SUPPLEMENTARY DATA

Figure S1: Developmental RT-PCR of genes involved in PPR specification

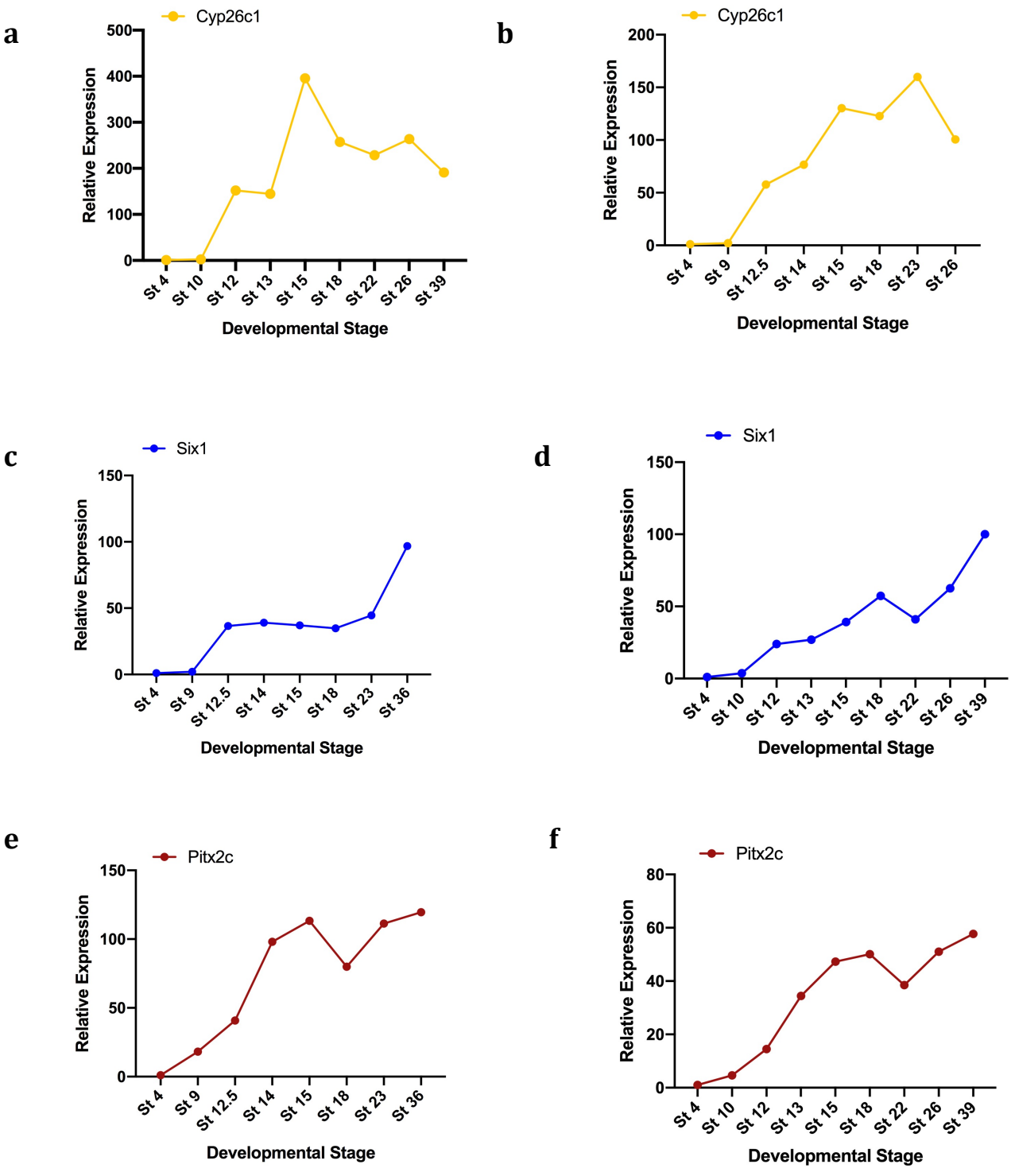

Figure S1: Developmental qRT-PCR expression profile *cyp26c1* (a,b), *six1* (c, d) and *pitx2c* (e, f) in two biological replicates (a, c, e and b, d, f) of WT *Xenopus* embryos. NF stages are indicated on x-axis, values are normalized to ODC.

Figure S2: Endogenous retinoid detection in the whole embryo by LC-MS/MS

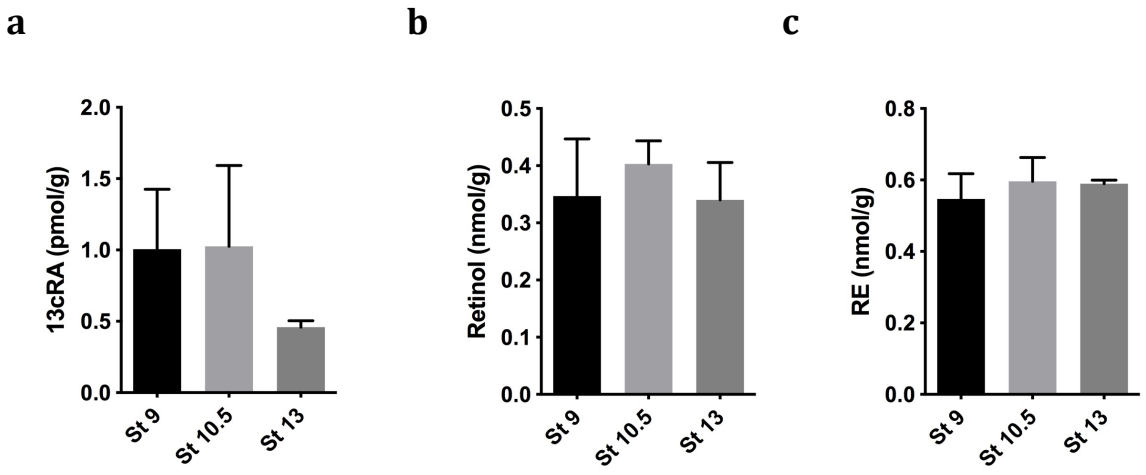

Figure S2: Endogenous retinoids in the whole embryo at stage 9, 10.5 and 13. (a) 13-*cis*-RA, (b) retinol, and (c) retinyl ester (RE). Data are for 130 embryos per group and are shown as mean ± SD, n=3 groups per timepoint and NF stages are indicated on the x-axis.

Figure S3: Cyp26c1 knockdown in Animal Caps Results in loss of Six1 and Pitx2c

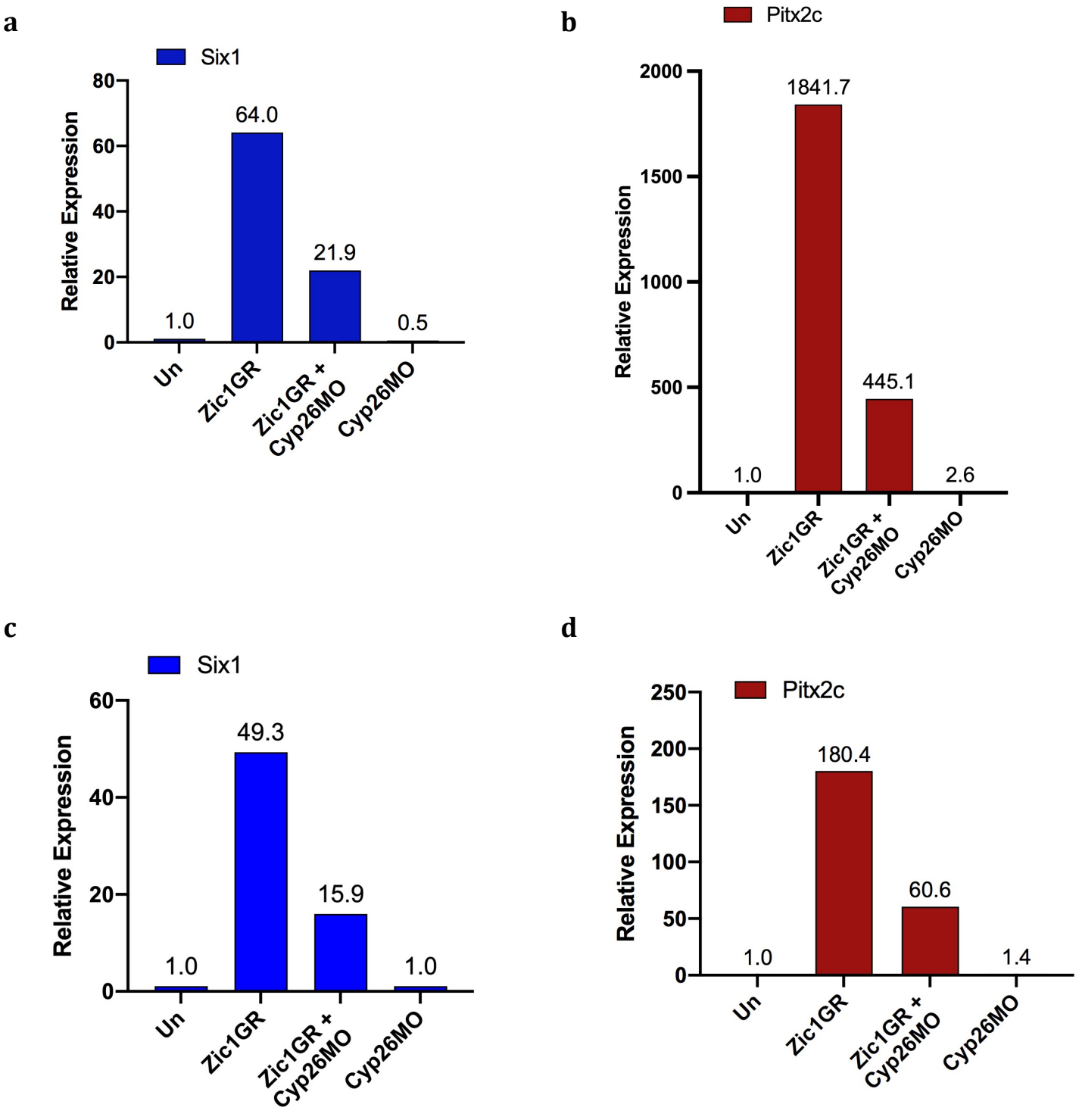

Figure S3: qRT-PCR analysis of *six1* (a, c) and *pitx2c* (b, d) expression in Zic1GR and Zic1GR+Cyp26MO injected animal caps from two biological replicates (a, b and c, d). Expression levels are normalized to ODC.

Figure S4: Cyp26c1 CRISPR/Cas9 Knockout validated by Direct Sequencing of PCR Product (DSP) Assay

Figure S4: Verification of sgRNA-mediated knockout of Cyp26c1 via DSP assay. Sanger sequencing of genomic DNA (gDNA) obtained from single embryos (1 to 3) injected with either Cas9 alone or with SL1 (a) or SL2 (b). For each sgRNA, the sequence of Cyp26c1 S form (Cyp26c1.S) was used as reference, annotated with the respective sgRNA target (gray bar) and PAM sequence (red bar). Sequencing traces of gDNA obtained from single embryos injected with both sgRNA (SL1, a; SL2, b) show occurrence of indels (insertion or deletion of bases) around the PAM site, which are absent in gDNA obtained from embryos injected with Cas9 only. The base and amino acid mismatches are indicated. Alignments were compiled using Geneious Prime.

Figure S5: Zic1 mediated induction of Six1 and Pitx2c is dependent on RA

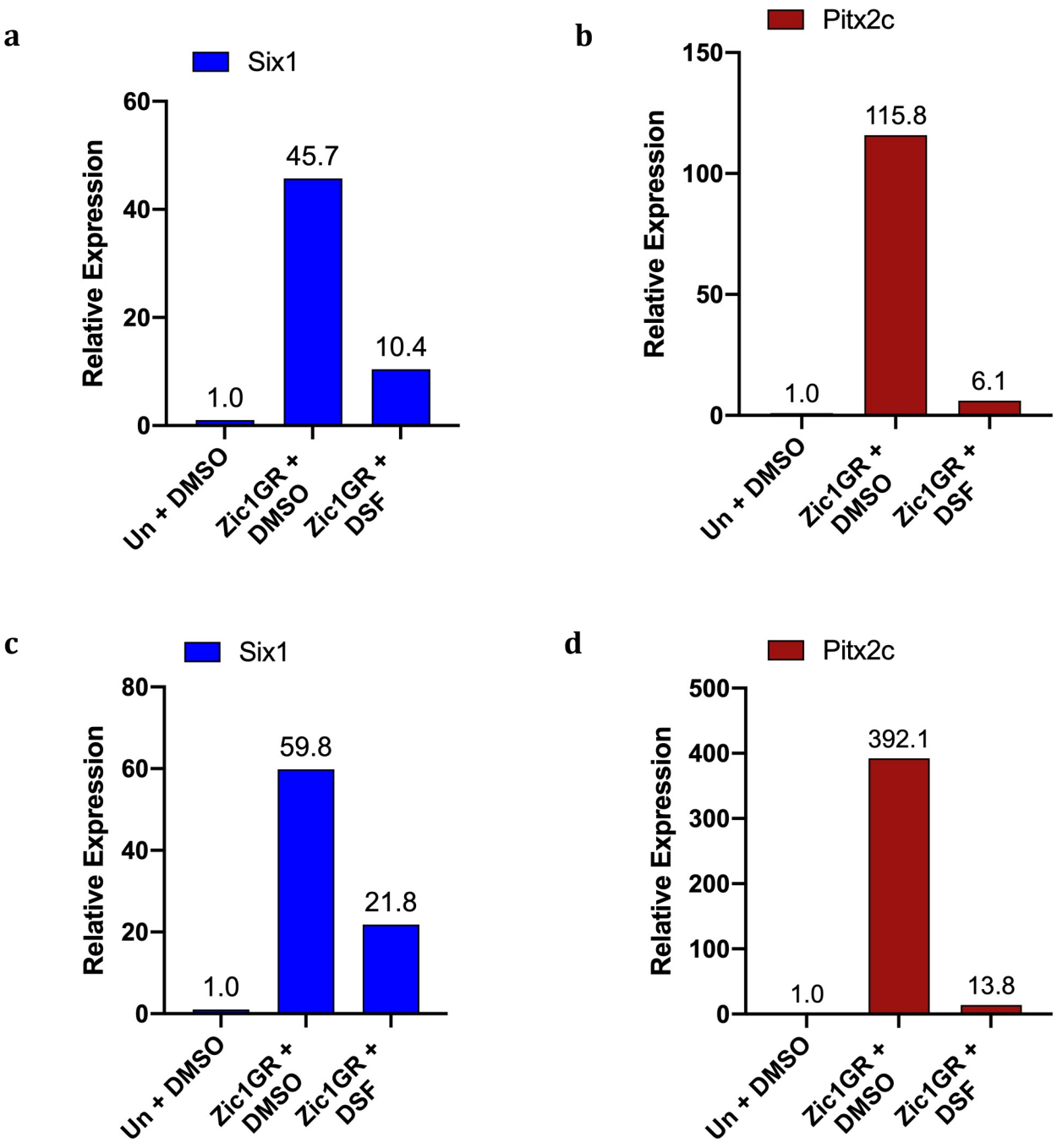

Figure S5: qRT-PCR analysis of *six1* (a, c) and *pitx2c* (b, d) expression) expression in animal cap explants injected with Zic1GR mRNA and cultured for 8 hours with or without Disulfiram (DSF; 100µM). Two biological replicates are shown (a, b and c, d), with values normalized to ODC.

Figure S6: Validation of Pitx2c splice-blocking morpholino

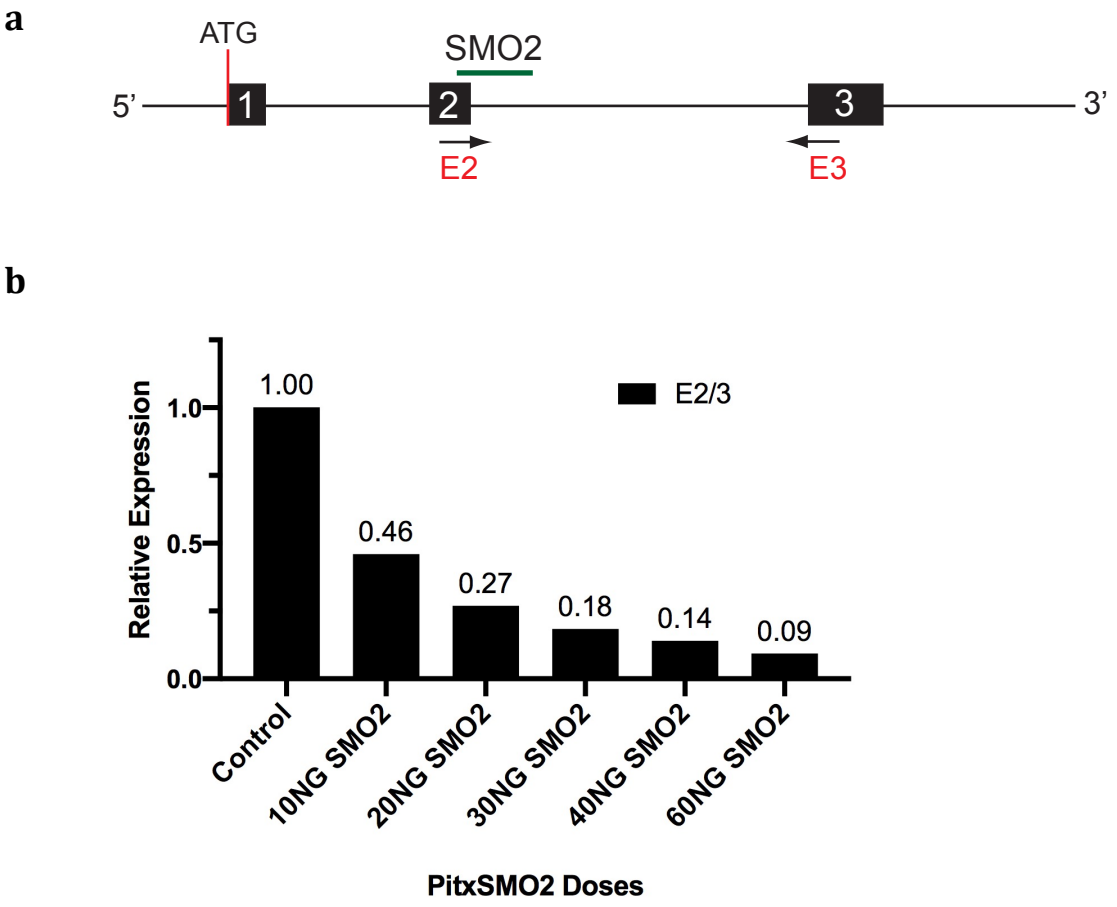

Figure S6: (a) Schematic representation of the *pitx2c* gene structure, indicating the target site for the splice-blocking (SMO2; green bar) morpholino. The primers used to detect exon skipping are indicated in red (E2, E3). (b) qRT-PCR analysis of total RNA from embryos bilaterally injected with increasing doses of PitxSMO2 as shown.

Figure S7: Pitx2c induces Cyp26c1 and Fgf8a

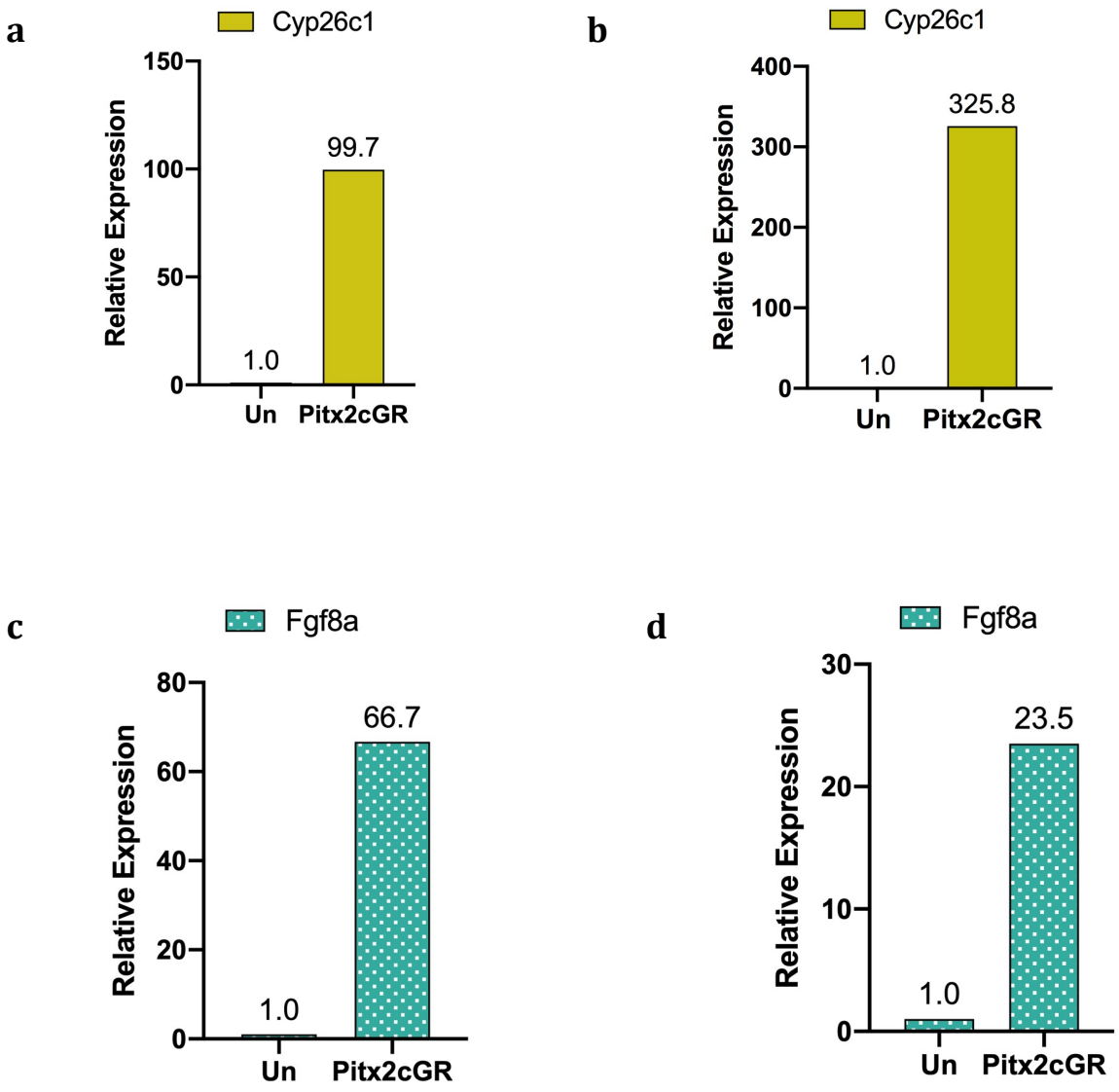

Figure S7: qRT-PCR analysis of *cyp26c1 and fgf8* expression in animal cap explants injected with Pitx2cGR mRNA and cultured for 8 hours and 4 hours, respectively. Two independent biological replicates are shown for cyp26c1 (a, and b) and fgf8 (c, and d). Values are normalized to ODC.

Figure S8: Pitx2c mis-expression in the whole embryo leads to cyp26c1 expansion and Fgf8 reduction

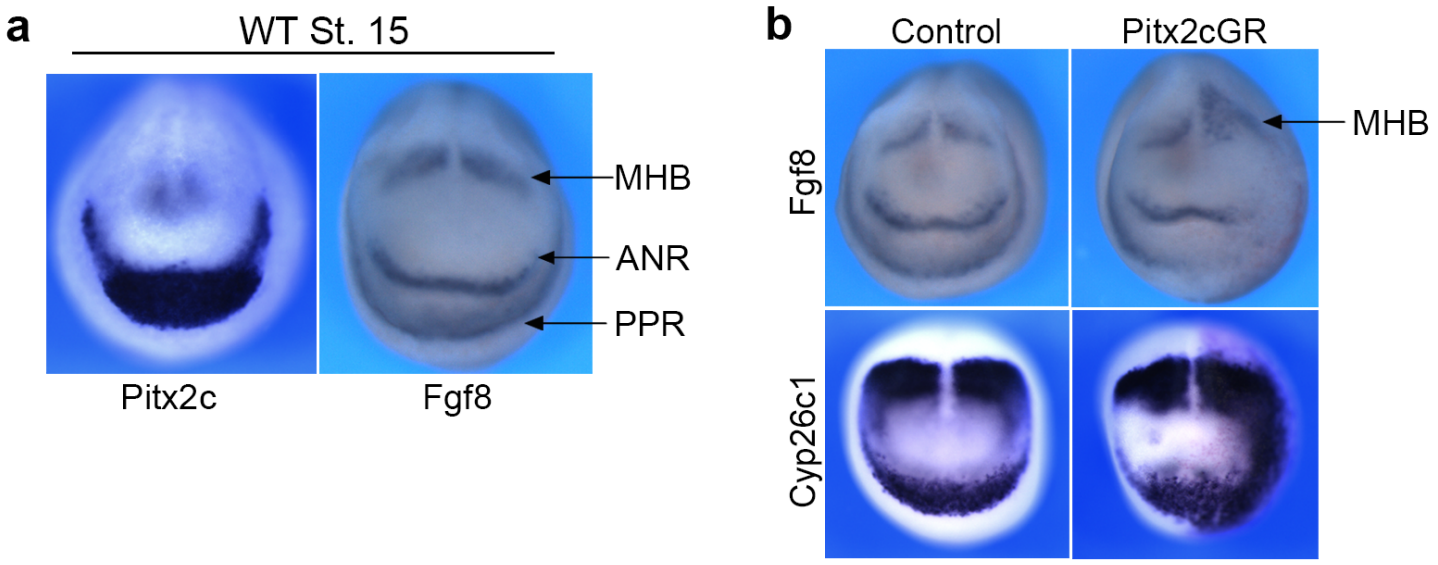

Figure S8: (a) Fgf8 is expressed in three nested domains: the prospective midbrain/hindbrain boundary (MHB), the anterior neural ridge (ANR) and the PPR (Ahrens and Schlosser, 2005), and the most anterior domains partially overlap with Pitx2c. (b) Pitx2c mis-expression in the whole embryo induces a posterior shift of Fgf8 MHB expression domain and a reduction of its ANR and PPR domains (upper panels), associated with a massive expansion of cyp26c1 expression domain (lower panels). Images show anterior views, dorsal to top.

Figure S9: SU5402 blocks the induction of Cyp26c1 by Pitx2c

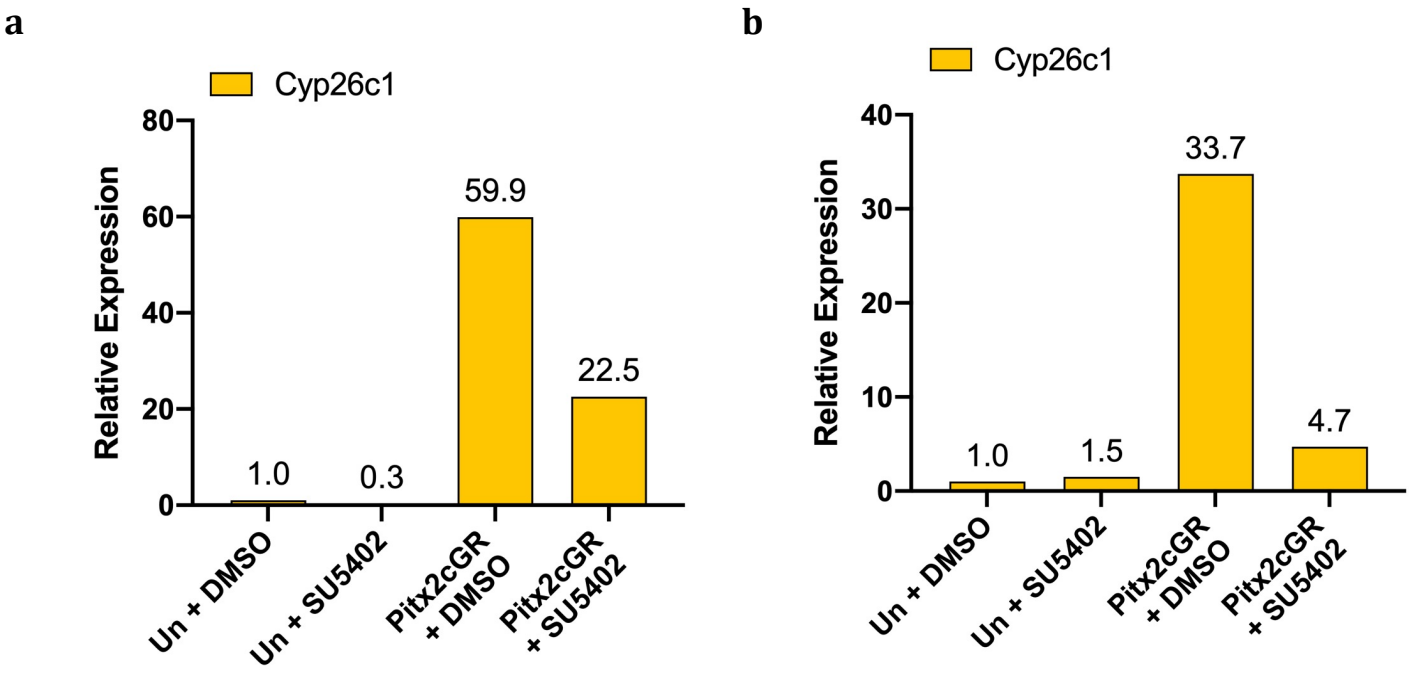

Figure S9: qRT-PCR analysis of *cyp26c1* expression in animal cap explants injected with Pitx2cGR mRNA and cultured for 8 hours with DMSO or SU5402 (25μM). Two independent experiments are shown (a, and b). Values are normalized to ODC.

Figure S10: Loss of Cyp26c1 does not affect the rate of cell death or proliferation in the ectoderm

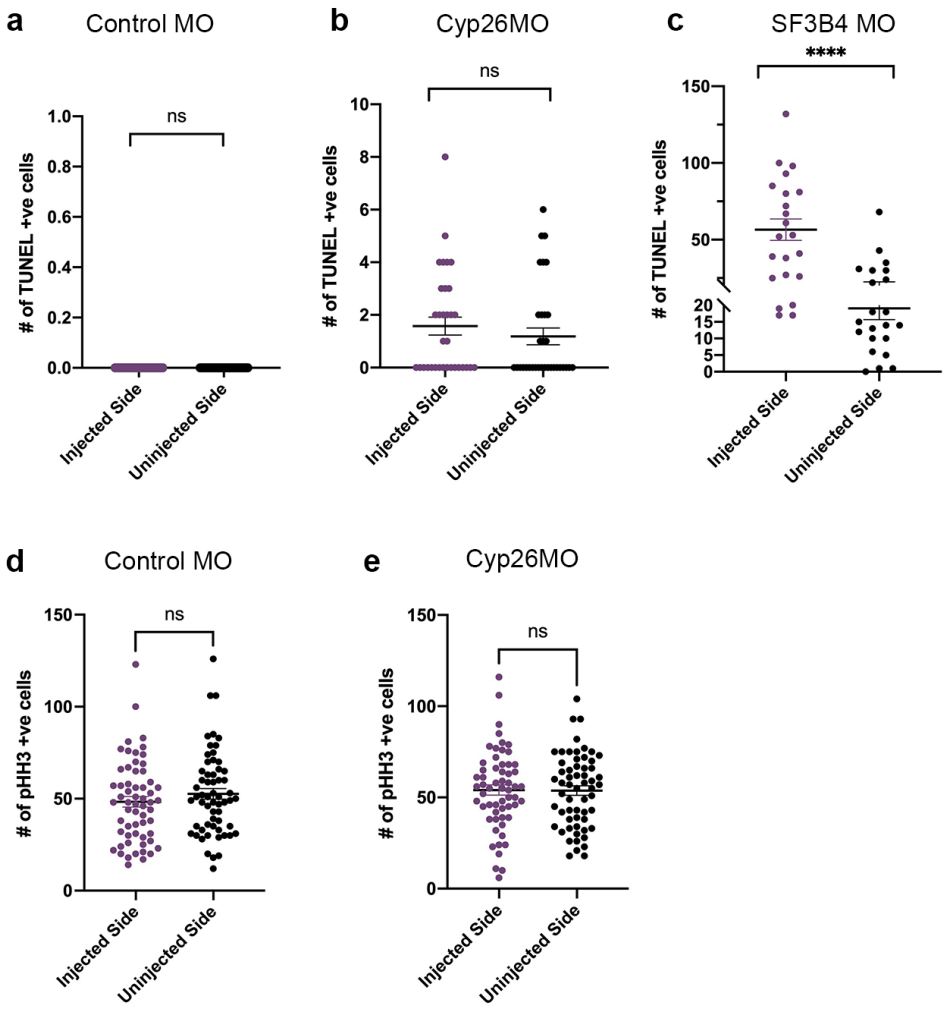

Figure S10: TUNEL (a-c) and pHH3 (d-e) staining of Cyp26c1-depleted *Xenopus* embryos. Embryos injected with CoMO (a) and Cyp26cMO (b) show no difference in the number of TUNEL-positive cells on injected vs. control sides. Injection of SF3B4MO (c) was used as a positive control (Devotta et al., 2016). A representative experiment of three biological replicates is shown. Similarly, embryos injected with CoMO (d) and Cyp26cMO (e) show no difference in the number of pHH3-positive cells on injected vs. control sides. Combined values from three biological replicates are shown. Each dot represents one embryo. p-values were calculated using unpaired t-test, **** $p<0.0001$, ns: not significant.

Figure S11: Cyp26c1 knockdown results in loss of *pitx2c* expression

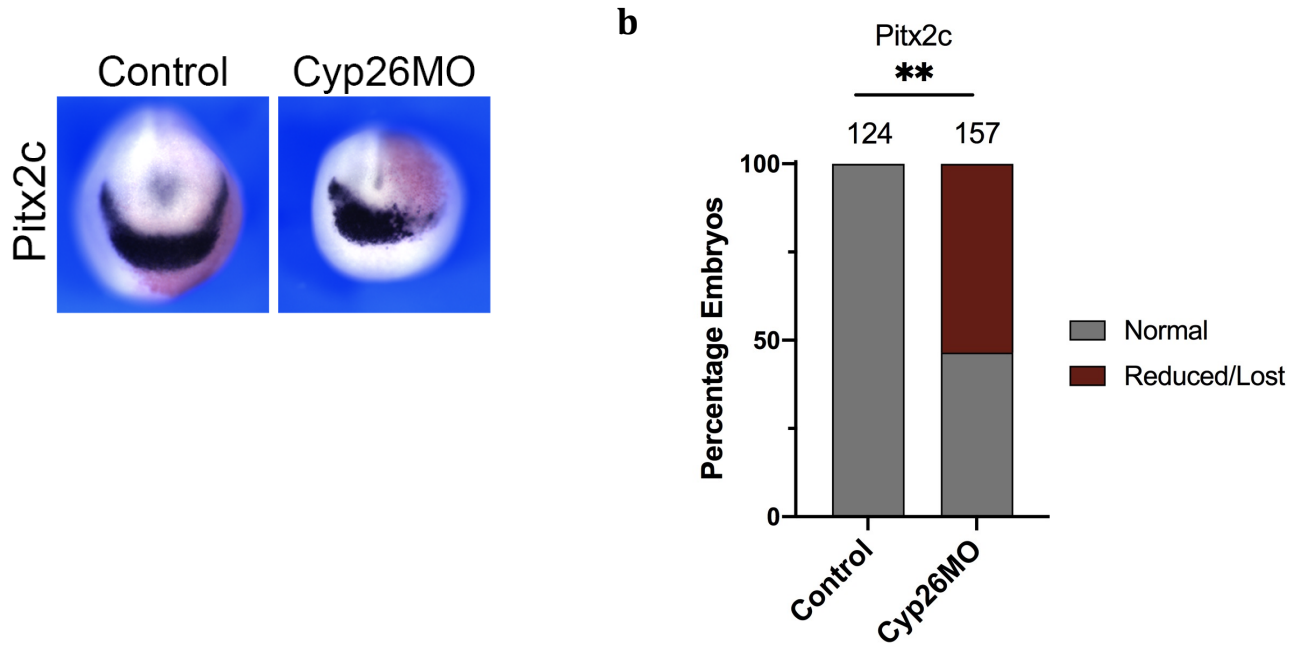

Figure S11: (a) ISH for *pitx2c* expression in control or Cyp26c1MO-injected embryos. The injected side is on the right showing Red-Gal. b) Quantification of the phenotypes for control versus Cyp26MO injected embryos. The total number of embryos (n) is indicated on the top of each bar. Data are from at least three biological replicates. p-values were calculated using unpaired t-test for major phenotype, ** p≤0.01. Images show anterior views with dorsal to top.

Table S1. Primers

Gene	Forward primer	Reverse primer
six1	5′ CTGGAGAGCCACCAGTTCTC 3′	5′ AGTGGTCTCCCCCTCAGTTT 3′
pitx2c	5′ ACTGTCCTCCCAGAGTATGT 3′	5′ GTTGCTGAGATTGTTCAGGTTATT 3′
cyp26c1	5′ ACAGTTCCAGGAGAGAGAAGTA 3′	5′ AGGCTGTGTTCTCCCAATAAG 3′
fgf8a	5′ GACTGCGTCTTCTCGGAAAT 3′	5′ CCCTTCTTGTGAAAGCCATAAAC 3′
ODC	5′ ACATGGCATTCTCCCTGAAG 3′	5′ TGGTCCCAAGGCTAAAGTTG 3′