Supplemental Data

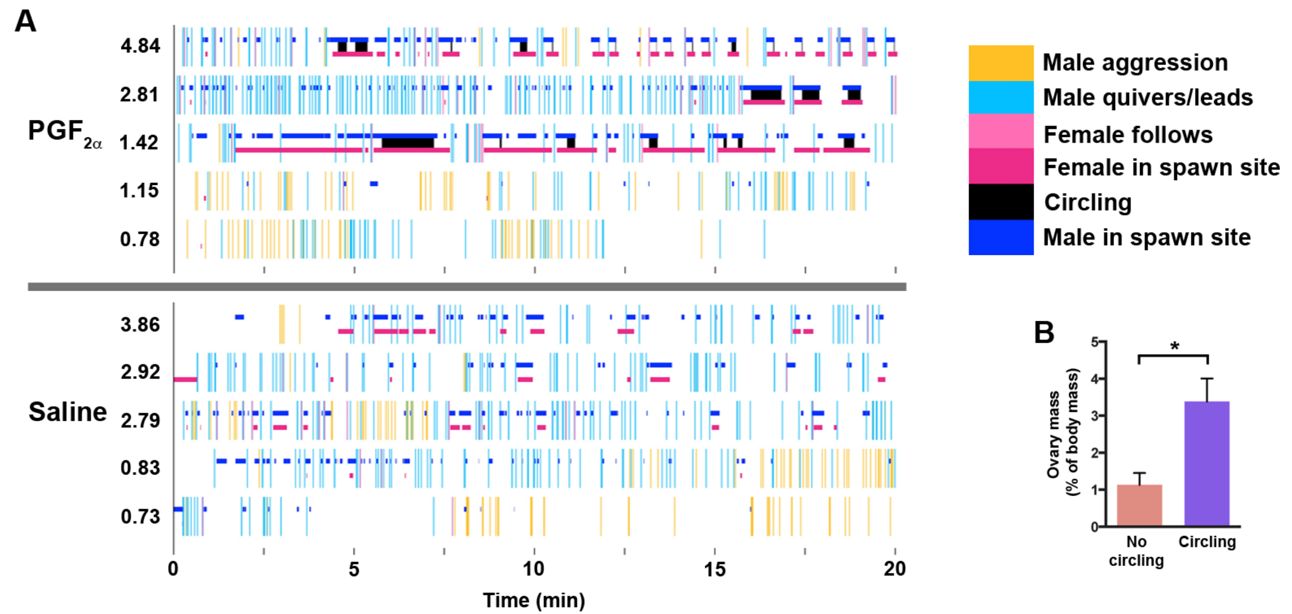
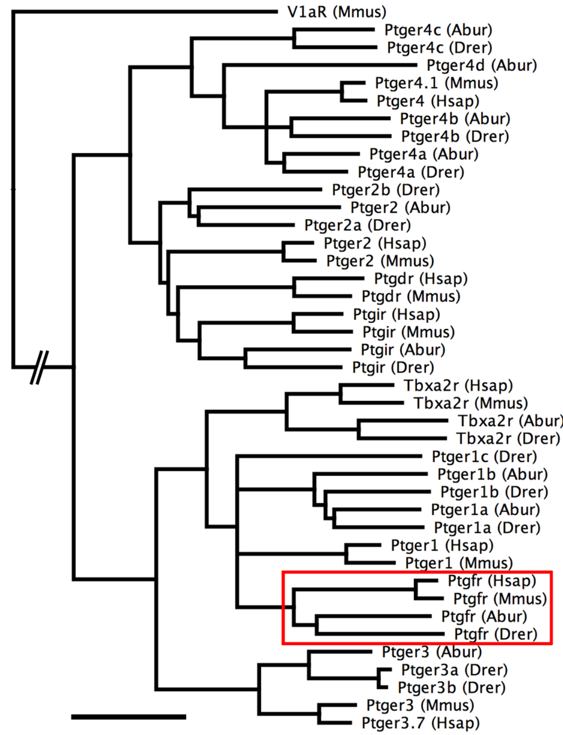


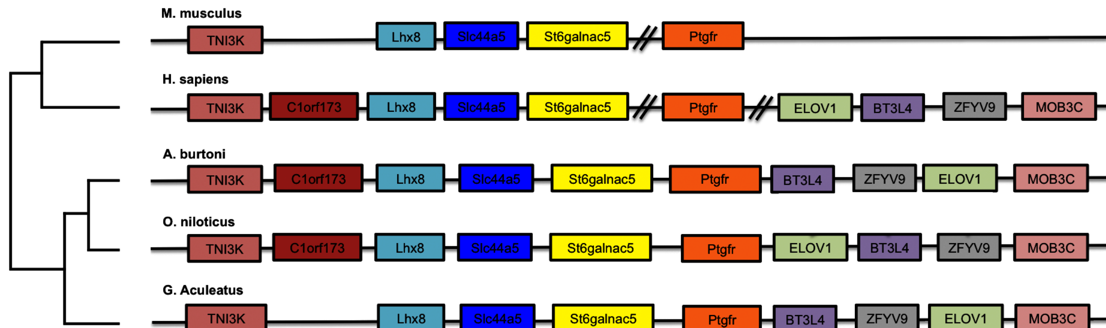
Figure S1 | Reproductive behaviors observed in spawning assays. Related to Fig. 1.

A, Representative raster plots depict the timing of male and female behaviors during a 20 minute portion of behavioral assays. Assays are arranged in descending order of normalized ovary mass (GSI). Time is measured from introduction of injected female to male tank. **B**, Females that proceeded to circle after $PGF_{2\alpha}$ injection had larger ovaries than those that did not spawn. *, *P* = 0.0021, Mann-Whitney test; n = 7-9 females per behavior group.

A



B



C

```
      1        10        20        30        40        50        60        70        80        90
Hsap  MSMNNSKQLVSPAAALLSNTTCQTENRLSVFFSVIFMTVGILSNSLAIAILMKAYQRFRQKSKASFLLLASGLVITDFFGHLINGAIAVFVYASDKEWI
Mmus  MSMNNSKQPVSPAAGLIANTTCQTENRLSVFFSIIFMTVGILSNSLAIAILMKAYQRFRQKSKASFLLLASGLVITDFFGHLINGIAVFVYASDKDWI
Abur  MSANGSESSCRSEVRPTNNTC-FQKELSITASVISMTVGIFSNSLALFILLKSYSSIRTKSRASFLLFASSLVITDLLGHLINGSLVLFVYSAEKKWE
Drer   MSHNRSAGCLMSSNVCNGSC-PKREVSVTSTGISMTVGIVSNTLALFILIKAYHRFRYKSKAAFLLFASGLVITDFLGHLINGSLALYVYVSQKEWE

      100       110       120       130       140       150       160       170       180       190
Hsap  RFDQSNVLCSIFGICMVFSGLCPLLLGSVMAIERCIGVTKPIFHSTKITSKHVKMMLSGVCLFAVFIALLPILGHRDYKIQASRTWCFYNTEDIKDWED
Mmus  RFDQSNILCSIFGISMVFSGLCPLFLGSAMAIERCIGVTNPIFHSTKITSKHVKMILSGVCMFAVFVAVLPILGHRDYQIQASRTWCFYNTEHIEDWED
Abur  NFDPYQIICTIFGACMVFFGLSPLFLGSAMAVERCIGVTKPIFHSTVLASHHMKRLLGVTWLLAALVAALPVMLSKPYKVQRSRSWCFFLIEQTKDWLD
Drer  TFDNHKSLCDFFGVCMAFFGLTPLLLGCLMAVERCIGVTRPLFHTTALGSHHVKRLLGVTWLLGLLVALLPVLFQRSYQVQRSRSWCFFRLQGPRDWMD

      200       210       220       230       240       250       260       270       280       290
Hsap  RFYLLLFSFLGLLALGVSLLCNAITGITLLRVKFK----SQQHRQGRSHHLEMVIQLLAIMCVSCICWSPFLVTMANIGINGNHSLETCETT-LFALRM
Mmus  RFYLLFFSFLGLLALGVSFSCNAVTGVTLLRVKFR----SQQHRQGRSHHLEMIIQLLAIMCVSCVCWSPFLVTMANIAINGNNSPVTCETT-LFALRM
Abur  VLLPLLFSLLGLLALLISIACNTLTGCVLIQAKLR----HKHHCRSMPYHIEMICQLLAIMLVSCICWGPLLIHVTILS--TRATDESPSFS-LLTIRM
Drer  TLLPILFSALGLLALLVSLLCNTMTFLTLLRSRVQLDRNNHRHSRKASHHSEMVCQLLAIMLVSCVCWGPILITAIIFSLQDRGEDQTSYTHMLLVVRM

      300       310       320       330       340       350       360       370       380       390 393
Hsap  ATWNQILDPWVYILLRKAVLKNLYKLASQCCGVHVISLHIWELSSIKNSLKVAAIS----------ESPVAEKSAST
Mmus  ATWNQILDPWVYILLRKAVLRNLYKLASRCCGVNIISLHIWELSSIKNSLKVAAIS----------ESPAAEKESQQASSEAGL
Abur  ATWNQILDPWVYILLRKAVLRKIFVLLHGCCGSKFYTLHRWQRSMLHSSAETSSSS--GSSDCRCLGKTPVHDVVIKSIT
Drer  ATWNQILDPWVYILLRKAVLRRLFKVALRLCSRHLKFTFQWQSSNLGSSTMEASSSVISRPDFTPLDGTFLPDTAIKLAHLPRASLGDTRSQFTDI
```

Figure S2 | Prostaglandin F receptor sequence search. Related to Figure 2.

A, Known prostaglandin receptor protein sequences from human (Hsap), mouse (Mmus), and zebrafish (Drer) were aligned with predicted *A. burtoni* (Abur) sequences using MUSCLE, and assembled into a phylogenetic tree. Red box highlights *Ptgfr* clade. Scale bar represents 0.3 substitutions per site.

B, Synteny analysis identifies conservation of blocks of genes surrounding vertebrate *Ptgfr* and a putative *A. burtoni Ptgfr*. Not to scale.

C, *A. burtoni* predicted Ptgfr protein sequence exhibits high identity with Ptgfr from other vertebrate species, including conservation of residues crucial for folding and signaling. Residues in black are identical in 3-4 species, while residues in gray are shared by ≤2 species. Seven predicted transmembrane regions are indicated by red bars. Putative N-glycosylation sites are indicated by gray boxes, while cysteine residues important for disulfide bonds are boxed in blue. Residues implicated in ligand binding and downstream signaling are indicated by green. These include residues implicated in $PGF_{2\alpha}$ binding (His-81, Ser-262), and signal transduction (Asp-77, Arg-296, Asn-301, and Asp-305), including the ERC motif (132-134). Numbering corresponds to human Ptgfr.

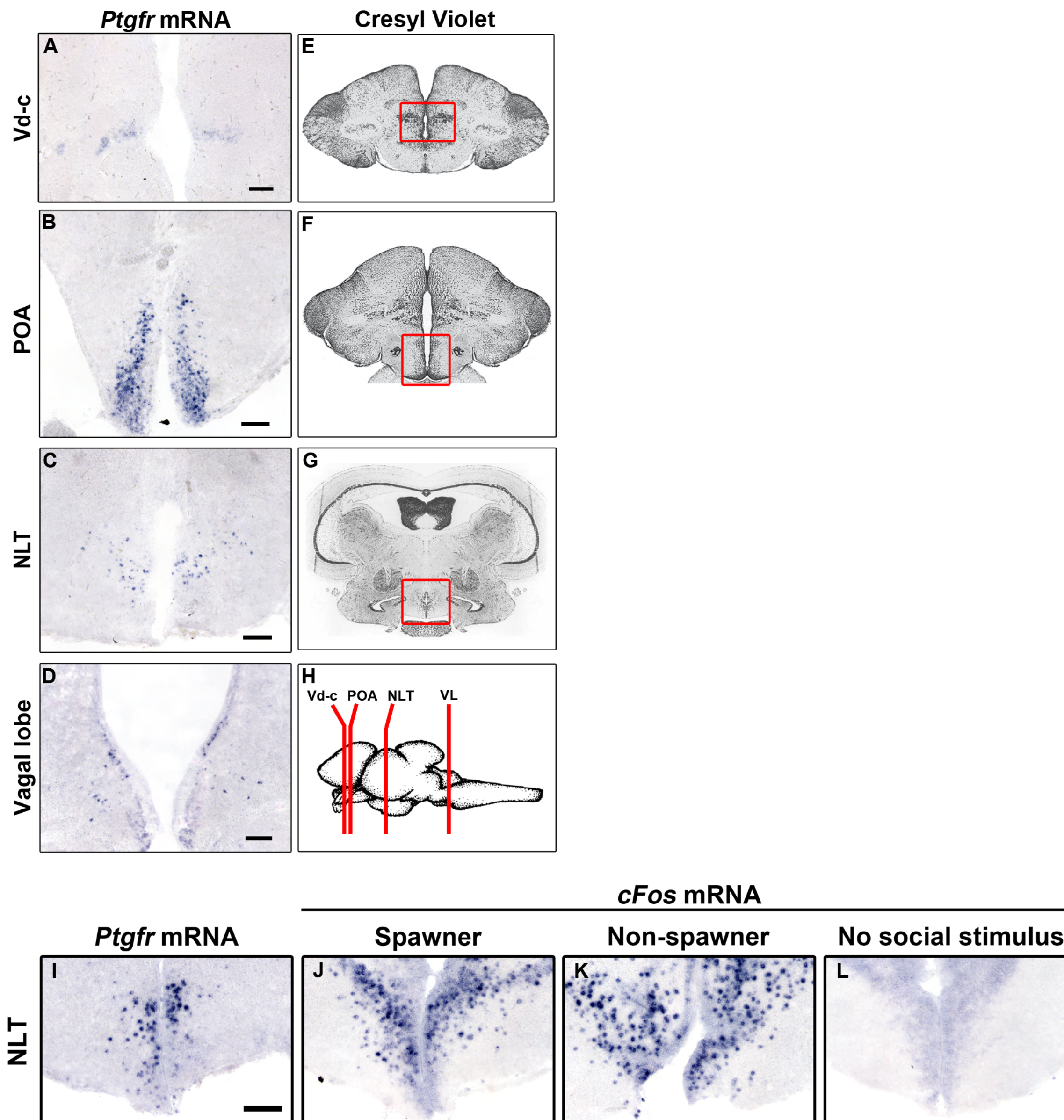
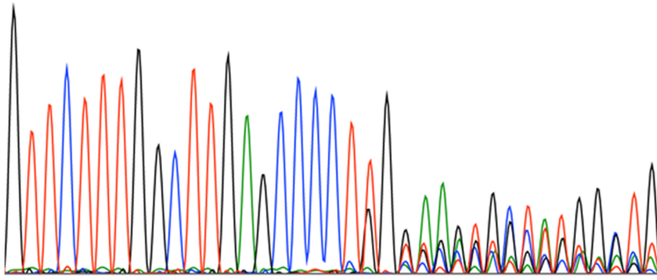


Figure S3 | *Ptgfr* mRNA is expressed in discrete regions of *A. burtoni* brain. Related to Figure 2.

A-D, Cells expressing *Ptgfr* mRNA were labeled using ISH in coronal brain sections. **E-G**, Cresyl violet-stained sections that indicate the location of the Vd-c, POA, and NLT [S1, S2]. **H**, Profile view of *A. burtoni* brain schematic delineates location of coronal sections. **I-L**, The *Ptgfr*+ region of NLT (**I**) in uninjected females expresses *cFos* mRNA after being allowed to spawn naturally when exposed to a male (**J**), as do that of females that were courted by a male, but did not spawn (**K**). **L**, Females not exposed to an acute social stimulus do not express *cFos*. Scale bars, 100 µm; n $\geq$ 3 females per condition.

A



B

Sequence	Mutation	Number
GTTCTTTGGCTTGAGCCCCTTGTTCCTGGGAAGTGCTAT	*Ptgfr*	
GTTCTTTGGCTTGAGCCCCTTGTTCCTTCCCCTGGGAAG	ins6	10
GTTCTTTGGCTTGAGCCCCTTG-------GAAGTGCTAT	del7	4
GTTCTTTGGCTTGAGCCCCTTGTTGCCCCTTCCCCCTGG	ins10	9
GTTCTTTGGCTTGAGCCCCTTG------GGAAGTGCTAT	del6	2
GTTCTTTGGCTTGAGCCCCT-------GGGAAGTGCTAT	del7	5
GTTCTTTGGCTTGAGCCCCTTGTTGGAGCCCCTGGGAAG	ins6	4
GTTCTTTGGCTTGAGCCCCTTGTT--------GTGCTAT	del8	3
GTTCTTTGGCTTGAGCCCCTTGT-----GGAAGTGCTAT	del5	2
GTTCTTTGGCTTGAGCCCC------CTGGGAAGTGCTAT	del6	1
GTTCTTTGGCTTGAGCCCCTTGT----GGGAAGTGCTAT	del4	1
GTTCTTTGGCTTGAGCCCCTTGTTCCTTCTTCCCCTTGG	ins10	2
GTTCTTTGGCTTGAGCCCCTTGAGCCCCTTGAGCCCCTG	del2,ins13	3
GTTCTTTGGCTTGAGCCCCTTGTT---GGGAAGTGCTAT	del3	2
--------------//---------CCTGGGAAGTGCTAT	del177	3

Figure S4 | Sequence analysis of CRISPR/Cas9-injected fish and germline transmission. Related to Figure 4.

A, PCR amplification and Sanger sequencing of *Ptgfr* locus from G_0 fish reveals extensive mutation and mosaicism.

B, Germline-transmitted *Ptgfr* alleles identified among G_1 fish. Arrowhead indicates Cas9 cut site, underlined sequence indicates protospacer-adjacent motif, mutated allele sequence is shown in red. To the right are the type of mutation detected, and the number of each allele recovered among G_1 fish.

Table S1 | Sequences reported. Related to Figure 2.

Gene name	Human	Mouse	Zebrafish	*A. burtoni*
Ptgfr	NP_000950	NP_032992	NP_001172000	XP_005946570
Ptger1	NP_000946	NP_038669	NP_001159805 NP_001159763 NP_001159802	XP_005924986 XP_005926783
Ptger2	NP_000947	NP_032990	NP_956929 NP_001025421	XP_005945213
Ptger3	NP_942011	NP_035326	XP_001340725	XP_005923427
Ptger4	NP_000949	NP_001129551	NP_001121839 NP_001034718	XP_005919111 XP_005917286 XP_005939395 XP_005934982
Ptgir	NP_000951	NP_032993	XP_689903	XP_005915826
Ptgdr	NP_000944	NP_032988	-	-
Tbxa2r	NP_001051	NP_033351	NP_001272464.1	XP_005920964

Table S2 | Allele frequencies in F_1 generation. Related to Figure 4.

Allele	# alleles detected	n alleles	Allele frequency	n broods
+	203	382	0.53	11
del8 (CCTGGGAA)	3	8	0.38	1
del7 (TTCCTGG)	21	102	0.21	4
del7 (TGTTCCT)	11	44	0.25	2
ins10 (GCCCCTTCCC)	53	250	0.21	6
ins6 (TCCCCT)	13	56	0.23	2
del177	62	248	0.25	5
ins6 (GGAGCC)	11	34	0.32	1
ins10 (TCTTCCCCTT)	5	22	0.23	1
All mutant alleles	179	764	0.23	11

Supplemental Experimental Procedures

Animals

Fish were bred and maintained at Stanford University from a colony derived from Lake Tanganyika [S3]. All animal procedures followed AAALAC standards. Aquarium water with cichlid salt and Tanganyika buffer (Seachem) was 28ºC and pH 8, and full-spectrum lights illuminated the tanks in a 12-hour light:12-hour dark cycle. Fish were fed each morning with cichlid flakes and pellets (AquaDine). Fish were housed separately by sex in 60 liter aquaria prior to behavioral assays: males were housed singly, while females were housed in groups of 10-20 fish.

Spawning behavioral assay

$PGF_{2\alpha}$ (Sigma) was resuspended in DMSO at 30 µg/µl, aliquoted, and stored at -20ºC until use. On the day of injection, $PGF_{2\alpha}$ or DMSO vehicle was thawed, diluted 1:10 in saline (0.6% NaCl), and Fast Green FCF (Santa Cruz Biotechnology) was added for visualization at a final concentration of 0.01%. Between 1:00 and 3:00 pm, non-gravid females were visually identified by the absence of any abdominal distension due to ovary growth. They were injected intraperitoneally with a 26 gauge needle on a Hamilton syringe by an experimenter blind to treatment with 2-4 µl of $PGF_{2\alpha}$ or vehicle, commensurate with female size. This results in a dose of ~1.5 µg per g body weight, a dose based on prior work [S4-S6]. Injected females were introduced into a male's tank immediately after injection. Behaviors were video recorded for 30 minutes for later coding, beginning when the female entered the tank. The assay was observed by the experimenter from behind a curtain to determine whether spawning occurred. The female was removed from the male's tank 30 minutes after spawning began in order to collect tissue samples. If no spawning was observed, females were removed at 60 minutes after the female was introduced into the male's tank. Naturally spawning females in Figures 2 and S3 were collected by allowing a male access to a community that contained uninjected females of various reproductive stages. Brains were dissected from females 30 minutes after observing spawning behavior, and simultaneously from a control female from the same tank that did not spawn. Brains were also collected from females in a stable community tank not perturbed by a behavioral assay to assess basal expression of *cFos*. For $PGF_{2\alpha}$ injections of CRISPR fish, all females were tested, regardless of ovarian status.

Behavior assays were coded by an observer blind to treatment using a custom software package written in MATLAB [S7]. We recorded the timing of male quivers, leads, bites, and pot entries; female follows, pot entries,

and spawn-like behavior. Behaviors were identified as in [S3]; spawning behavior was identified when both male and female entered the pot, and took turns (≥ 2 times) quivering while the other pecked at the anal fin.

Ovariectomy (OVX) and 17α,20β-dihydroxyprogesterone (DHP) treatment

Adult female *A. burtoni* were prepared for surgery by an initial 20-30 second anesthetization in tricaine mesylate, (MS-222; 0.1 g/ 200 mL). After the fish were fully sedated, as determined by unresponsiveness to a tail pinch, an incision was made along their ventral surface between the pectoral fins and the cloaca. Ovaries were detached at their anterior and posterior ends. Fish were then sutured, revived in oxygenated water, and allowed to recover in isolation for approximately 1 week in 32 L recovery tanks treated with methylene blue anti-fungal (1 mg/L stock; Sigma-Aldrich) and Furan-2 antibiotic (1 packet of 60 mg Nitrofurazone and 25 mg Furazolidone per 10 gallons; API). Females were then group housed with other operated females in a 64 L tank until hormone injections at 1-2 weeks post-surgery. OVX females were injected intraperitoneally with a 26 gauge needle on a syringe (Hamilton) with DHP at 125 ng/g body weight (Sigma-Aldrich; 10 mM stock in DMSO was diluted 1:10 in 0.6% saline) or DMSO/saline vehicle, then returned to their tanks. Brains were collected at 3 hours post-injection and processed for ISH as described below. We also checked at sacrifice for residual ovary tissue, and eliminated from analysis any females with detectable tissue.

Statistics

We analyzed all two-group data using Prism (GraphPad). As continuous behavioral data could not be confirmed as normally distributed (Lillefors' test, MATLAB), we applied Mann-Whitney tests. Latencies were compared using the Mantel-Cox test. Fractions of assays with spawning were compared using a Fisher's Exact test. Raster plots and transitional probabilities were generated using a custom software package in R (http://fernaldlab.stanford.edu/resources). We used Mann-Whitney U-tests for two-group comparisons of continuous data and Fisher's Exact test for categorical data. Transitional probabilities were calculated by dividing the total number of each behavior by the number of instances in which the subsequent behavior occurred. Arrow weights in Figure 1J are only shown for transitions with probability ≥4%. We selected 5 $PGF_{2\alpha}$-injected females with a similar number of circling bouts to compare with 5 naturally spawning females, and matched 5 vehicle-injected females by their comparable GSI. We used Mann-Whitney U-tests to compare transition probabilities across groups, with a

Bonferroni-corrected cutoff of $\alpha=0.0027$ to correct for 19 transitions we observed in Figure 1J. Comparison of *Ptgfr* mRNA staining levels between females of various reproductive conditions was performed using a Kruskal-Wallis test, followed by Dunn's post-hoc test. Ovary sizes are normalized by body weight to give gonadosomatic index (GSI; ovary weight / body weight * 100).

Nucleic acid sequence analysis

To identify prostaglandin receptor homologs, we used tBLASTn [S8] to search for loci in the *A. burtoni* genome (v1; Broad Institute, Cambridge USA) with high homology to mammalian prostaglandin receptors. We used protein sequences from the mouse and human receptors (Table 1) to query. We generated phylogenetic trees of the prostaglandin receptors (Table 1; Geneious; MUSCLE alignment followed by tree assembly using Jukes-Cantor algorithm with neighbor end joining, 1000 bootstraps). To predict orthologs by synteny conservation, we used Bouillabase (T. Kocher, University of Maryland) to determine the genes neighboring *A. burtoni Ptgfr*, and the UCSC genome browser [S9] and Genomicus [S10] to determine the genes neighboring *Ptgfr* in other species.

In situ *hybridization (ISH)*

We used RT-PCR to amplify a portion of coding sequence from *Ptgfr* and *cFos* (NM_001286320), and subcloned products into pCR-TOPO4 (Life Technologies). Ptgfr forward, 5'-AAC CAA AGA CTG GCT GGA TG-3'; Ptgfr reverse, 5'-AAA TTT CGA GCC ACA ACA GC-3'; cFos forward, 5'-AAT TGG ATC CAA GCC CAG ATC TTC AGT GG-3'; cFos reverse, 5'-AAT TGA ATT CAT AGC CCT GTG ATC GGC AC-3'. Antisense RNA probe was transcribed using T3 (*Ptgfr*; Ambion) or T7 (*cFos*; Promega) RNA polymerase. Dissected brains were fixed in 4% paraformaldehyde (PFA) overnight, cryoprotected for 1-2 days in 30% sucrose (in PBS; Gibco), embedded in Neg50 (Thermo) and stored at -80ºC until cryostat sectioning at 30 µm. We performed ISH on slides that contained every fourth section. Slides were fixed in 4% PFA before Proteinase K (10 µg/ml; Life Technologies) treatment, followed by a second fixation in PFA. We acetylated sections, incubated in prehybridization buffer at 62ºC for 1-3 hours, and incubated in hybridization buffer with 0.2 ng/µl digoxygenin-labeled probe overnight at 62ºC. The next day, slides were washed in 50% formamide and 2x SSC at 62ºC, followed by washes in 2x SSC at 37ºC and a 30 minute treatment with RNase (200 ng/ml; Qiagen) in 2x SSC at 37ºC. Next, slides were washed in maleic acid buffer (MABT; 100 mM Maleic Acid, 150 mM NaCl, 0.1% Tween-20), then blocked in MABT plus 2% BSA for 1-3

hours. Anti-digoxygenin antibody Fab fragments conjugated to alkaline phosphatase (Roche; 1:5000) were diluted in MABT plus BSA, and incubated on slides at 4ºC overnight. On the third day, slides were washed in MABT prior to detection of alkaline phosphatase with NBT (37.5 µg/ml; Roche) and BCIP (94 µg/ml; Roche) for 1.5 hours (*cFos*) or 5 hours (*Ptgfr*) at 37ºC. A detailed protocol is available upon request.

Sections were imaged with brightfield optics on a Zeiss Axioskop microscope using SPOT software (Diagnostic Instruments). Quantification of ISH staining was performed in ImageJ (NIH) by an experimenter blind to experimental conditions. For *Ptgfr* mRNA, a convex polygon was drawn around all stained cells within the POA. For *cFos* mRNA, a region of interest was drawn around the POA, as defined in [S1]. Then, we inverted the image color, so that more intense staining was reflected in increased pixel values. We obtained the area bounded by the polygon, and the mean pixel value within it. This procedure was repeated independently for each hemisphere and each section with staining. To adjust for background noise, on each section a polygon was drawn in a neighboring brain region, and the mean pixel count was subtracted from the values of the stained region of interest. This adjusted mean pixel count was multiplied by the area to obtain a measure of the total signal above background. This value was added across all sections in a hemisphere containing staining, and then averaged between hemispheres.

Generation of Ptgfr *mutant fish*

Mutations of *Ptgfr* were induced by injection of a single guide RNA (sgRNA) targeting the second transmembrane domain, such that a frameshift mutation would result in a premature stop that deletes ~54% of the protein. We annealed the oligonucleotides gPtgfrF, 5' – TAG GCT TGA GCC CCT TGT TCC T – 3', and gPtgfrR, 5' – AAA CAG GAA CAA GGG GCT CAA G – 3', and ligated the product into pT7-gRNA [S11, S12]. Plasmid was linearized with BamHI (NEB) and transcribed using T7 polymerase (NEB) and purified. To produce mRNA encoding zebrafish codon-optimized Cas9 with two nuclear localization sequences (nls-zCas9-nls), we linearized pT3TS [S12] with XbaI, transcribed mRNA using T3 polymerase, and purified with a RNeasy mini column (Qiagen). Injection and raising of embryos followed prior work that generated transgenic *A. burtoni* [S13, S14]. Briefly, we group housed 10-15 females in a 168 x 46 x 30 cm tank, separated by a barrier from a stud male. Upon removing the barrier and allowing the females to interact with the male, we watched for a spawning female. If a female spawned with the male, we waited 30 min for fertilization, and then collected eggs for injection at the single-cell stage. We delivered to the cell of each fertilized egg ~1 nL of a cocktail containing 12 ng/µL *Ptgfr* sgRNA, 60

ng/µL *nls-zCas9-nls* mRNA, and 0.3% Texas Red-conjugated dextran (3000 MW, Life Technologies). After injection, fertilized eggs were transferred into individual wells of 6-well plates containing tank water and 1 mg/L methylene blue antifungal, where they were allowed to develop until ~10 days post-fertilization (dpf). These developing G_0 fry were housed in 1.5 L tanks until ~5 weeks of age, when we tested a finclip for *Ptgfr* mutations. We PCR amplified a 554 bp amplicon spanning the sgRNA binding site with the primers PtgfrFlankF, 5' – CTT CTC CAA CAG CCT TGC TC – 3' and PtgfrFlankR, 5' – CAC AGC CTG TTA GCG TGT TG – 3', and Sanger sequenced the product with PtgfrFlankF (ElimBio). Those fish showing evidence of mutant *Ptgfr* alleles were transferred to 32 L tanks. At reproductive maturity (as judged by the appearance of male-typical coloration), fish were separated by sex, and then wild-type fish of the opposite sex were added to those tanks. We observed tanks regularly to watch for the appearance of mouthbrooding females, and then collected those embryos (G_1) at 5-12 dpf. When G1 embryos reached 4-6 weeks of age, we genotyped a tailfin sample for mutations at *Ptgfr* as described above. Those G_1 fish carrying an indel predicted to result in a frameshift mutation were raised and then intercrossed. Offspring of G_1 intercrosses (F_1) were genotyped, and we saved fish carrying biallelic mutations of *Ptgfr* and homozygous wild-type fish, housed in 32-L tanks. We followed the development of these fish, noting when they showed signs of sexual maturity: bright coloration in males with eyebar and egg spots [S3], the onset of abdominal distention due to ovary growth, and mouthbrooding in females. 3-8 days after noting mouthbrooding, we collected a finclip from the female for later genotyping and removed her eggs. 40-90 days after the first observation of mouthbrooding, we tested behavior of females after injection with $PGF_{2\alpha}$, as detailed above. Additional information and protocols can be found at http://fernaldlab.stanford.edu/resources.

Supplemental References

S1. Burmeister, S.S., Munshi, R.G., and Fernald, R.D. (2009). Cytoarchitecture of a cichlid fish telencephalon. Brain Behav Evol *74*, 110-120.

S2. Fernald, R.D., and Shelton, L.C. (1985). The organization of the diencephalon and the pretectum in the cichlid fish, Haplochromis burtoni. J Comp Neurol *238*, 202-217.

S3. Fernald, R.D., and Hirata, N.R. (1977). Field-Study of Haplochromis-Burtoni - Quantitative Behavioral Observations. Anim Behav *25*, 964-975.

S4. Stacey, N.E. (1976). Effects of indomethacin and prostaglandins on the spawning behaviour of female goldfish. Prostaglandins *12*, 113-126.

S5. Liley, N.R., and Tan, E.S.P. (1985). The Induction of Spawning Behavior in Puntius-Gonionotus (Bleeker) by Treatment with Prostaglandin-Pgf2a. J Fish Biol *26*, 491-502.

S6. Cole, K.S., and Stacey, N.E. (1984). Prostaglandin induction of spawning behavior in Cichlasoma bimaculatum (Pisces cichlidae). Horm Behav *18*, 235-248.

S7. Wu, M.V., Manoli, D.S., Fraser, E.J., Coats, J.K., Tollkuhn, J., Honda, S.I., Harada, N., and Shah, N.M. (2009). Estrogen Masculinizes Neural Pathways and Sex-Specific Behaviors. Cell *139*, 61-72.

S8. Altschul, S.F., Madden, T.L., Schaffer, A.A., Zhang, J., Zhang, Z., Miller, W., and Lipman, D.J. (1997). Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res *25*, 3389-3402.

S9. Kent, W.J., Sugnet, C.W., Furey, T.S., Roskin, K.M., Pringle, T.H., Zahler, A.M., and Haussler, D. (2002). The human genome browser at UCSC. Genome Res *12*, 996-1006.

S10. Louis, A., Muffato, M., and Crollius, H.R. (2013). Genomicus: five genome browsers for comparative genomics in eukaryota. Nucleic Acids Res *41*, D700-D705.

S11. Varshney, G.K., Pei, W., LaFave, M.C., Idol, J., Xu, L., Gallardo, V., Carrington, B., Bishop, K., Jones, M., Li, M., et al. (2015). High-throughput gene targeting and phenotyping in zebrafish using CRISPR/Cas9. Genome Res *25*, 1030-1042.

S12. Jao, L.E., Wente, S.R., and Chen, W.B. (2013). Efficient multiplex biallelic zebrafish genome editing using a CRISPR nuclease system. Proc Natl Acad Sci U S A *110*, 13904-13909.

S13. Ma, Y., Juntti, S.A., Hu, C.K., Huguenard, J.R., and Fernald, R.D. (2015). Electrical synapses connect a network of gonadotropin releasing hormone neurons in a cichlid fish. Proc Natl Acad Sci U S A *112*, 3805-3810.

S14. Juntti, S.A., Hu, C.K., and Fernald, R.D. (2013). Tol2-Mediated Generation of a Transgenic Haplochromine Cichlid, Astatotilapia burtoni. PLoS One *8*, e77647.