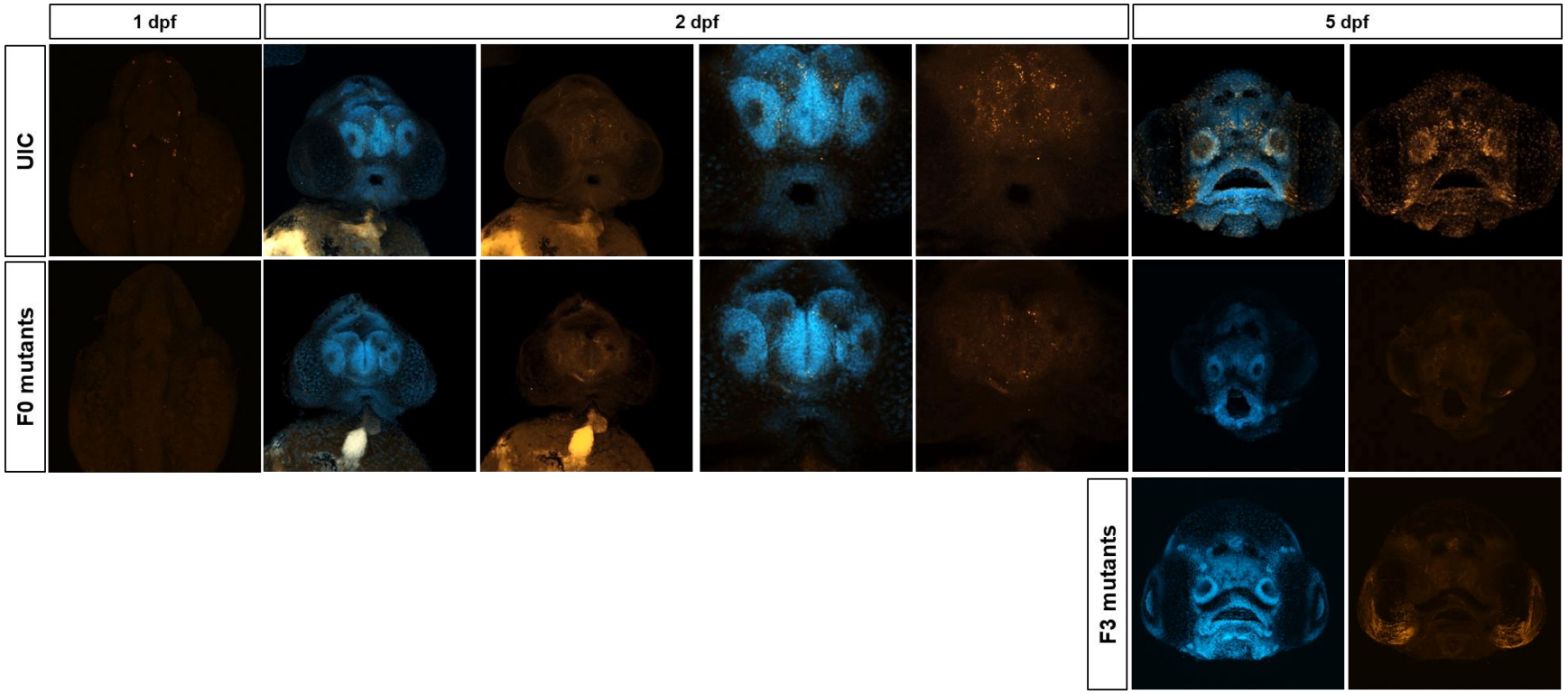
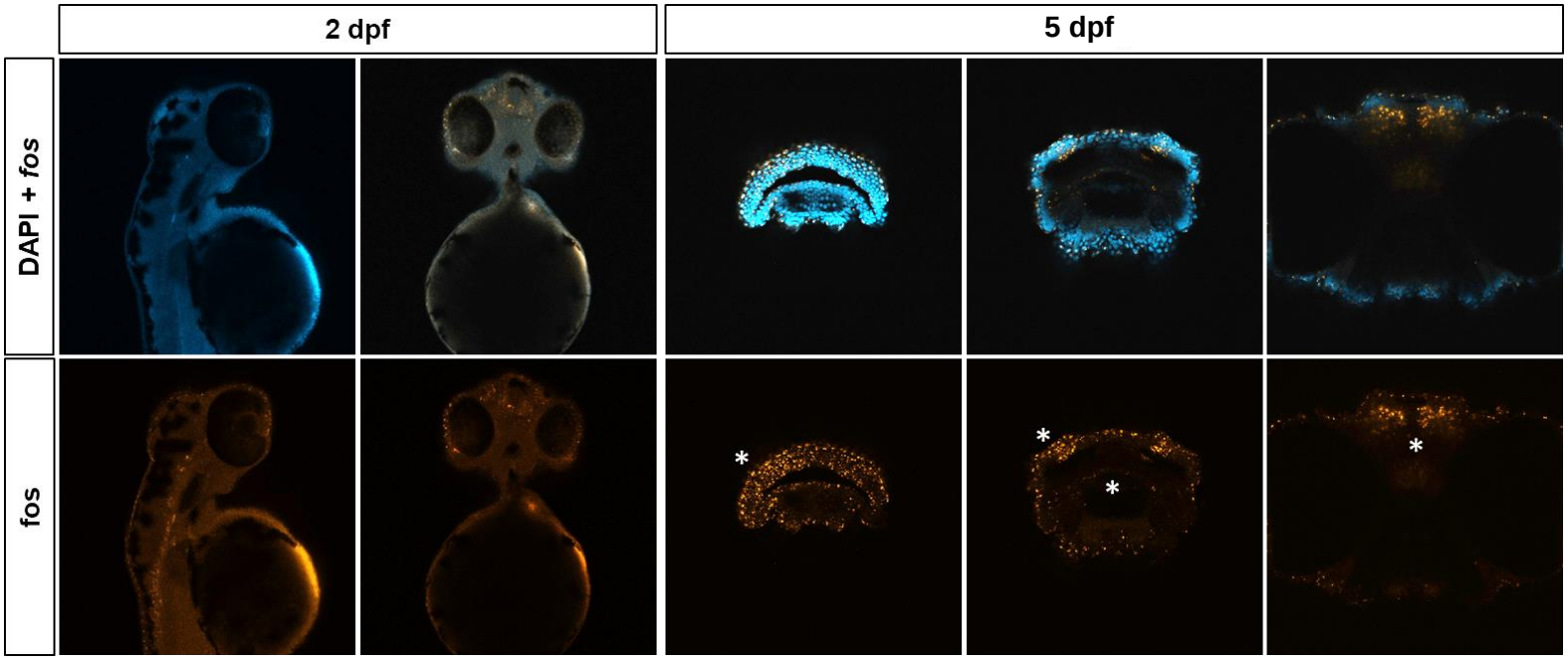
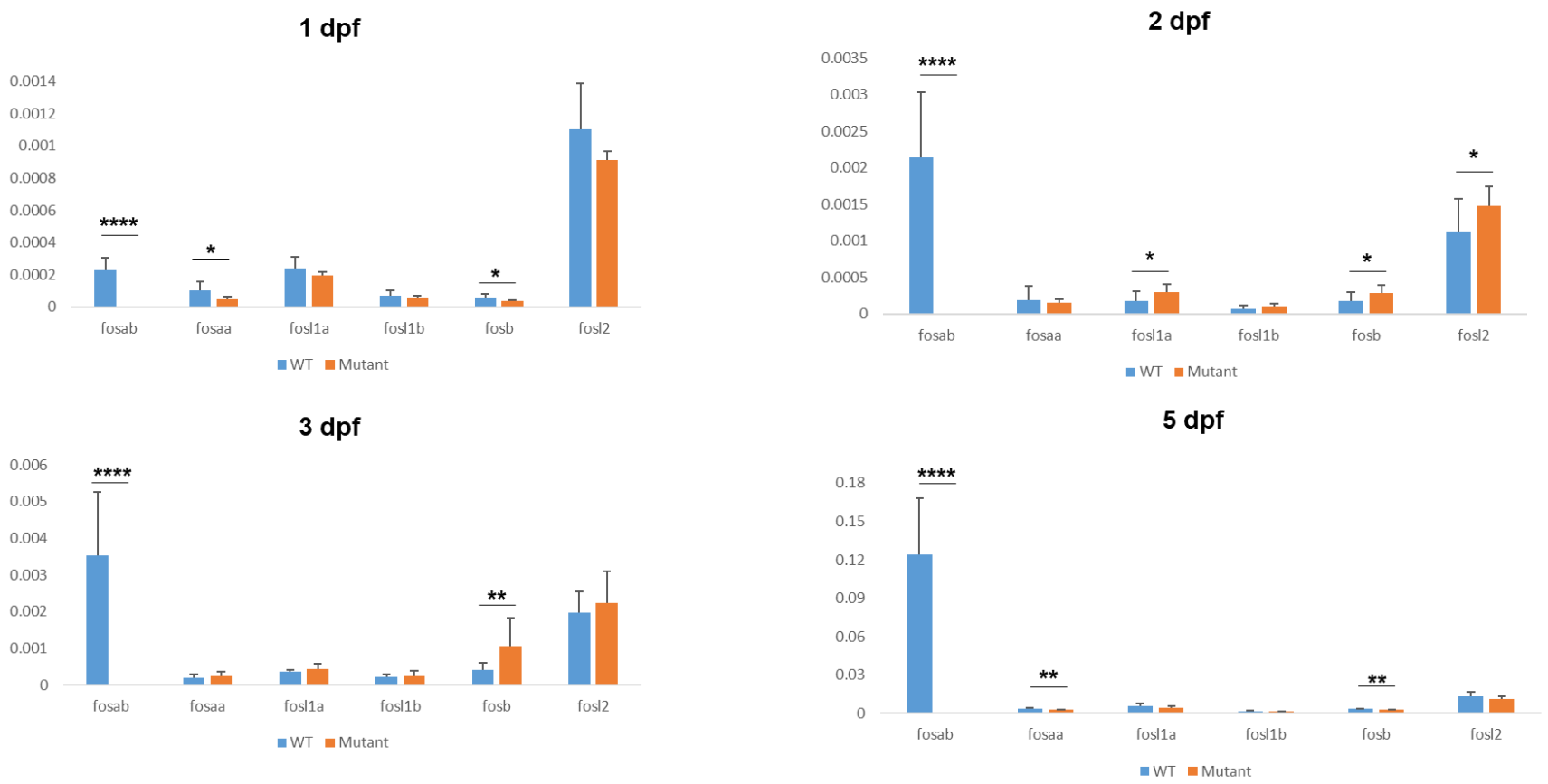


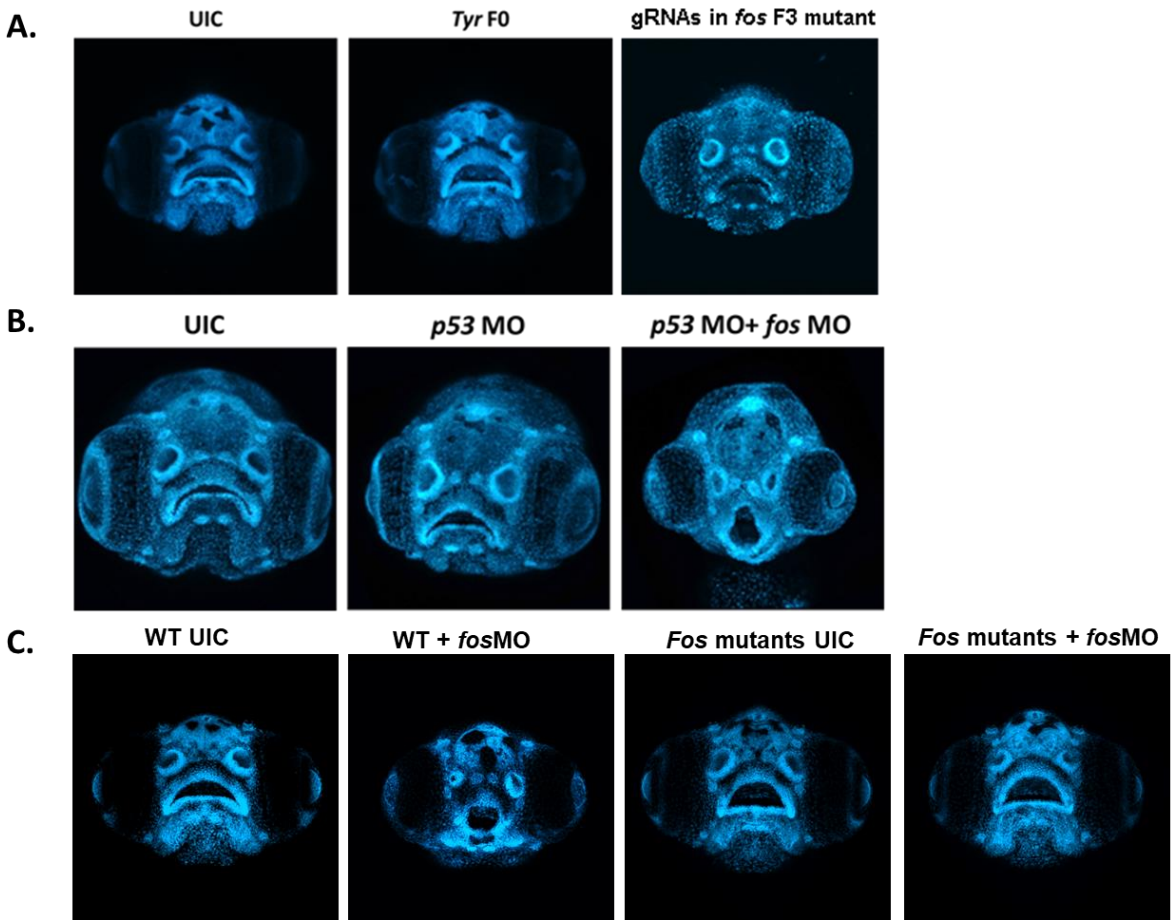

**Supplemental Figure 1**. ***Fos*** **expression in controls and** ***fos*** **F0 mutants (crispants).** Hybridization chain reaction (HCR) in situ with zebrafish *fos* mRNA probe set showed that *fos* is regionally expressed at 1 dpf (dorsal view shown). Expression was reduced in *fos* crispants at all developmental time points evaluated compared to controls. Stable F3 mutants showed absent *fos* mRNA expression at 5 dpf. UIC, uninjected control; F0, crispant; MO, morphant; MM, mismatch control morpholino



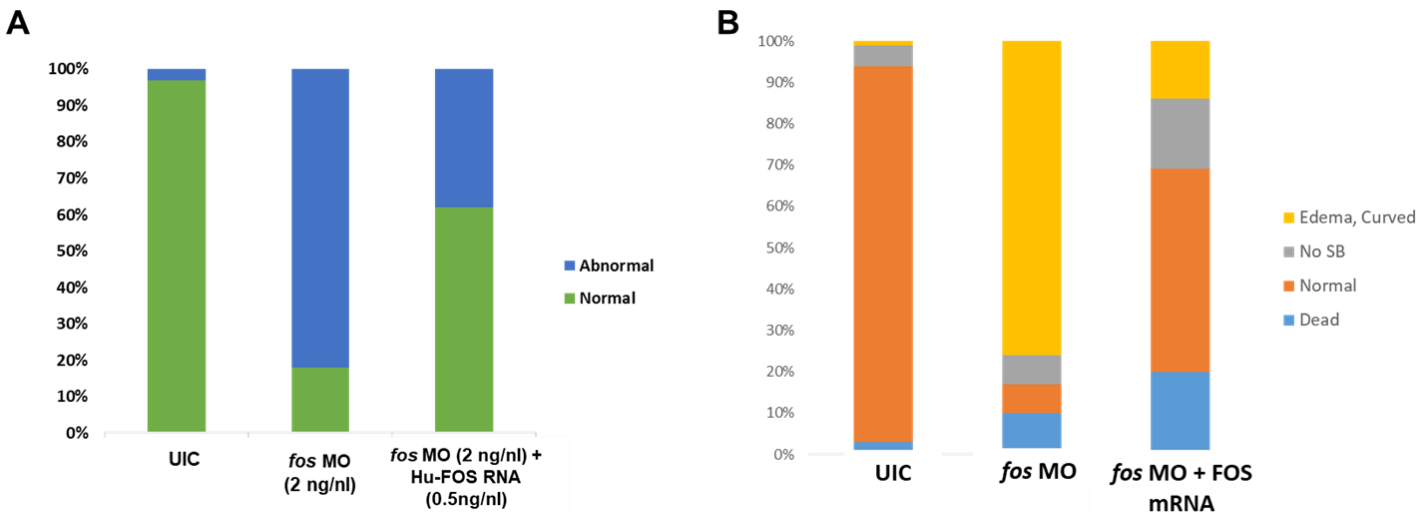

**Supplemental Figure 2.** Examination of individual confocal sections showed *fos* mRNA expression both in the inner as well as more superficial tissues at both 2 and 5 dpf. Interestingly, at 5dpf, strong expression was found in the brain (slice 45/60), palate and olfactory placodes (slice 17/60), and periderm (slice 4/60) (asterisks).



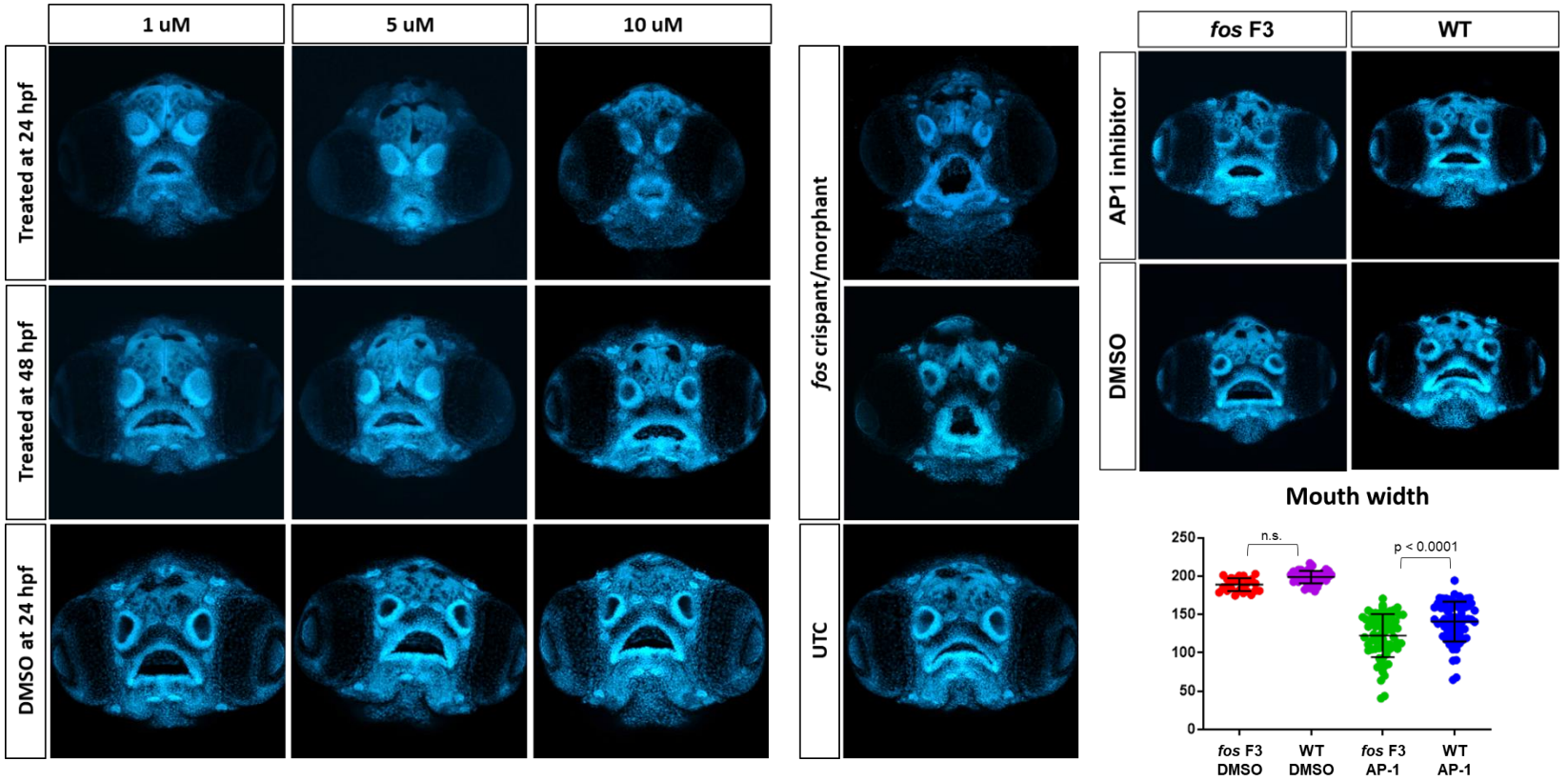

**Supplemental Figure 3.** RNA transcripts of *fos*-related genes in *fos* mutant embryos at 1-5 dpf. Student's t-test * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$ . WT, wild type; mutant, *fos* stable mutant.



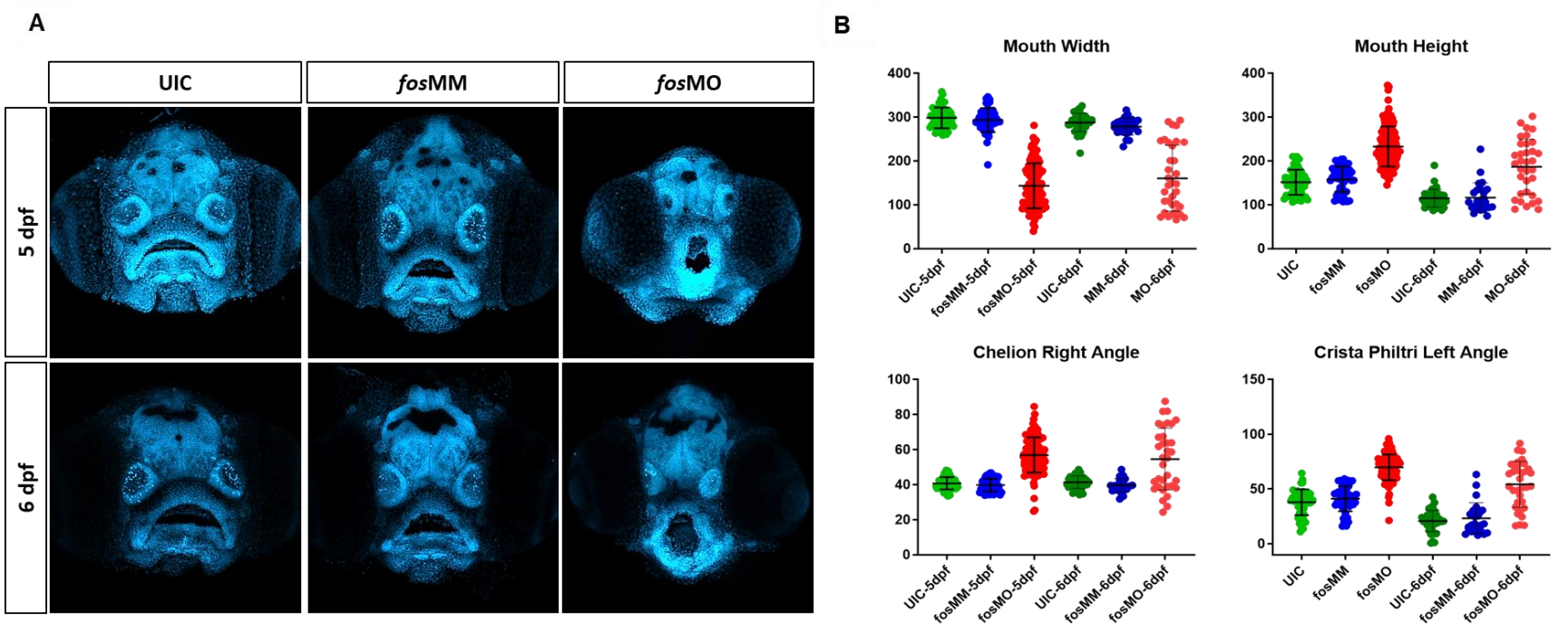

**Supplemental Figure 4. Phenotypic effects are specific to *fos* perturbation. A)** Tyrosinase control crispants (tyr F0) have a normal orofacial phenotype as do F3 mutants injected with the same crispr guides. **B**) *Fos* morphant phenotype is not rescued by p53 morpholino co-injection, indicating that p53-related neurotoxicity is not contributing to the observed craniofacial abnormalities in *fos* morphants. **C)** Injection of *fos* MO in stable fos F3 mutants did not lead to a craniofacial phenotype, further supporting *fos* MO specificity. Approximately 50 1-cell stage embryos were injected and a minimum of n = 10 were imaged for each condition. UIC, uninjected control, tyr, tyrosinase guide RNA (gRNA) injected; MO, morpholino;



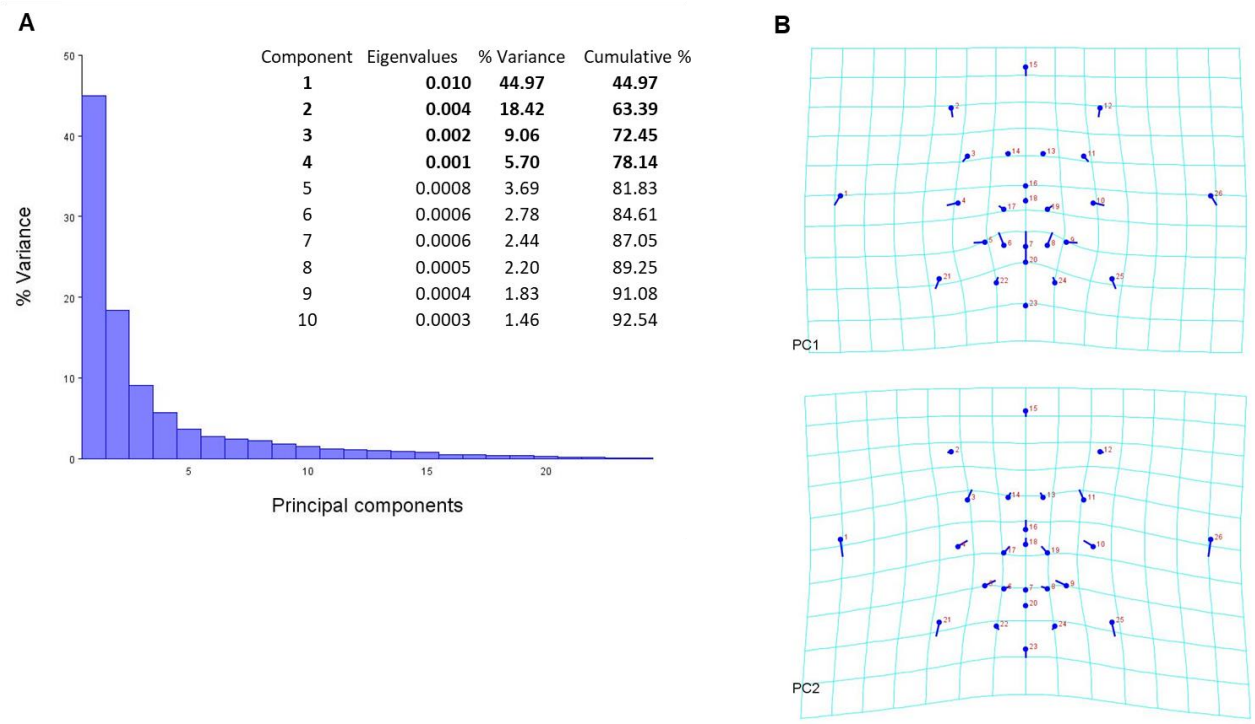

**Supplemental Figure 5**. ***Fos* morphant phenotype was rescued with human *FOS* mRNA injection. A)** Human *FOS* mRNA (Hu-FOS RNA) co-injection rescue decreased abnormal embryos by 45%. **B**) The edema and curved body phenotypes were also rescued by co-injection. Approximately 50 1-cell stage embryos were injected and a minimum of n = 20 were imaged and phenotypically examined for each condition. UIC, uninjected control; MO, morphant.




**Supplemental Figure 6**. **AP-1 inhibitor treatment alters craniofacial morphology.** Embryos were continuously treated with SR 11302 starting at 24 and 48 hpf and collected at 5 dpf. Dishes of n=50 larvae were treated for each condition and a minimum of n=15 for each condition were imaged. Earlier treatment at all doses led to more anomalies, including a horizontally constricted face, small mouth, abnormal nasal pits and merged neuromasts. When treatment was started at 48 hpf and a concentration of 10 uL, mouth shape was the most severely affected while at a concentration of at 1 uM the mouth was normal. Interestingly, when stable fos F3 mutants were treated with AP-1 inhibitor, they showed a more severe phenotype Test and p value (significantly decreased mouth width measurement is shown) suggesting they are more sensitive to AP-1 perturbation. The number of zebrafish analyzed was: drug treated fos F3 mutants= 70, drug treated wild type (WT) controls = 68, DMSO treated mutants = 29, DMSO treated controls = 40. UTC, untreated control; DMSO, vehicle control.

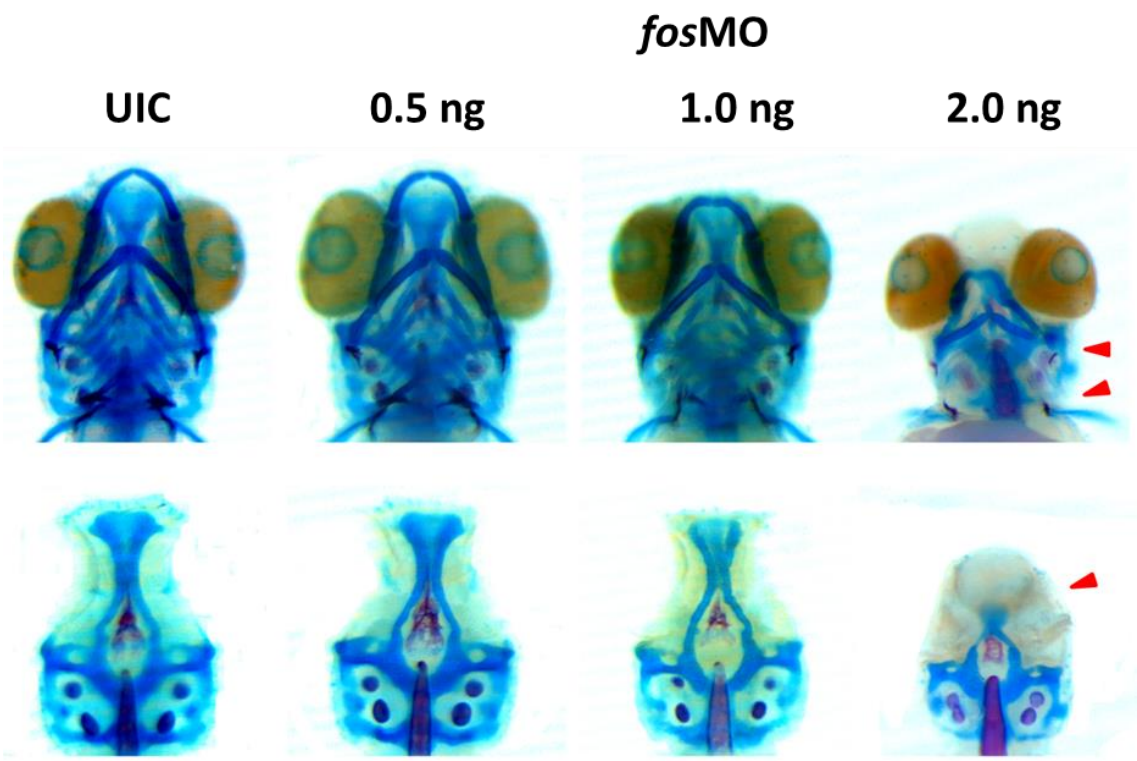



**Supplemental Figure 7**. ***Fos*** **morphants have altered mouth measurements at 6 dpf. A**) Representative DAPI images of 5 and 6 dpf embryos for each condition. **B**) *Fos* knockdown perturbs facial development with altered measurements for mouth width, mouth height, chelion right and crista philtra left angles, among others, at both 5 and 6 dpf, supporting that the facial phenotype is not due to developmental delay. ANOVA and p values The number of embryos analyzed were as follows: 5dpf- UIC = 98, MM = 113, MO = 138, 6dpf- UIC = 38, MM = 26, MO = 35. UIC, uninjected control; MO, *fos* morpholino, MM, mismatch control morpholino.

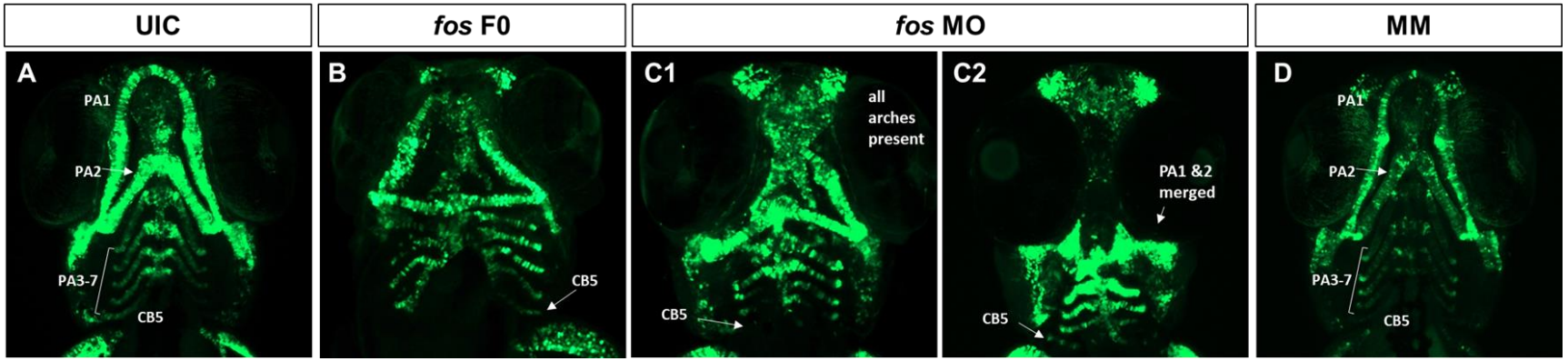



| Component | Eigenvalues | % Variance | Cumulative % |
|---|---|---|---|
| **1** | **0.010** | **44.97** | **44.97** |
| **2** | **0.004** | **18.42** | **63.39** |
| **3** | **0.002** | **9.06** | **72.45** |
| **4** | **0.001** | **5.70** | **78.14** |
| 5 | 0.0008 | 3.69 | 81.83 |
| 6 | 0.0006 | 2.78 | 84.61 |
| 7 | 0.0006 | 2.44 | 87.05 |
| 8 | 0.0005 | 2.20 | 89.25 |
| 9 | 0.0004 | 1.83 | 91.08 |
| 10 | 0.0003 | 1.46 | 92.54 |

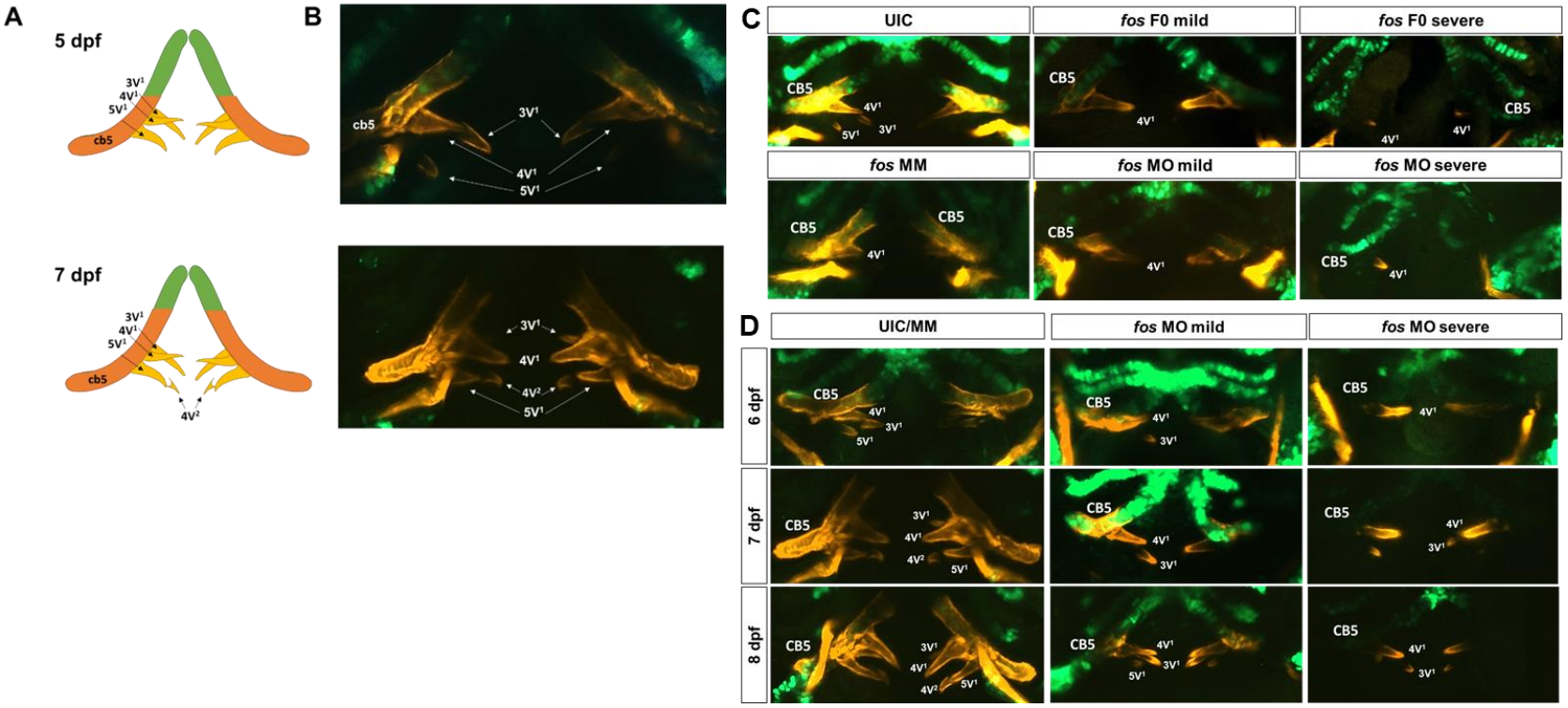
**Supplemental Figure 8**. **Principal component analysis results after Procrustes transformation. A)** Scree plot depicting eigenvalues of all principal components (PCs), with the first 4 explaining 78% of the variance in the combined dataset. **B**) Transformation grids with lollipop graphs showing vectors of change for each landmark along the first two components, PC1 and PC2. The number of embryos analyzed in each group was untreated controls, n=93, *fos* F0 mutants n=103, *fos* morphants n=138, and mismatch morpholino controls, n = 47.



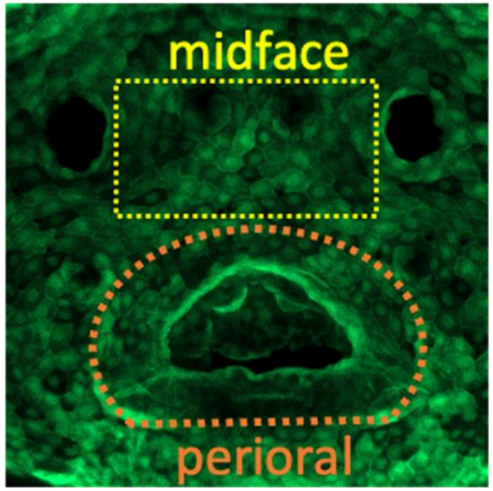

**Supplemental Figure 9. Bone and cartilage phenotype severity increases with higher doses of *fos* morpholino.** At the highest dose, embryos displayed a severely reduced primary palate, missing lower jaw cartilages and lack of pharyngeal teeth (red triangles). A minimum of n=50 1-cell stage embryos were injected for each dose and stained and n>=10 embryos were dissected and imaged for upper and lower jaw skeletal examination. UIC, uninjected control; MO, *fos* morpholino



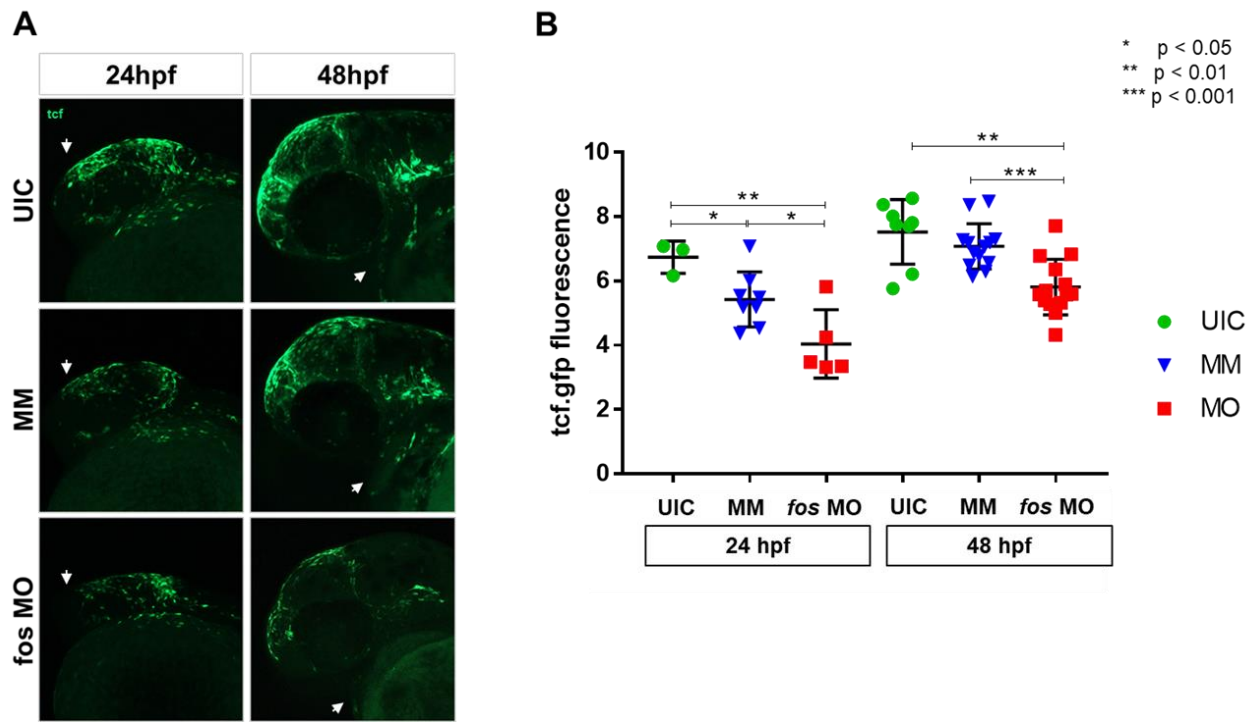

**Supplemental Figure 10**. **A**) Pharyngeal arches PA 1-7 at 5dpf in UIC. **B**) Abnormal arches in *fos* F0 mutants. **C**) The range of abnormalities observed in morphants compared to **D**) MM controls. UIC, uninjected control; F0, crispant; MO, morphant, MM, mismatch morpholino control. A minimum of n=28 zebrafish were imaged for this analysis.




**Supplemental Figure 11. Zebrafish pharyngeal tooth development is affected in *fos* morphants**. **A**) Schematic representation of the 5th ceratobranchial arch (CB5) and pharyngeal teeth (3V1, 4V1, and 5V1) at 5 and 7dpf and **B**) confocal images of Alizarin red stained embryos at the same time points. **C**) *Fos* mutants and morphants were examined at 5 dpf and the range of tooth phenotypes is represented for each group. The number of zebrafish analyzed was UIC n=9, F0 n=10, MO n=42, MM n=15. **D**) Morphants were followed up to 8 dpf (5dpf UIC= 18, MO=84, MM =30; 6dpf UIC=8, MO= 60, MM =8; 7dpf UIC=22, MO=24, MM=16; 8dpf UIC=15, MO = 58, MM=10); defects in tooth attachment to CD5 and in tooth morphogenesis were observed throughout developmental stages. UIC, uninjected control; F0, crispant; MO, morphant, MM, mismatch morpholino control.



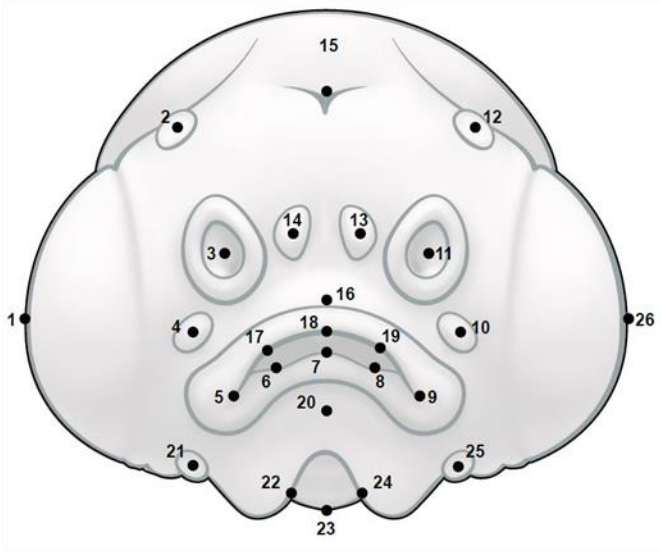

**Supplemental Figure 12. Quantitation of periderm cell size.** Schematic of regions where cells were measured. Cell size was compared for all 4 groups (UIC, crispant, morphant and MM control) at 3 and 5 dpf. Midface and perioral cells were analyzed separately due to existing differences in cell size.




**Supplemental Figure 13. Wnt/β-catenin pathway activation is significantly reduced in *fos* morphants. A)** *Fos* knockdown in a Wnt biosensor *Tg(7xTCF-Xla.Siam:GFP)* line showed significant reduction of β-catenin responsive cells (arrows) at 24 and 48 hpf in *fos* morphants as compared to MM and UIC. **B**) Fluorescence quantification using ImageJ showed significantly reduced gfp signal in *fos* morphants at both time points. Test and p-values The number of zebrafish analyzed was 24hpf UIC =3, MM=9, MO=5; 48hpf UIC=8, MM=14, MO=13. UIC, uninjected control; MO, morphant; MM, mismatch morpholino control.

## Appendix: Landmarks and measurements used in morphometric analysis.

| Feature Name | Landmarks used in measurement |
|---|---|
| Olfactory Distance | 3 to 11 |
| Upper Lip Width | 16 to 18 |
| Lower Lip Width | 7 to 20 |
| Mouth Width | 5 to 9 |
| Olfactory to Mouth | 11 to 3 to 16 |
| Olfactory to Mouth 2 | 3 to 11 to 16 |
| Difference | |
| Olfactory to Mouth 3 | 3 to 16 to 11 |
| Chin Width | 21 to 25 |
| Mouth to Chin | 16 to 23 |
| Alternate Height | Midpoint of 2 to 12 to point 23 |
| Mouth Height | 16 to 20 |
| Neuromast Angle 1 | 2 to 4 to 16 |
| Neuromast Angle 2 | 12 to 10 to 16 |
| Neuromast Height | Midpoint of 2 to 12 to midpoint of 4 to 10 |
| Neuromast Width | Midpoint of 2 to 4 to midpoint of 12 to 10 |
| Mid Neuromast Width | 13 to 14 |
| Avg Length Olfactory to Mouth | Avg of 3 to 16 and 11 to 16 |
| Area Top | Area of 2 to 12 to 10 to 4 |
| Area Bottom | Area of 4 to 10 to 25 to 21 |
| Area Combined | Area top + area bottom |
| Mid Olfactory to Chin Height | Midpoint of 3 to 11 to point 23 |
| Mouth Area | |
| Mouth Perimeter | |
| Libiale Superius Angle | 5 to 18 to 9 |
| Chelion Left Angle | 5 to 9 to 18 |
| Chelion Right Angle | 9 to 5 to 18 |
| Chelion Angle Difference | Difference |
| Labiale Inferius Angle | 17 to 7 to 19 |
| Crista Philtri Left Angle | 17 to 19 to 7 |
| Crista Philtri Right Angle | 19 to 17 to 7 |
| Crista Philtri Angle Difference | Difference |
| Labiale Superius Mid Angle | 6 to 18 to 8 |
| Labiale Inferius Left Angle | 6 to 8 to 18 |
| Labiale Inerius Right Angle | 8 to 6 to 18 |
| Labiale Inferius Angle Difference | Difference |



**Supplemental Table 1**. **zFACE analysis results at 6 dpf.** Shown in bold are parameters consistently altered at both 5 and 6 dpf.

| zFACE feature | 5dpf results | UIC vs fosMO | fosMM vs fosMO |
|---|---|---|---|
| **Width** | lower | *** | *** |
| Height | | **** | **** |
| **Olfactory Distance** | lower | **** | **** |
| Upper Lip Width | higher | | |
| Lower Lip Width | higher | **** | ** |
| **Mouth Width** | lower | **** | **** |
| **Olfactory to Mouth** | higher | **** | **** |
| Olfactory to Mouth 2 | higher | | |
| Difference | | **** | **** |
| **Olfactory to Mouth 3** | lower | **** | **** |
| Chin Width | lower | | |
| Mouth to Chin | | | |
| Alternate Height | | **** | **** |
| **Mouth Height** | higher | **** | **** |
| **Neuromast Angle 1** | higher | **** | **** |
| **Neuromast Angle 2** | higher | **** | **** |
| Difference | higher | | |
| Neuromast Height | | | |
| **Neuromast Width** | lower | **** | **** |
| **Mid Neuromast Width** | lower | *** | * |
| Avg Length Olf mouth | | **** | **** |
| Area Top | lower | | |
| Area Bottom | lower | | |
| Area Combined | lower | | |
| Mid Olf. to Chin height | | **** | **** |
| Mouth Area | | | |
| **Mouth Perimeter** | lower | **** | **** |
| **Libiale Superius Angle** | lower | **** | **** |
| **Chelion Left Angle** | higher | **** | **** |
| **Chelion Right Angle** | higher | **** | **** |
| **Chelion Diff** | bigger | **** | **** |
| **Labiale Inferius Angle** | lower | **** | **** |
| **Crista Philtri Left Angle** | higher | **** | **** |
| **Crista Philtri Right Angle** | higher | **** | **** |
| Crista Philtri Diff | higher | | |
| **Labiale Superius Mid Angle** | lower | **** | **** |
| **Labiale Inferius Left Angle** | higher | **** | **** |
| **Labiale Inferius Right Angle** | higher | **** | **** |
| Labiale Inferius Diff | | | |

**Supplemental Table 2**. **Fifth ceratobranchial (CB) arch mineralization and pharyngeal tooth development is affected in *fos* morphant embryos.** Pharyngeal teeth were counted in embryos at 5-8 dpf and tabulated the developmental timeline they appear (4V first and 4V2 last). While UIC and MM embryos had completely mineralized CB5s and developed most of the pharyngeal teeth, *fos* morphants showed only partially mineralized CB5s by 8dpf and failed to develop specific rows of teeth. UIC, uninjected control; MO, morphant; MM, mismatch morpholino control

| Day post fertilization (dpf) | Tooth Id. | UIC | | *fos*MM | | *fos*MO | |
|---|---|---|---|---|---|---|---|
| | | Left | Right | Left | Right | Left | Right |
| 5dpf | **5th arch** | **100%** | | **100%** | | **24%** | |
| | 4V | 100 | 100 | 100 | 100 | 91 | 93 |
| | 3V | 89 | 78 | 73 | 54 | 10 | 2 |
| | 5V | 67 | 0 | 60 | 33 | 7 | 0 |
| 6dpf | **5th arch** | **100%** | | **100%** | | **47%** | |
| | 4V | 100 | 75 | 100 | 100 | 100 | 100 |
| | 3V | 100 | 75 | 75 | 75 | 47 | 47 |
| | 5V | 100 | 75 | 75 | 50 | 27 | 37 |
| 7dpf | **5th arch** | **100%** | | **100%** | | **58%** | |
| | 4V | 91 | 91 | 100 | 100 | 100 | 100 |
| | 3V | 90 | 73 | 100 | 100 | 75 | 50 |
| | 5V | 73 | 73 | 100 | 100 | 25 | 17 |
| | 4V2 | 64 | 55 | 88 | 88 | 0 | 0 |
| 8dpf | **5th arch** | **100%** | | **100%** | | **69%** | |
| | 4V | 100 | 100 | 100 | 100 | 93 | 100 |
| | 3V | 100 | 86 | 100 | 100 | 69 | 66 |
| | 5V | 86 | 86 | 100 | 100 | 41 | 45 |
| | 4V2 | 71 | 86 | 80 | 100 | 7 | 10 |