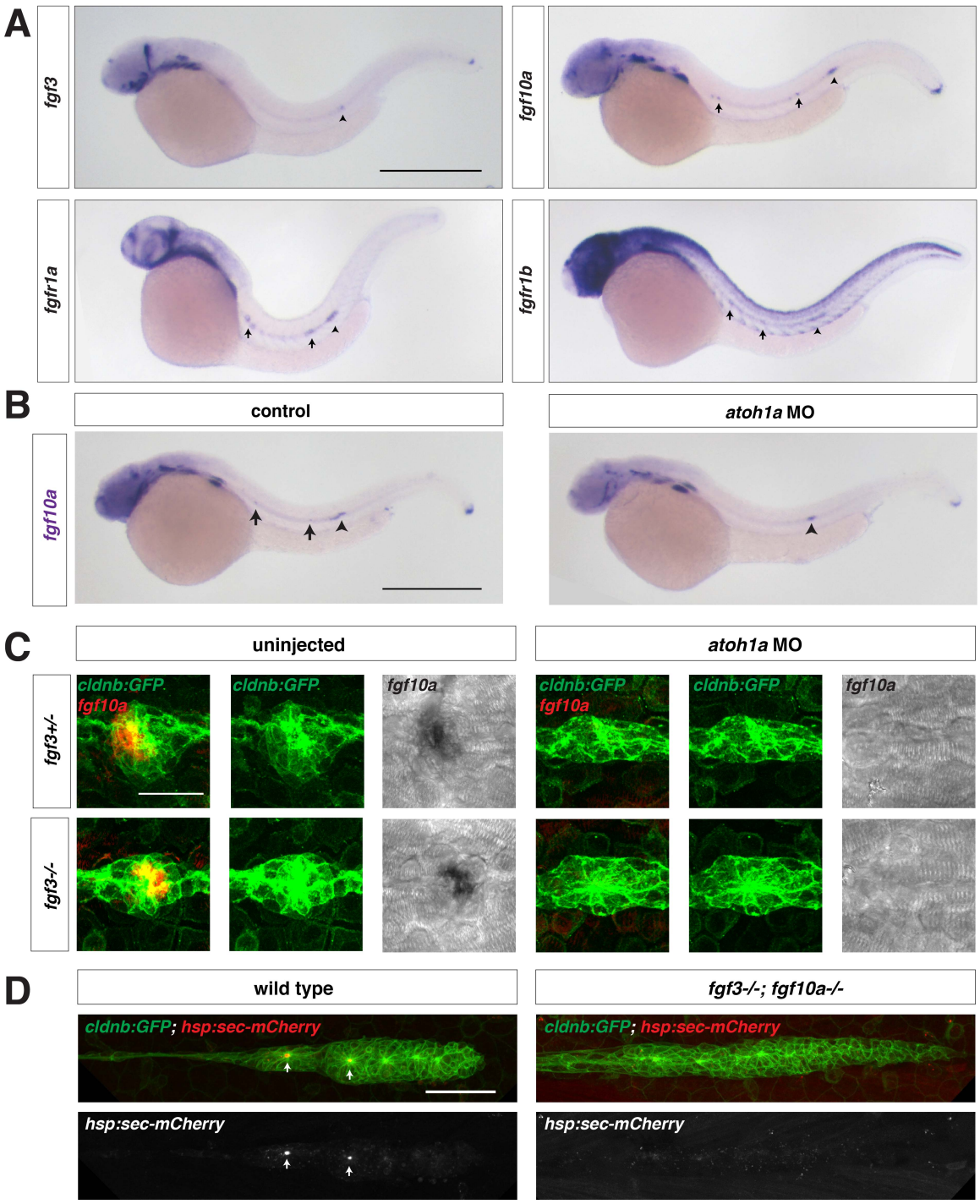

A
fgf3
fgf10a
fgfr1a
fgfr1b
B
control
atoh1a MO
fgf10a
C
uninjected
atoh1a MO
fgf3+/-
fgf3-/-
cldnb:GFP
fgf10a
D
wild type
fgf3-/-; fgf10a-/-
cldnb:GFP; hsp:sec-mCherry
hsp:sec-mCherry

Figure S1. Fgf ligand expression in wild-type and *atoh1a* MO-injected embryos, Related to Figure 1.

(A) Expression of *fgf3*, *fgf10a*, *fgfr1a*, and *fgfr1b* mRNA (purple) at 32 hpf. Arrows indicate expression in neuromasts and arrowhead denotes the position of the primordium. Scale bar = 500 μm.

(B) *fgf10a* mRNA expression at 32 hpf in a control embryo (left) and an embryo injected with a morpholino against *atoh1a* (right). Arrows indicate *fgf10a* expression in central cells of deposited neuromasts, and arrowheads indicate *fgf10a* expression in the front of the primordium. Scale bar = 500 μm.

(C) Maximum intensity projection of neuromast 1 in embryos of the indicated genotypes. Left, composite of GFP from *cldnb:lyn$_2$GFP* (green) and *fgf10a* mRNA (false-colored red). Middle, GFP only. Right, *fgf10a* mRNA only. Scale bar = 25 μm.

(D) Maximum intensity projection of the primordium in live, heat-shocked embryos of the indicated genotypes. Top, composite of GFP from *cldnb:lyn$_2$GFP* (green) and secreted mCherry from *hs:sec-mCherry* (red). Bottom, mCherry only. Scale bar = 50 μm.

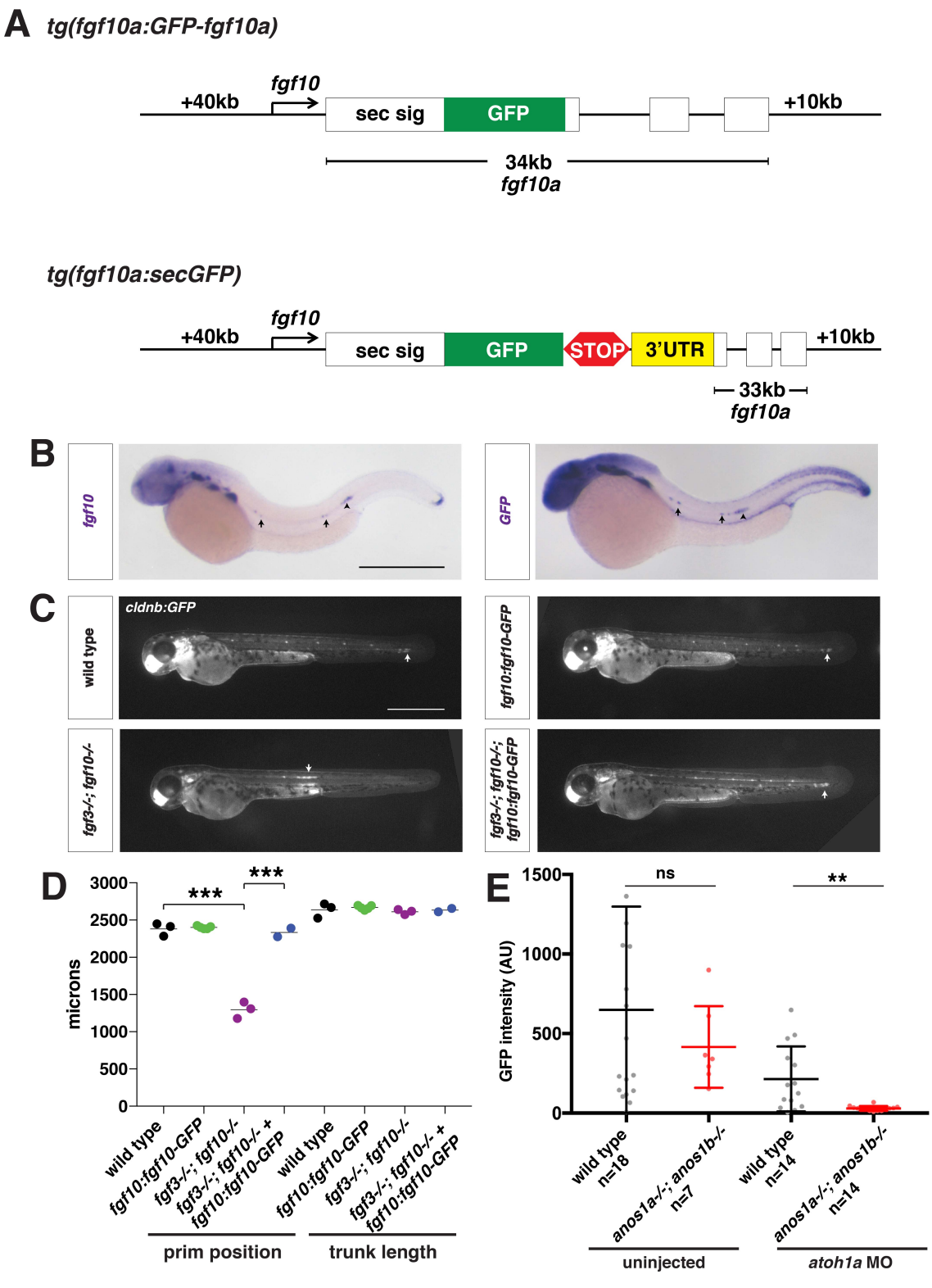

A
tg(fgf10a:GFP-fgf10a)
+40kb
fgf10
sec sig
GFP
+10kb
34kb
fgf10a
tg(fgf10a:secGFP)
+40kb
fgf10
sec sig
GFP
STOP
3'UTR
+10kb
33kb
fgf10a
B
fgf10
GFP
C
cldnb:GFP
wild type
fgf10:fgf10-GFP
fgf3-/-; fgf10-/-
fgf3-/-; fgf10-/-; fgf10:fgf10-GFP
D
microns
3000
2500
2000
1500
1000
500
0
***
***
wild type
fgf10:fgf10-GFP
fgf3-/-; fgf10-/-
fgf3-/-; fgf10-/- + fgf10:fgf10-GFP
wild type
fgf10:fgf10-GFP
fgf3-/-; fgf10-/-
fgf3-/-; fgf10-/- + fgf10:fgf10-GFP
prim position
trunk length
E
GFP intensity (AU)
1500
1000
500
0
ns
**
wild type n=18
anos1a-/-; anos1b-/- n=7
wild type n=14
anos1a-/-; anos1b-/- n=14
uninjected
atoh1a MO

Figure S2. Characterization of the *fgf10a:fgf10a-GFP* transgenic line, Related to Figure 2.

(A) Schematic of *fgf10a:fgf10a-GFP* and *fgf10a:sec-GFP* BAC transgenes. White boxes represent exons, and black lines in-between represent introns. STOP represents the stop codon after the *GFP* coding sequence and 3'UTR indicates the *fgf10a* 3'UTR fused directly to GFP in exon1. The same *fgf10a* 3'UTR sequence is also present in exon 3.

(B) mRNA *in situ* hybridization against *fgf10a* in a wild-type embryo (left) and against *GFP* in a *fgf10a:fgf10a-GFP* embryo (right). Arrows indicate expression in neuromasts and arrowhead indicates expression in the primordium. Scale bar = 500 μm.

(C) Neuromast and primordium labeled by GFP in *cldnb:lyn$_2$GFP* embryos of the indicated genotypes at 50 hpf. Arrow indicates position of the primordium. Scale bar = 500 μm.

(D) Quantification of the position of the primordium at 50 hpf and trunk length (distance from the posterior edge of the otic vesicle to the front of the primordium or the tip of the tail, respectively) for the indicated genotypes. Black lines represent the mean and data points are individual embryos. *** = $p < 0.001$.

(E) Total Fgf10a-GFP intensity in mature microlumina at the fourth apical constriction from the front in uninjected embryos (left) and embryos injected with *atoh1a* morpholino of the indicated genotypes (right). Dark black and dark red lines are means and data points are individual embryos. n.s. = $p > 0.05$, ** = $p < 0.01$.

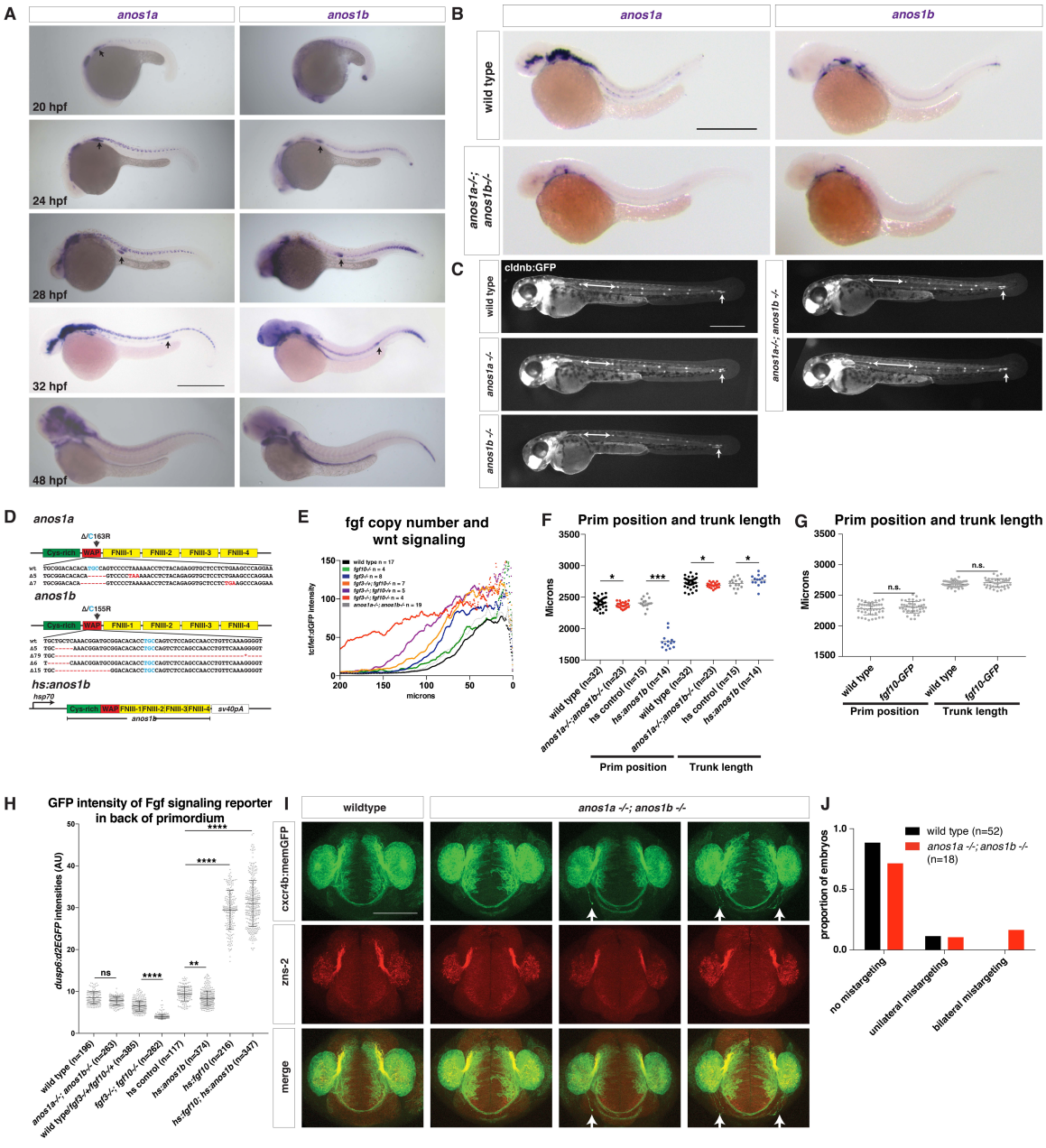

A
anos1a
anos1b
20 hpf
24 hpf
28 hpf
32 hpf
48 hpf
B
anos1a
anos1b
wild type
anos1a-/-; anos1b-/-
C
cldnb:GFP
wild type
anos1a -/-
anos1b -/-
anos1a-/-; anos1b -/-
D
anos1a
Δ/C163R
Cysrich
WAP
FNIII-1
FNIII-2
FNIII-3
FNIII-4
anos1b
Δ/C155R
hs:anos1b
hsp70
sv40pA
E
fgf copy number and wnt signaling
tcf/lef:d2GFP intensity
microns
F
Prim position and trunk length
Microns
wild type (n=32)
anos1a-/-;anos1b-/- (n=23)
hs control (n=15)
hs:anos1b (n=14)
Prim position
Trunk length
G
Prim position and trunk length
n.s.
fgf10-GFP
H
GFP intensity of Fgf signaling reporter in back of primordium
dusp6:d2EGFP intensities (AU)
ns
wild type (n=196)
anos1a-/-; anos1b-/- (n=263)
wild type/fgf3-/+/fgf10-/+ (n=385)
fgf3-/-; fgf10-/- (n=262)
hs control (n=117)
hs:anos1b (n=374)
hs:fgf10 (n=216)
hs:fgf10; hs:anos1b (n=347)
I
wildtype
anos1a -/-; anos1b -/-
cxcr4b:memGFP
zns-2
merge
J
proportion of embryos
wild type (n=52)
anos1a -/-; anos1b -/- (n=18)
no mistargeting
unilateral mistargeting
bilateral mistargeting

Figure S3. Characterization of the *anos1a* and *anos1b* mutant embryos, Related to Figure 3.

(A) Expression of *anos1a* and *anos1b* mRNA (purple) at indicated developmental stages. Arrow indicates the primordium. Scale bar = 500 μm.

(B) Expression of *anos1a* (left) or *anos1b* (right) mRNA in wild-type (top) and *anos1a-/-; anos1b-/-* (bottom) embryos (purple). Scale bar = 500 μm.

(C) Neuromast deposition and primordium migration in *anos1a* and *anos1b* single mutant and *anos1a; anos1b* double mutant embryos. Arrow denotes position of primordium and double-headed arrow indicates spacing between neuromast 1 and 2. The increased spacing between neuromast 1 and 2 in *anos1a; anos1b* double mutants is not fully penetrant. Scale bar = 500 μm.

(D) Schematic of *anos1a* and *anos1b* mutant alleles and *hsp70:anos1b* over-expression transgene. Top and middle, red dashes indicate missing nucleotides in the mutant alleles and red nucleotides denote premature STOP codons. Red asterisk (middle) marks the position of exon/intron boundary. Light blue cysteine C163 (top) and C155 (middle) are homologous to C172, which leads to Kallmann syndrome in humans when mutated to arginine. The codon for these cysteines is indicated in light blue. The premature STOP in the *anos1b Δ5* allele is 123 nt downstream of the deletion and outside of the region shown. All *anos1a-/-; anos1b-/-* embryos are *anos1a*$^{\Delta 5/\Delta 7}$*; anos1b*$^{\Delta 5/\Delta 79}$. Bottom, *anos1b* domains are encoded downstream of the *hsp70* promoter as a continuous stretch of cDNA followed by an SV40pA polyadenylation signal.

(E) Comparison of Wnt reporter (*tcf/lef-miniP:dGFP*) intensity in the primordia of embryos with different mutant copy numbers of *fgf3* and *fgf10a* and primordia of *anos1a-/-; anos1b-/-* mutant embryos. X-axis represents distance from the front of the

primordium. Data points indicate the mean. Error bars are not shown for clarity. n = number of individual embryos measured.

(F) Quantification of the position of the primordium and length of the trunk in embryos of the indicated genotype at 50 hpf measured from the posterior margin of the otic vesicle. Black lines indicate the average. Data points are individual embryos. * = $p < 0.05$, *** = $p < 0.001$.

(G) Quantification of the position of the primordium and length of the trunk in embryos of the indicated genotype at 50 hpf measured from the posterior margin of the otic vesicle. Black lines indicate the average. Data points are individual embryos. n.s. = $p > 0.05$.

(H) Quantification of the GFP intensities of the Fgf signaling reporter *dusp6:d2EGFP* in the back only of the primordia of the indicated genotypes. Black lines indicate the average. Gray data points are individual pixel measurements in the back of the primordia of the embryos averaged in Fig. 3D and 7F. **** n.s.= $p > 0.05$, ** = $p < 0.01$., and **** = $p < 0.0001$. ANOVA $p<0.0001$.

(I) Olfactory axon targeting in wild-type and *anos1a-/-; anos1b-/-* embryos at 28 hpf. Olfactory axons are labeled with the *cxcr4b:cxcr4b-Kate2-ires-GFP-CAAX* transgene and stained with the zns-2 antibody that labels olfactory pioneer neuron cell membranes (Trevarrow et al., 1990). Arrows indicate mistargeted axons.

(J) Quantification of olfactory axon targeting defects in wild-type and *anos1a-/-; anos1b-/-* embryos. Defects were grouped as indicated and represented as fraction of the total number of embryos.

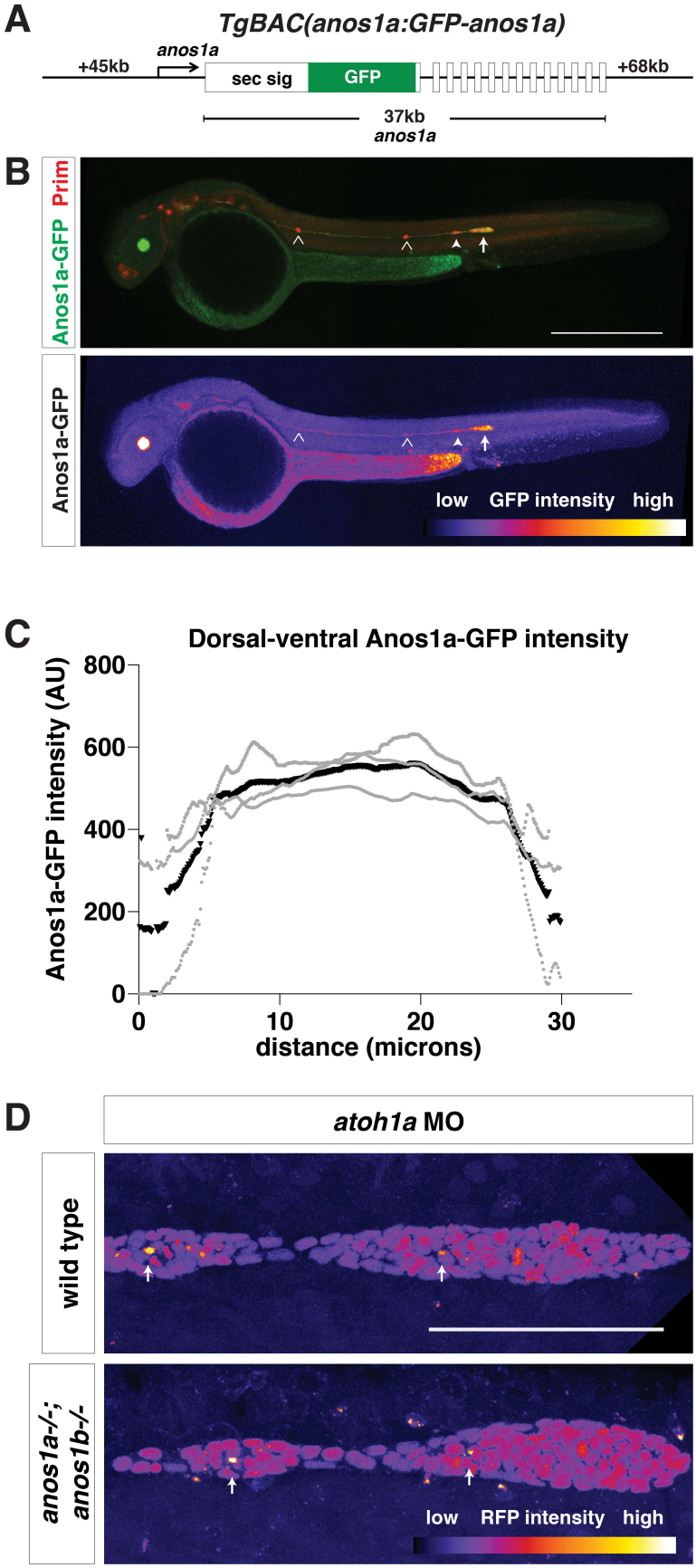

A
TgBAC(anos1a:GFP-anos1a)
anos1a
+45kb
sec sig
GFP
+68kb
37kb
anos1a
B
Anos1a-GFP Prim
Anos1a-GFP
low GFP intensity high
C
Dorsal-ventral Anos1a-GFP intensity
Anos1a-GFP intensity (AU)
800
600
400
200
0
0
10
20
30
distance (microns)
D
atoh1a MO
wild type
anos1a-/-; anos1b-/-
low RFP intensity high

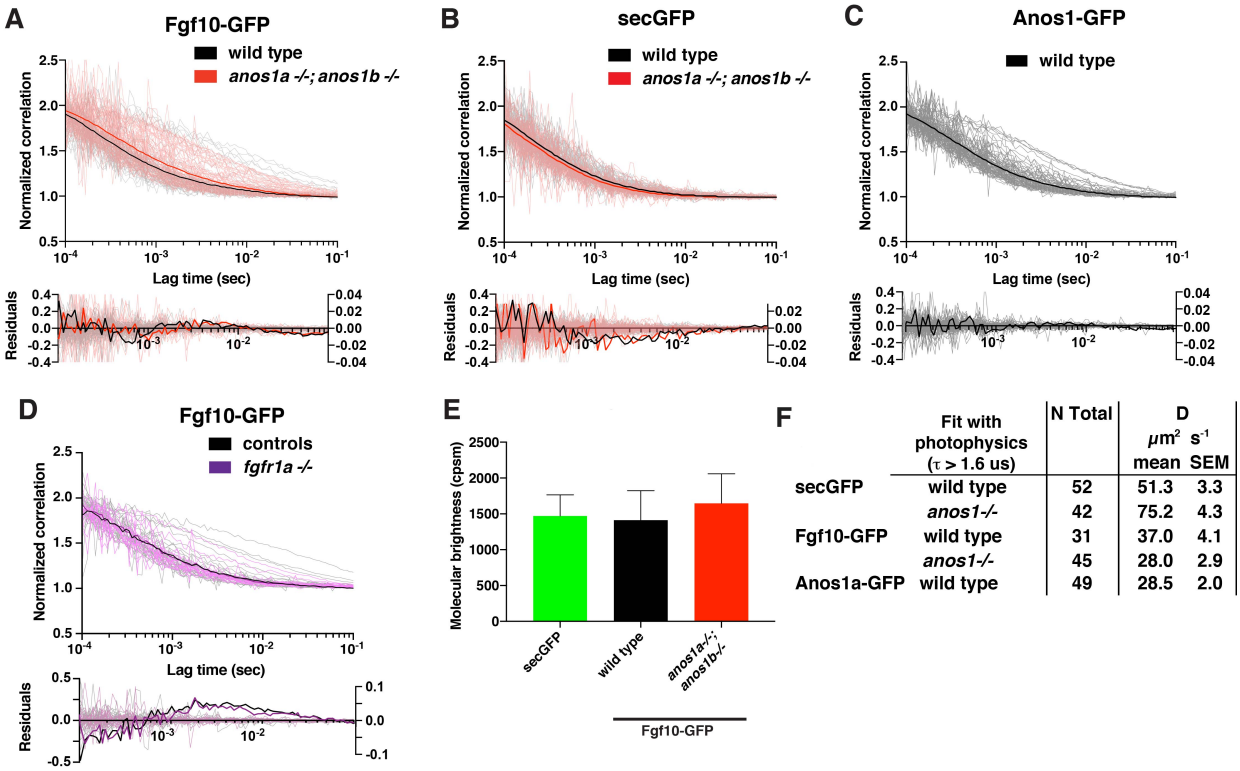
Figure S4. Characterization of *anos1a:anos1a-GFP* transgenic line Related to Figure 4.

(A) Schematic of the *anos1a* BAC transgene. GFP was placed behind the Anos1a secretion signal in exon 1 of the *anos1a* locus. Numbers to the left and right indicate length of BAC upstream of exon 1 and downstream of exon 14 (the last exon), and number below indicates the distance between exon 1 and 14. White rectangles represent exons, and solid black lines in-between represent introns.

(B) Top, immunostaining against GFP (green) and mCherry (red) of *anos1a:GFP-anos1a*; *prim:lyn$_2$mCherry* embryos. Bottom, false-coloring of immunostaining against Anos1a-GFP with intensity scale below. YFP expression in the lens originates from the transgenesis marker *cryaa:citrine* on the *anos1a:anos1a-GFP* transgene. Arrowheads indicate neuromasts with Anos1a-GFP signal, hollow arrowheads indicate neuromasts without Anos1a-GFP signal and the arrow indicates the primordium. Scale bar = 500 μm.

(C) Anos1a-GFP intensity along the dorsal-ventral axis of the primordium. Gray lines represent individual embryos and the black line indicates the average.

(D) Lumen integrity in embryos of genotypes indicated as assessed by accumulation of *hsp70:sec-mCherry* (arrows) in a primordium with nuclei marked by *cxcr4b:H2A-mCherry*. Image is a maximum intensity projection of a Z-stack and is false colored based on fluorescence intensity using the fire look-up table (bottom). Sec-mCherry is distinguished from H2A-mCherry based on anatomical position, smaller size and higher signal intensity of microlumina compared to nuclei. Scale bar = 100 μm.



F

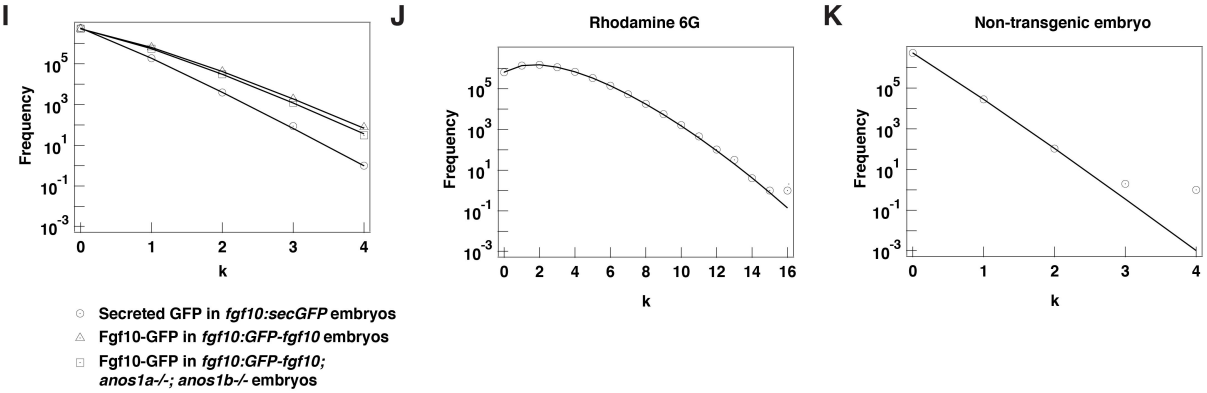
	Fit with photophysics (τ > 1.6 us)	N Total	D μm^2 s^{-1} mean	D μm^2 s^{-1} SEM
secGFP	wild type	52	51.3	3.3
	anos1-/-	42	75.2	4.3
Fgf10-GFP	wild type	31	37.0	4.1
	anos1-/-	45	28.0	2.9
Anos1a-GFP	wild type	49	28.5	2.0

G

	Fit without photophysics (τ > 50 us)	N Total	% 1-comp	D μm^2 s^{-1} mean	D μm^2 s^{-1} SEM	% 2-comp	% fast comp mean	% fast comp SEM	D_fast μm^2 s^{-1} mean	D_fast μm^2 s^{-1} SEM	D_slow μm^2 s^{-1} mean	D_slow μm^2 s^{-1} SEM	N particles per confocal mean	N particles per confocal SEM	Conc nM mean	Conc nM SEM
secGFP	wild type	86	98.8%	56.0	3.0	1.2%	84.7%	NaN	59.7	NaN	3.7	NaN	3.8	0.3	27.1	2.0
	anos1-/-	67	98.5%	69.2	3.0	1.5%	91.8%	NaN	98.1	NaN	5.5	NaN	5.1	0.4	36.6	2.6
Fgf10-GFP	wild type	60	65.0%	43.9	4.1	35.0%	88.2%	2.3%	40.2	5.0	1.7	0.6	33.8	2.6	242.3	18.3
	anos1-/-	83	62.7%	31.6	2.7	37.3%	74.3%	3.2%	33.3	4.4	1.8	0.3	33.2	3.0	238.1	21.2
Anos1a-GFP	wild type	76	75.0%	32.0	1.5	25.0%	75.6%	4.3%	31.1	4.9	2.7	0.7	7.6	0.5	54.7	3.6

H

	Mol. brightness	N (embryos)	N (time)
Secreted GFP	1647 ± 412	3	1300 sec
Fgf10-GFP wild type	1412 ± 412	5	2100 sec
Fgf10-GFP *anos1-/-*	1471 ± 294	7	4000 sec
Non-transgenic	412	1	100 sec
Rhodamine 6G	18824 ± 4706	-	200 sec



○ Secreted GFP in *fgf10:secGFP* embryos
△ Fgf10-GFP in *fgf10:GFP-fgf10* embryos
□ Fgf10-GFP in *fgf10:GFP-fgf10; anos1a-/-; anos1b-/-* embryos

Figure S5. Autocorrelation function (ACF) and photon counting histogram (PCH) analysis, Related to Figure 5.

(A-D) Top, autocorrelation curves for individual measurements (gray, light red or magenta) and average two-component fit (black, red or purple) for proteins and genotypes indicated. Bottom, residuals for individual fits (gray, light red or magenta) and average fit (black, red or purple). Left Y-axis is for individual fit residuals, and right Y-axis is for average fit residuals.

(E) Molecular brightness of secreted GFP and Fgf10a-GFP. Error bars represent standard error of the mean. Differences are not statistically significant.

(F) Table of fits including short time-lags. N total = total number of individual traces, D = diffusion coefficient.

(G) Table of fits excluding short time-lags. N total = total number of individual traces, % 1-comp = percentage of traces where the F test failed to reject a one-component fit, % 2-comp = percentage of traces where the F test rejected a one-component fit and did not reject a 2-component fit, % fast comp = % of molecules in the fast component, D = diffusion coefficient, N particles per confocal = number of molecules within the confocal volume, Conc = concentration of molecules within the confocal volume.

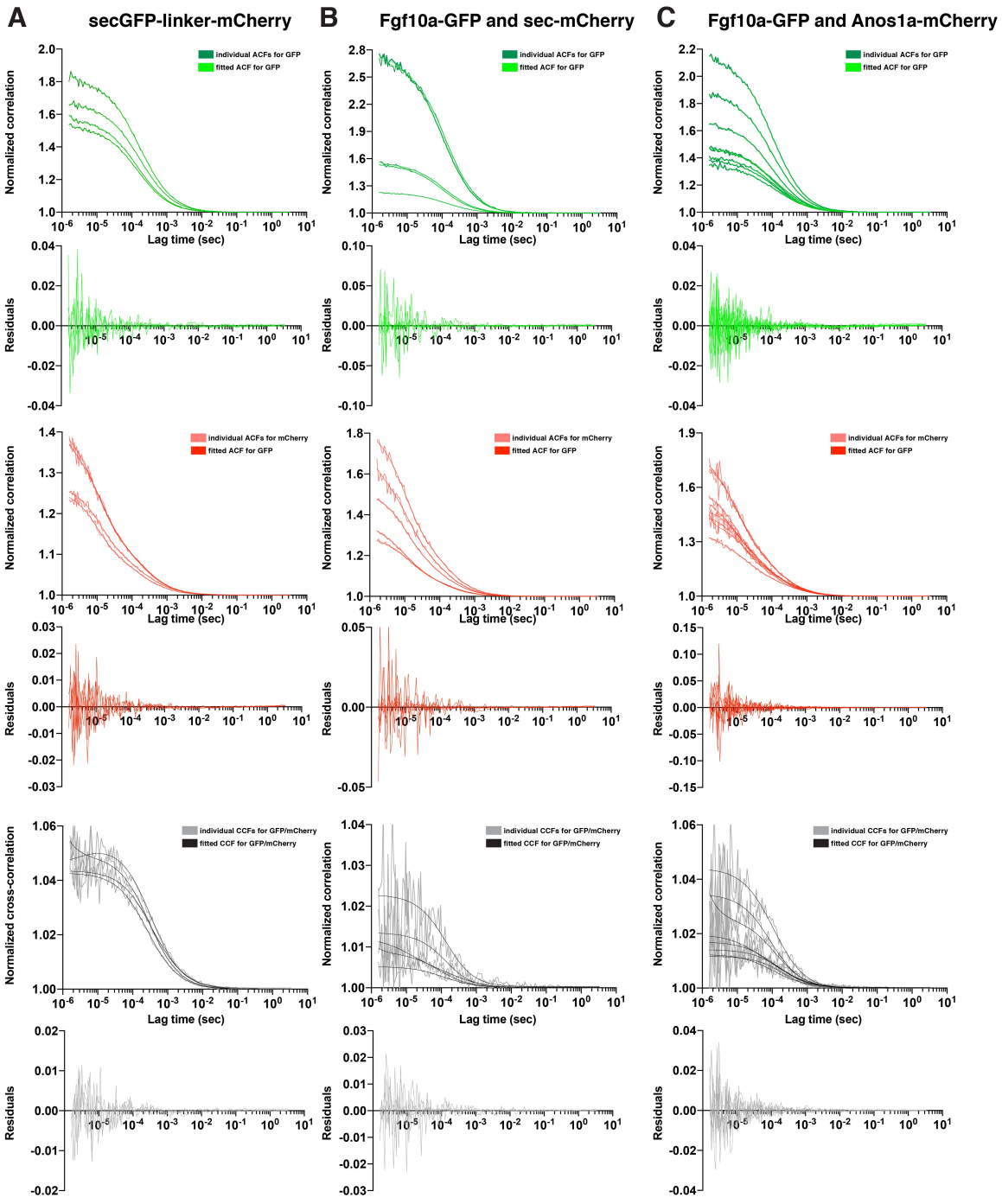
(H) Table of molecular brightness for indicated molecules and genotypes. Molecular brightness = counts per molecule per second, N (embryos) = number of embryos measured, N (total collection time) = total measurement duration.

The *anos1a*-/-; *anos1b*-/- genotype is abbreviated as *anos1*-/- in D and E.

(I) Photon counting histograms (circles, squares and triangles) and fits (lines) for secreted GFP and Fgf10a-GFP expressed from the *fgf10a* promoter in the indicated genotypes. k = photon counts.

(J) Photon counting histogram (circles) and fit (line) of Rhodamine 6G in water.

(K) Photon counting histogram (circles) and fit (line) of non-transgenic embryos measured with the same settings used for secreted GFP and Fgf10a-GFP expressed from the *fgf10a* promoter to measure and correct for background autofluorescence.



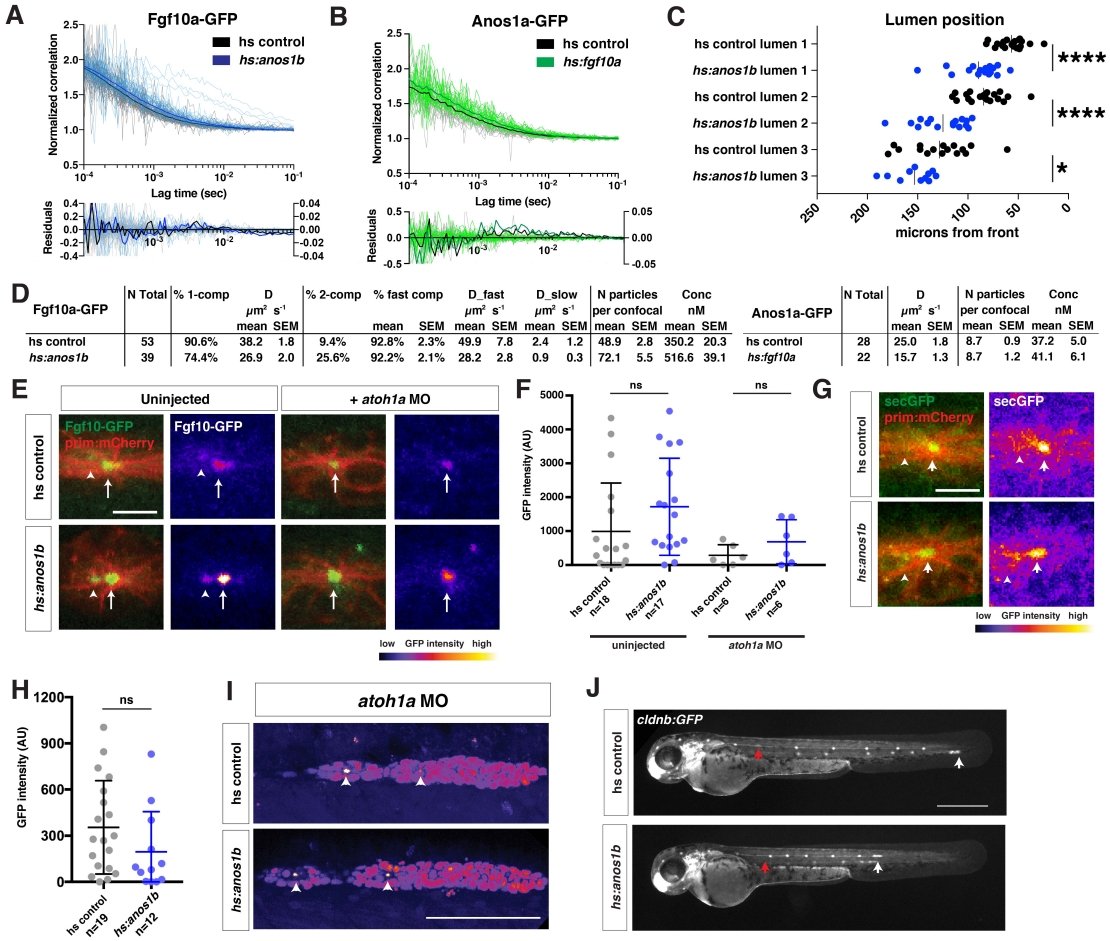
D

	low laser powers			high laser powers		
	ACF GFP D in um²/s	ACF RFP D in um²/s	CCF D in um²/s	ACF GFP D in um²/s	ACF RFP D in um²/s	CCF D in um²/s
secGFP-linker-mCherry	131.9±11.9 (n=4)	108±18.2 (n=4)	166.4±10.6 (n=4)	91.6±11.6 (n=4)	137.7±12.3 (n=4)	115.5±20.0 (n=4)
Fgf10a-GFP and sec-mCherry	78.0±16.7 (n=7)	99.4±35.7 (n=4)	*360.5±105.5 (n=5)*	128.0±21.6 (n=5)	224.0±34.3 (n=5)	*390.9±149.7 (n=5)*
Fgf10a-GFP and Anos1a-mCherry	106.5±11.5 (n=4)	102.9±46.7 (n=3)	252±93.9 (n=3)	106.3±12.1 (n=8)	194.4±46.0 (n=8)	336.2±77.7 (n=8)

Figure S6. Autocorrelation and cross-correlation analysis, Related to Figure 6.

(A-C) Autocorrelation curves and residuals for GFP and mCherry (top and middle) and cross-correlation curves for GFP/mCherry for secreted GFP-mCherry dimers (A), Fgf10a-GFP and secreted mCherry (B) and Fgf10a and Anos1a-mCherry (C). Curves for individual measurements (dark green, light red and gray) and one-component fitted curves for individual measurements (light green, dark red and black) are shown. Measurements were collected with high laser powers.

(D) Table of fits for high and low laser power FCCS measurements. n = number of individual traces, D = diffusion coefficient, mean and standard deviation are shown.



Fgf10a-GFP	N Total	% 1-comp	D $\mu m^2\ s^{-1}$		% 2-comp	% fast comp		D_fast $\mu m^2\ s^{-1}$		D_slow $\mu m^2\ s^{-1}$		N particles per confocal		Conc nM	
			mean	SEM		mean	SEM	mean	SEM	mean	SEM	mean	SEM	mean	SEM
hs control	53	90.6%	38.2	1.8	9.4%	92.8%	2.3%	49.9	7.8	2.4	1.2	48.9	2.8	350.2	20.3
hs:anos1b	39	74.4%	26.9	2.0	25.6%	92.2%	2.1%	28.2	2.8	0.9	0.3	72.1	5.5	516.6	39.1

Anos1a-GFP	N Total	D $\mu m^2\ s^{-1}$		N particles per confocal		Conc nM	
		mean	SEM	mean	SEM	mean	SEM
hs control	28	25.0	1.8	8.7	0.9	37.2	5.0
hs:fgf10a	22	15.7	1.3	8.7	1.2	41.1	6.1

Figure S7. Characterization of the Fgf10a localization and diffusion in Anos1b over-expressing embryos, Related to Figure 7.

(A) Top, autocorrelation curves for individual measurements (gray and light blue) and average two-component fit (black and blue) for genotypes indicated. Bottom, residuals for individual fits (gray and light blue) and average fit (black and blue). Left Y-axis is for individual fit residuals, and right Y-axis is for average fit residuals.

(B) Top, autocorrelation curves for individual measurements (gray and light green) and average two-component fit (black and green) for genotypes indicated. Bottom, residuals for individual fits (gray and light green) and average fit (black and green). Left Y-axis is for individual fit residuals, and right Y-axis is for average fit residuals.

(C) Position of microlumina in the primordium as scored by ZO-1 presence. X-axis represents distance from the front of the primordium. Vertical lines indicate the average. Each data point represents an individual embryo. * = $p<0.05$, **** = $p < 0.0001$. ANOVA $p<0.0001$.

(D) Tables of fits. N total = total number of individual traces, % 1-comp = percentage of traces where the F test failed to reject a one-component fit, % 2-comp = percentage of traces where the F test rejected a one-component fit and did not reject a 2-component fit, % fast comp = % of molecules in the fast component, D = diffusion coefficient, N particles per confocal = number of molecules within the confocal volume, Conc = concentration of molecules within the confocal volume. Note, Anos1a-GFP was fitted to a 1-component model only.

(E) Live imaging of microluminal Fgf10a-GFP (green) with membrane marker (red) and false coloring of Fgf10a-GFP signal only (fire look-up table, scale below) in uninjected embryos (left) and embryos injected with *atoh1a* morpholino (right). Arrows indicate Fgf10a-GFP signal in microlumina and arrowheads (left) indicate Fgf10a-GFP-producing

central cells adjacent to the microlumen. Central cells are missing in embryos injected with *atoh1a* morpholino (right). Scale bar = 10 μm.

(F) Total Fgf10a-GFP intensity in mature microlumina at the fourth apical constriction from the front in uninjected embryos (left) and embryos injected with *atoh1a* morpholino (right). Black lines are means and data points are individual embryos. n.s. = $p > 0.05$.

(G) (Left) Secreted GFP from the *fgf10a* promoter (green) in microlumina of the primordium (red) in the indicated genotypes at 36 hpf. (Right) Secreted GFP from the *fgf10a* promoter only (intensity scale on lower right). Hotter colors represent higher intensity values. Arrows indicate secreted GFP in microlumina and arrowheads indicate GFP-producing central cells. Scale bar = 10 μm.

(H) Quantification of total secreted GFP signal in mature microlumina as defined by the fourth apical constriction from the front of the primordium at 36 hpf for the indicated genotypes. Lines represent the mean, vertical lines the standard deviation and data points represent individual embryos.

(I) Lumen integrity in embryos of genotypes indicated as assessed by accumulation of *hsp70:sec-mCherry* (arrows) in a primordium with nuclei marked by *cxcr4b:H2A-mCherry*. Image is a maximum intensity projection of a Z-stack and is false colored based on fluorescence intensity using the fire look-up table (bottom). Sec-mCherry is distinguished from H2A-mCherry based on anatomical position, smaller size and higher signal intensity of microlumina compared to nuclei. Scale bar = 100 μm.

(J) Live images of heat-shocked control embryo (right) and heat-shocked *hsp70:anos1b* embryo (left) transgenic for *cldnb:lyn$_2$GFP* at 48 hpf. Red arrow indicates position of primordium at the start of 1-hour heat shock, and white arrow indicates position of the primordium at 48 hpf. Scale bar = 500 μm.

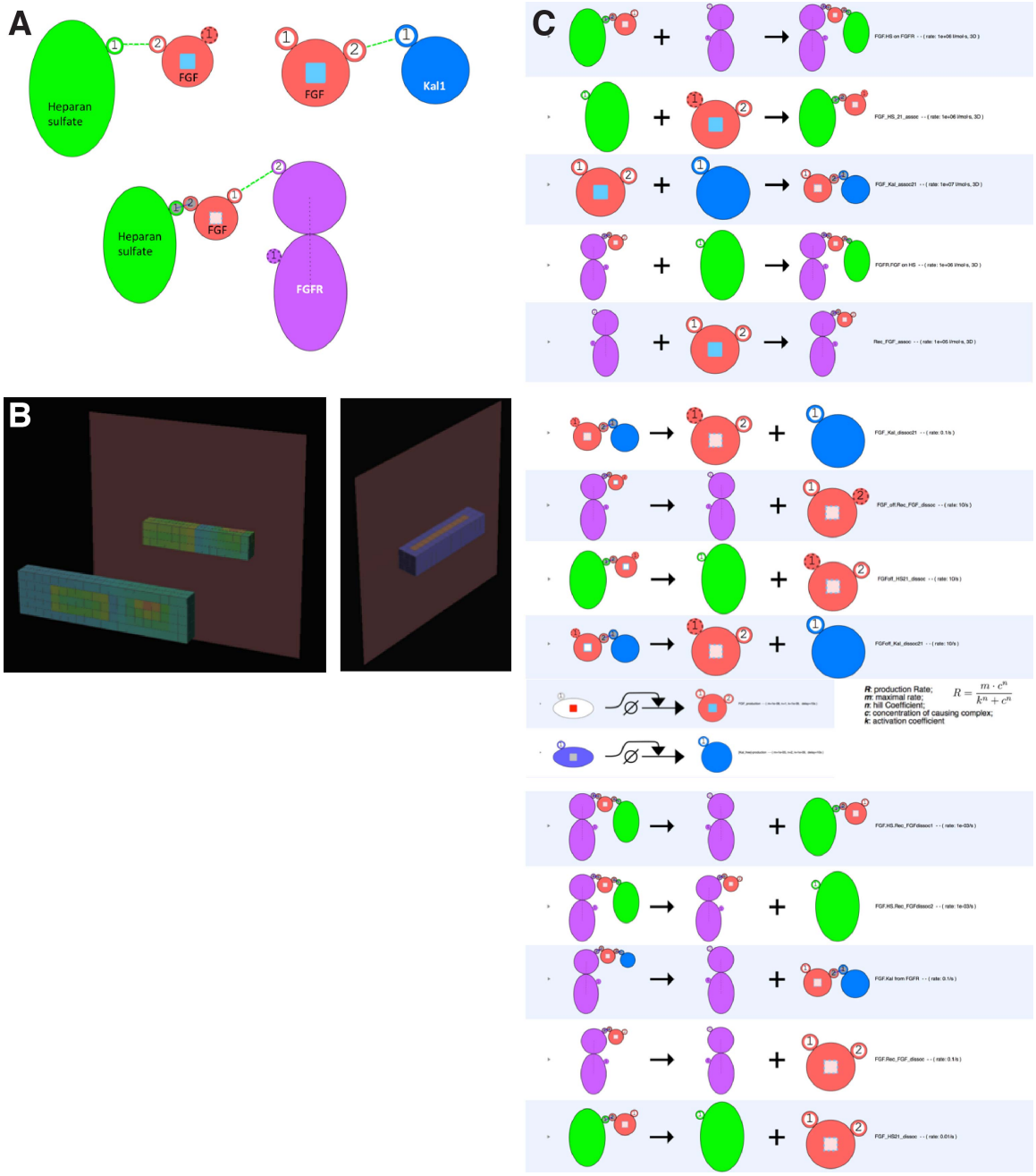

A
Heparan sulfate
FGF
FGF
Kal1
Heparan sulfate
FGF
FGFR
B
C
R: production Rate;
m: maximal rate;
n: hill Coefficient;
c: concentration of causing complex;
k: activation coefficient
$R = \frac{m \cdot c^n}{k^n + c^n}$

Figure S8. Parameters for model of FGF shuttling by Anos1, Related to Figure 8.

(A) Iconographic representation of the molecular interaction among Heparan Sulfate, FGF and the FGF receptor. The (only) binding site of Heparan Sulfate binds to one of the binding sites of FGF (upper left panel). This binding site of FGF also interacts with Anos1 (upper right panel). This means that FGF can be bound either to HS or to Anos1. FGF, when bound to HS, binds to the FGF receptor with a high affinity (lower panel). Simmune translates these representations (complemented by their rates, see Fig. S8D) into sets of differential equations that can be simulated with the Simmune Simulator.

(B) Screenshots of the 3D simulation interface of the Simmune Simulator. Left panel: Color-coded spatial distribution of FGF (Red: high concentration, Blue: low concentration). The screenshot shows – in the right region of the primordium – the cells secreting FGF with the highest concentration of the molecule while the cells in the left region that express the FGF receptor also show a substantial accumulation (receptor-bound FGF). Right panel: Location of cells that secrete Anos1.

(C) Molecular reactions and rates for associations, dissociations and productions. Production of FGF and Anos1: The production rates are controlled by a Hill function that translates the concentration c of an initiator complex (such as a transcription factor) into the production of a complex (here FGF and Anos1). The Hill functions comprise a maximal rate m (in mol/(l*sec)) and an activation coefficient k (a concentration) that determines the concentration of the initiator complex at which the production rate is half of its maximum.

Movie 1. Time lapse of heat-shocked control embryos, heat-shocked *hsp70:fgf10a* embryos and heat-shocked *hsp70:dnFgfr1* embryos, Related to Figure 1.

The primordium is marked with *prim:lyn$_2$mCherry* false-colored with fire look-up table. Warmer colors indicate higher mCherry fluorescence intensities (scale in top left corner). Scale bar = 100 μm, time stamp in hrs:min. The movie starts 90 min from start of a 60 min heat shock at 35 hpf.

Movie 2. Z-stack of Fgf10a-GFP in wild-type embryos, Related to Figure 2.

Z-stack of the primordium from apical (first frame) to basal (last frame) collected on the light sheet microscope Zeiss Z.1 for Fgf10a-GFP and Leica SP5 II confocal microscope for secGFP expressed from the *fgf10a* promoter. Top, Fgf10a-GFP and secGFP are displayed as green and mCherry from *prim:lyn$_2$mCherry* is displayed as red. Bottom, Fgf10a-GFP and secGFP are false-colored with fire look-up table. Warmer colors indicate higher GFP fluorescence intensities. Scale bar = 50 μm. In the first part of the movie, white arrows indicate microlumen with surrounding patch of Fgf10a-GFP, and arrowheads indicates patch of Fgf10a-GFP above an apical constriction where the microlumen has not yet formed. Arrowhead indicates patch of Fgf10a-GFP above an apical constriction where the microlumen has not yet formed. Green arrows indicate the central cells, in which intracellular Fgf10a-GFP is visible. In the second part of the movie, white arrows indicate secGFP in microlumen and green arrows indicate the central cells, in which intracellular secGFP is visible.

Movie 3. Z-stack of mosaic primordium with Fgf10a-GFP-secreting cells in the front, Related to Figure 2.

Z-stack of the primordium from apical (first frame) to basal (last frame) collected on a Leica SP5 II confocal microscope. Top and bottom left, host-derived nuclei of the primordium are labeled with mCherry from the *cxcr4b:H2A-mCherry* transgene (red), the donor-derived primordium cells are labeled with membrane GFP from the *cldnB:lyn$_2$-GFP* transgene (green). Note, the *cldnB:lyn$_2$-GFP* transgene also labels donor-derived cells in the lateral line nerve that trails the primordium and is located underneath the primordium and donor-derived cells in the skin above the primordium. The membrane mCherry expression of donor-derived primordium cells from the *prim:lyn$_2$-mCherry* transgene is not visible due to the high levels of membrane GFP expression from the *cldnB:lyn$_2$-GFP* transgene. Top left, GFP intensity of Z-stack scaled to show the membrane GFP expression from the *cldnB:lyn$_2$-GFP* transgene. Bottom left, GFP intensity of Z-stack scaled to show accumulated Fgf10a-GFP in microlumen (arrow). The Fgf10a-GFP intensity is about 10-fold lower than the membrane GFP intensity from the *cldnB:lyn$_2$-GFP* transgene. There are two reasons for this. First, the *fgf10a:GFP-fgf10a* BAC transgene encompasses the entire genomic *fgf10a* locus and recapitulates endogenous expression levels while the *cldnB:lyn$_2$-GFP* transgene consists of a 8-kb DNA fragment from the *cldnB* promoter and was selected for high expression. Second, *fgf10a* is expressed at low levels while *cldnB* is expressed at high levels. Right, close-up of the microlumen indicated by a square in the overview on the bottom left. Fgf10a-GFP intensities are pseudo-colored with a heat-map to visualize intensity differences. Host-derived primordium nuclei are pseudo-colored with cyan to avoid overlap with heat-map color scale. Scale bar is 25 μm for overview image on left and 2 μm for close-up on right. Section thickness is indicated on top left.

Movie 4. Time lapse of wild-type and *anos1a-/-; anos1b-/-* embryos, Related to Figure 4.

Time lapse imaging of wild-type (top) and *anos1a-/-; anos1b-/-* embryos (bottom). The primordium is marked with *cldnb:lyn$_2$GFP* false-colored with the fire look-up table (scale in top left corner). Warmer colors indicate higher GFP fluorescence intensities. Scale bar = 100 μm, time stamp in hrs:min. The movie starts at 35 hpf

Movie 5. Z-stack of Anos1a-GFP protein distribution in the primordium, Related to Figure 4.

Z-stack of the primordium from apical (first frame) to basal (last frame) in *anos1a:GFP-anos1a*; *prim:lyn$_2$mCherry* 36 hpf embryo collected on the light sheet microscope Zeiss Z.1. Top, *Anos1a-GFP* is displayed as green and *prim:lyn$_2$mCherry* is displayed as red. Middle, *prim:lyn$_2$mCherry* is displayed as greys. Bottom, heatmap of *Anos1a-GFP* intensities, with higher intensities represented by hotter colors. Scale bar = 10 μm, Z-slice = 0.65 μm.

Movie 6. Z-stack of Fgf10a-GFP in heat-shocked control and heat-shocked *hsp70:anos1b* embryos, Related to Figure 7.

Z-stack of the primordium from apical (first frame) to basal (last frame) in a heat-shocked control embryo (left) or heat-shocked *hsp70:anos1b* embryo (right) collected on the light sheet microscope Zeiss Z.1. Top, *Fgf10a-GFP* is displayed as green and *prim:lyn$_2$mCherry* is displayed as red. Bottom, heatmap of *Fgf10a-GFP* intensities, with higher intensities represented by hotter colors (scale in bottom left corner). Arrow indicates microlumen with surrounding patch of Fgf10a-GFP, and arrowhead indicates

patch of Fgf10a-GFP above an apical constriction where the microlumen has not yet formed (this is absent in the *hsp70:anos1b* embryo). Scale bar = 100 μm, Z-slice = 0.47 μm.

Movie 7. Time lapse of Cdh2-GFP in heat-shocked control embryos and heat-shocked *hsp70:anos1b* embryos, Related to Figure 7.

Time lapse of heat-shocked control embryos and heat-shocked *hsp70:anos1b* embryos carrying the *cdh2:cdh2-GFP* transgene. The primordium is marked with *prim:lyn$_2$mCherry* (red) and Cdh2-GFP (green). The second part of the movie shows the same images but with Cdh2-GFP false-colored with fire look-up table. Warmer colors indicate higher GFP fluorescence intensities. Scale bar = 100 μm, time stamp in min. Each time frame is a maximum projection of an individual Z-stack. The movie starts 105 min after start of a 60 min heat shock at 31 hpf.

Movie 8. Time lapse of heat-shocked control embryos and heat-shocked *hsp70:anos1b* embryos, Related to Figure 7.

Time lapse of heat-shocked control embryos and heat-shocked *hsp70:anos1b* embryos. The primordium is marked with *cldnb:lyn$_2$GFP* and false-colored with fire look-up table (scale in top left corner). Warmer colors indicate higher GFP fluorescence intensities (scale in top left corner). Scale bar = 100 μm, time stamp in hrs:min. The movie starts 90 min from start of a 60 min heat shock at 35 hpf.

Table S1. Oligonucleotides used in this study, related to STAR Methods.

Oligonucleotides	SOURCE	IDENTIFIER
anos1a probe cloning: 5'-GGTGGCACGGACATCAGAAGAACG-3', 5'-GAAGACCGGGAAGGCTGGCAAAAT-3'	Integrated DNA Technologies	Custom synthesis
anos1b probe cloning: 5'- TCCCGCTGAAGCCAAGAAGAGAC -3', 5'-CAGCAGGCCGGTAATCACAAAATG-3'	Integrated DNA Technologies	Custom synthesis
fgf3 probe cloning: 5'-ATGGTTATAATTCTGCTCTT-3', 5'-TTAAATGTCAGCCCTTCTGT-3'	Integrated DNA Technologies	Custom synthesis
fgfr1a probe cloning: 5'-AGGCGATGGGGATGGATAAAGAAA-3', 5'-TCCGGCGTCATGAGAAAACACT-3'	Integrated DNA Technologies	Custom synthesis
fgfr1b probe cloning: 5'-CTGGCGGAGTGATTTGTTTTGATT-3', 5'-ATGCTCCCGTATTCGTTCTCCACA-3'	Integrated DNA Technologies	Custom synthesis
fgf10a probe pcr template: 5'-CTACAACCCCACCAAAGGGAAC-3', 5'- GAAATTAATACGACTCACTATAGGCATGTG TAACCGATAGAATAGC-3'	Integrated DNA Technologies	Custom synthesis
EGFP probe pcr template: 5'-GTGAGCAAGGGCGAGGAGCTG-3', 5'- GAAATTAATACGACTCACTATAGGGCTTGT ACAGCTCGTCCATGCC-3'	Integrated DNA Technologies	Custom synthesis
anos1a genotyping primers: Outer PCR primers: 5'-AGCTGTGTGCAAAGTGTTGTAG-3' 5'-CATATAGTTTGATAGAGCGCTTGGAC-3' Inner PCR primers: 5'-AGATGAGAGTGTGTGTACTATGGC-3' 5'-CCCAGAAGCCACTTTGTGTG-3'	Integrated DNA Technologies	Custom synthesis

anos1b genotyping primers: Outer PCR primers (all alleles): 5'-GTGTGACGAGCGCTGAGTTCCTG-3' 5'-ATTTCACCTGTTTCTTTTAGTATG-3' Inner PCR primers (5 nt deletion allele): 5'- TGTGTGGAGAGCTGTGCGCGGGACCGCG AG-3' 5'- TAAATACCTTTATTGAAATTCCCCAGTATCA TCCAGCCGTGCTCTCCAGTGTG-3' Inner PCR primers (6, 15 and 79 nt deletion allele): 5'-TCGCTACGTGTGCAGAAGCAGGG-3' 5'-TAAATACCTTTATTGAAATTCC-3'	Integrated DNA Technologies	Custom synthesis
fgf3 genotyping primers: Outer PCR primers: 5'-ATCCCGCCATGCCACAAT-3' 5'-TCTCGTACCCCACATAAAACTGAC-3' Inner PCR primers: 5'-CTGCTCTTGTTGTTACTGAGC-3' 5'-CTCAAATATCAAACGGTTTACTCAC-3'	Integrated DNA Technologies	Custom synthesis
fgf10a genotyping primers: Outer PCR primers: 5'-TGCATCACCCTTTCTCCCATCCAG-3' 5'-TCGTCCTTGCTTTTGGTGCCATTG-3' Inner PCR primers: 5'- GCTCTTCCCAGTTTTCCGAGCTCCAGGACA ATGTGCAAATCG-3' 5'-TCCGTTCTTATCGATCCTGAG-3'	Integrated DNA Technologies	Custom synthesis
hsp70:anos1b genotyping primers: Outer PCR primers: 5'-TGAGCATAATAACCATAAATACTA-3 5'TCAGGCAGCGGGACACG-3' Inner PCR primers: 5'-AGCAAATGTCCTAAATGAAT-3' 5'-CGCAACGCTCACCTCAAAC-3'	Integrated DNA Technologies	Custom synthesis
Fgfr1a genotyping primers: PCR primers: 5'-TTTGCCGGTGAAATGGATGGCTCC-3' 5'-AGTCTTACAGCTCATGTGTGCATG-3'	Integrated DNA Technologies	Custom synthesis

hsp70:fgf10a genotyping primers: Outer PCR primers: 5'- TGAGCATAATAACCATAAATACTA-3'/5'- TCGTCCTTGCTTTTGGTGCCATTG-3' Inner PCR primers: 5'- AGCAAATGTCCTAAATGAAT-3'/5'- TCCGTTCTTATCGATCCTGAG-3'	Integrated DNA Technologies	Custom synthesis