL (200pg), *efnb1*-FL (200pg) and *efnb1*-MO (16ng) together, *efnb1*-FL-rescue (200pg) and *efnb1*-MO (16ng) together, or scramble-MO (16ng) at the one-cell stage. Embryos were also injected with *sox7*-MO (16ng), *sox7*dCEnR (*X. laevis*) (200pg), Xt*sox7* (*X. tropicalis*) (200pg), or *sox7*dCEnR (200pg) and Xt*sox7* (200pg) mRNAs at the one-cell stage. *X. laevis sox7* dominant negative construct (*sox7*dCEnR) and *X. tropicalis sox7* WT (Xt*sox7*) were generous gifts from Dr. Mike Klymkowsky (University of Colorado, Boulder) and Dr. Jing Yang (University of Illinois at Urbana), respectively. In sox7dCEnR the transacting domain was deleted and the engrailed transcriptional repression domain was inserted downstream of the HMG box (Zhang et al., 2005). Synthetic capped mRNAs for microinjection were obtained by *in vitro* transcription using the mMESSAGE mMACHINE SP6 or T3 Kit (Ambion). DNA templates were linearized then transcribed as follows: *efnb1*-FL (ApaI/SP6); *efnb1*-FL-rescue (ApaI/SP6); *sox7*dCEnR (XhoI/T3); Xt*sox7* (NotI/SP6).

To inhibit p300 activity, embryos were incubated with the small molecule inhibitor of p300, C646, or DMSO as a control. To prevent reported light induced retinopathy by C646 (Kawase et al., 2016), embryos were protected from light.

Injected and treated embryos were collected and fixed at stage 32-35 (tailbud), staging according to Nieuwkoop and Faber (1956), and PGCs were identified using WISH against *xpat* as previously described (Lai, et al., 2012). PGC number per embryo was calculated by counting PGCs on both sides of each embryo. An embryo was considered to have mis-localized PGCs if PGCs were located anterior to somite 5 or posterior to somite 11 as described in Tarbashevich et al. (2011). All results shown are representative of at least two independent experiments. The p(t)-values were determined using a two-tailed unpaired Student's t-test. P-values <0.05 were considered significant.

**Ethical Statements**

The animal protocols used were evaluated and approved by the Institutional Animal Care and Use Committee (IACUC) of the University of Miami. All activities are in compliance with federal, state and institutional regulations. The University was granted full accreditation by the Association for Assessment and Accreditation of Laboratory Animal Care, International (AAALAC) in February 2005 and received its current re-accreditation in October 22, 2013. In addition, University of Miami is licensed by the U.S. Department of Agriculture (USDA) and has filed a Letter of Assurance with the Office of Laboratory Animal Welfare (OLAW), U.S. Department of Health and Human Services (DHHS).

## Supplemental FIGURES

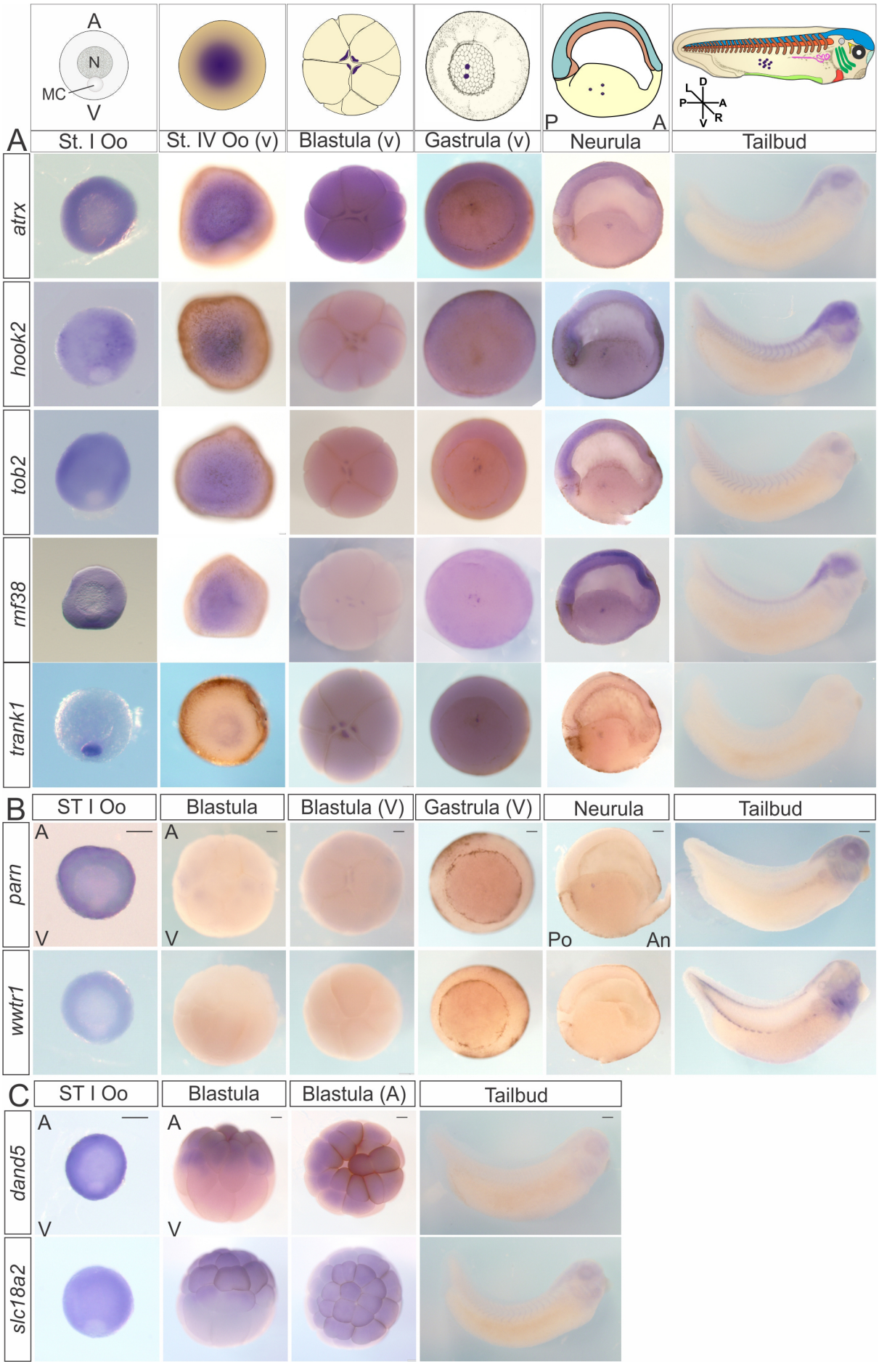

**Figure S1. Whole mount in situ hybridization (WISH) of select vegetal and animal pole transcripts.** Expression of a subset of RNAs enriched in the vegetal (A-B) and animal (C) poles were analyzed during oogenesis and development by WISH. Probes, developmental stages, and developmental structures are indicated: germplasm/PGCs (purple), pronephros (pink), ventral blood islands (lime green), eye (black), lens (white), otic vesicle (gray), cranial ganglia (yellow), brachial arches (green), nasal placodes (teal), inter-segmental region (brown), notochord (orange), brain and neural tube (blue). Transcripts detected in primordial germ cells at the tailbud stage are magnified and embedded in their respective images. Black bars represent 100um in st. I oocyte panels, and 200um in all other panels. Please refer to Fig. 3 for use of *xpat* probe as a positive control for germ plasm localization.

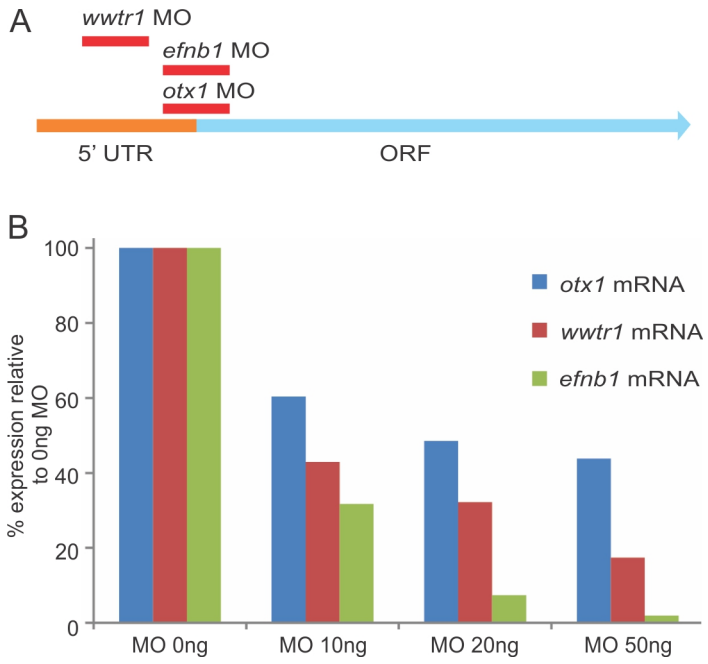


**Figure S2. Morpholinos (MOs) inhibit expression of their target RNAs.** A) Schematic of the *efnb1*, *otx1*, and *wwtr1* morpholino targeted regions are indicated in red. B) Wheat germ extracts were incubated with either *efnb1*-FL (1ug), *otx1*-FL (250ng), or *wwtr1*-FL (250ng) transcripts in the presence of increasing concentrations of their respective MOs and subject to anti-flag western blot analysis. Quantification of respective flag expression is shown.

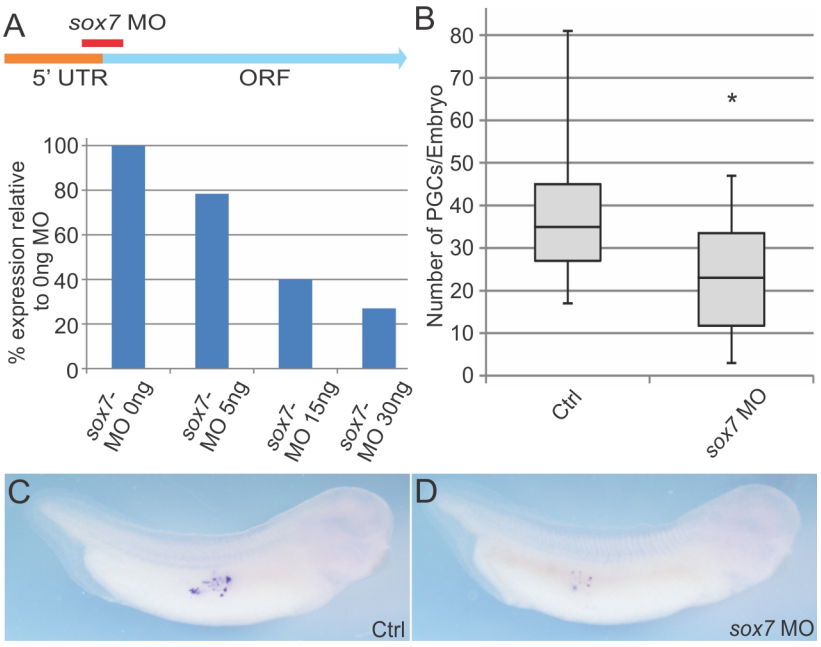


**Figure S3. Morpholino-mediated *sox7* inhibition reduces PGC number in tailbud embryos.** A) Schematic of the *sox7* morpholino target region (top). Wheat germ extract was incubated with *sox7*-FL (500ng) in the presence of increasing concentrations of *sox7*-MO and subject to anti-flag western blot analysis. Quantification of respective flag expression is shown (bottom). B-D) One-cell embryos were injected in the vegetal region with *sox7*-MO (16ng). Tailbud embryos were analyzed for *xpat* expression by WISH. The number of PGCs per embryo was quantified (B). Representative images are shown (C-D). Uninjected control (ctrl) n=36, *sox7*-MO n=43. * statistically significant compared to ctrl ($p<0.05$). Analysis based on at least two independent experiments.

**Supplemental Table 1**: Top 198 vegetally enriched transcripts identified by RNA-seq.

Click here to Download Table S1

**Supplemental Table 2**: All annotated transcripts identified using v6.0 and v7.1 *Xenopus laevis* scaffold sets.

Click here to Download Table S2

**Supplemental Table 3**: All un-annotated transcripts identified using v6.0 and v7.1 *Xenopus laevis* scaffold sets.

Click here to Download Table S3

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