Supplementary Information for

Prostaglandin signaling regulates renal multiciliated cell specification and maturation

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This PDF file includes:

Figs. S1 to S9
Captions for datasets S1 and S2

Other supplementary materials for this manuscript include the following:

Datasets S1 and S2

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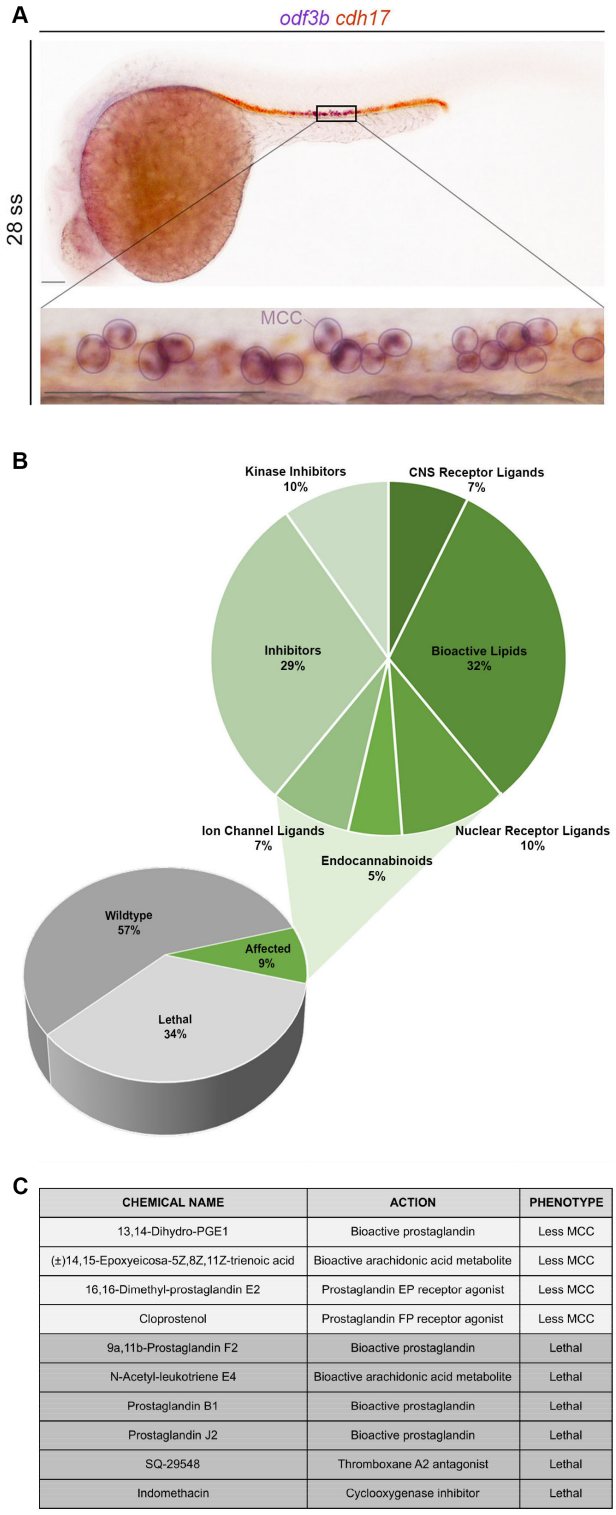

C

CHEMICAL NAME	ACTION	PHENOTYPE
13,14-Dihydro-PGE1	Bioactive prostaglandin	Less MCC
(±)14,15-Epoxyeicosa-5Z,8Z,11Z-trienoic acid	Bioactive arachidonic acid metabolite	Less MCC
16,16-Dimethyl-prostaglandin E2	Prostaglandin EP receptor agonist	Less MCC
Cloprostenol	Prostaglandin FP receptor agonist	Less MCC
9a,11b-Prostaglandin F2	Bioactive prostaglandin	Lethal
N-Acetyl-leukotriene E4	Bioactive arachidonic acid metabolite	Lethal
Prostaglandin B1	Bioactive prostaglandin	Lethal
Prostaglandin J2	Bioactive prostaglandin	Lethal
SQ-29548	Thromboxane A2 antagonist	Lethal
Indomethacin	Cyclooxygenase inhibitor	Lethal

Fig. S1. (A) Wild-type embryo at the 28 ss stained by WISH (lateral view), with nephron cells labeled by *cdh17* (orange) and maturing MCCs by *odf3b* (purple). (Bottom) Magnified view of the region outlined by the black box on the whole animal. Scale bar = 50 μm. (B) Pie chart of screen results. (C) List of prostaglandin pathway hits and lethal phenotypes.

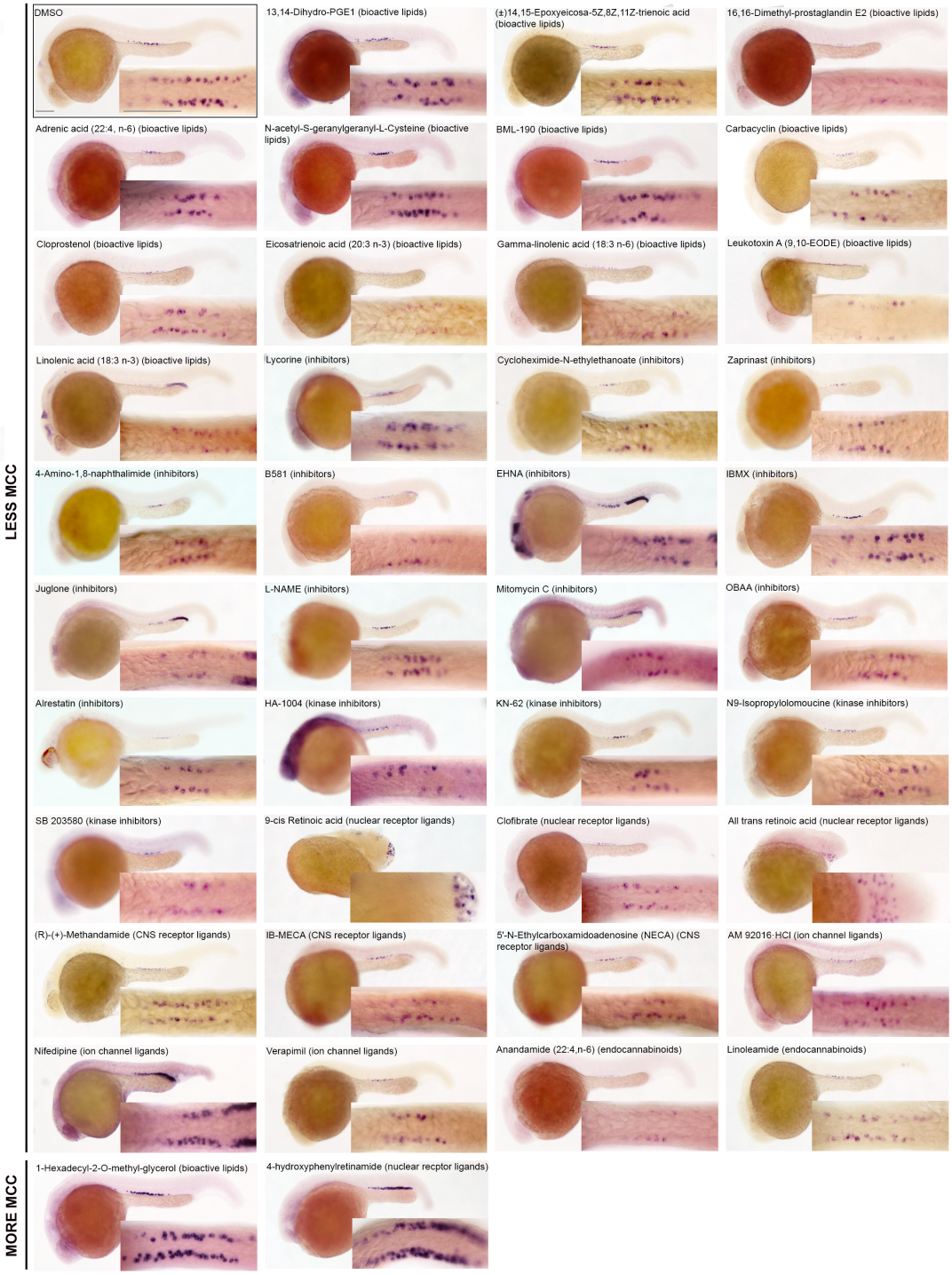

Fig. S2. Representative images of each "hit" from the chemical genetic screen. WISH was performed to detect *odf3b* transcripts (purple), and in some, transcripts encoding the DL segment marker *slc12a3* were labeled because riboprobe cocktails were sometimes used during the screen. Images are labeled with the hit and category. Lateral view shows whole embryos, and dorsal view shows the MCC field. Categories of less or more MCCs are as indicated, with the exception of the DMSO control (black box) which indicates standard MCC number. Scale bars = 50 μm.

Fig. S3. (A) Representative images of live embryos at 48 hpf shows brain swelling (black arrowhead) and pericardial edema (teal arrowhead) in *cox* morphants. (B) Percentage of animals with pericardial edemas for each experimental condition. Scale bar = 100 μm.

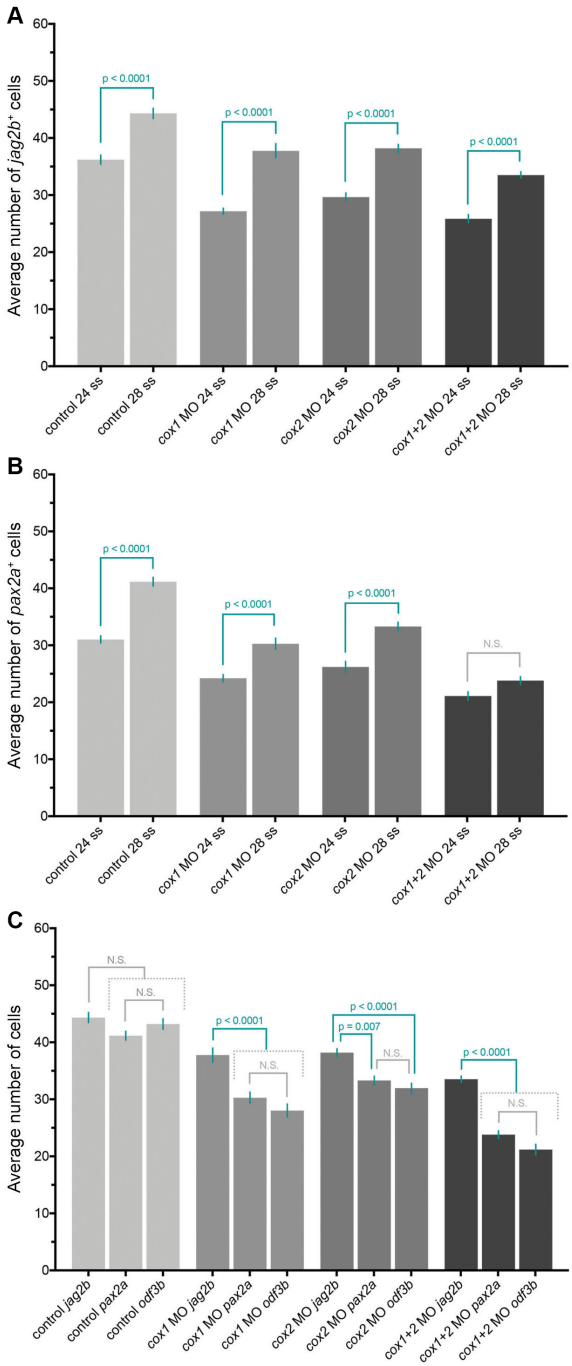

Fig. S4. Graphical presentation comparing the average number of *jag2b*$^{+}$ (A) and *pax2a*$^{+}$ (B) cells in the pronephros at the 24 and 28 ss for each experimental condition. (C) Graphical comparison of average number of *jag2b*$^{+}$, *pax2a*$^{+}$, and *odf3b*$^{+}$ cells in the pronephros at the 28 ss. Data are presented +/- SEM and ANOVA was used to determine significance.

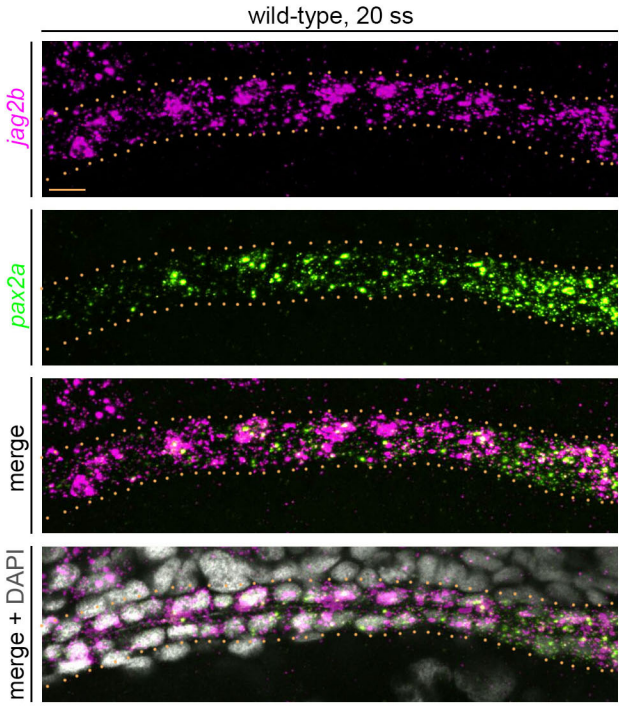

Fig. S5. Whole mount FISH analysis at the 20 ss displays *jag2b* (magenta) and *pax2a* (green) transcript expression, where DAPI labels nuclei in grey. Orange dotted lines outline the nephron tubule. Images are max image projections. Scale bar = 50 μm.

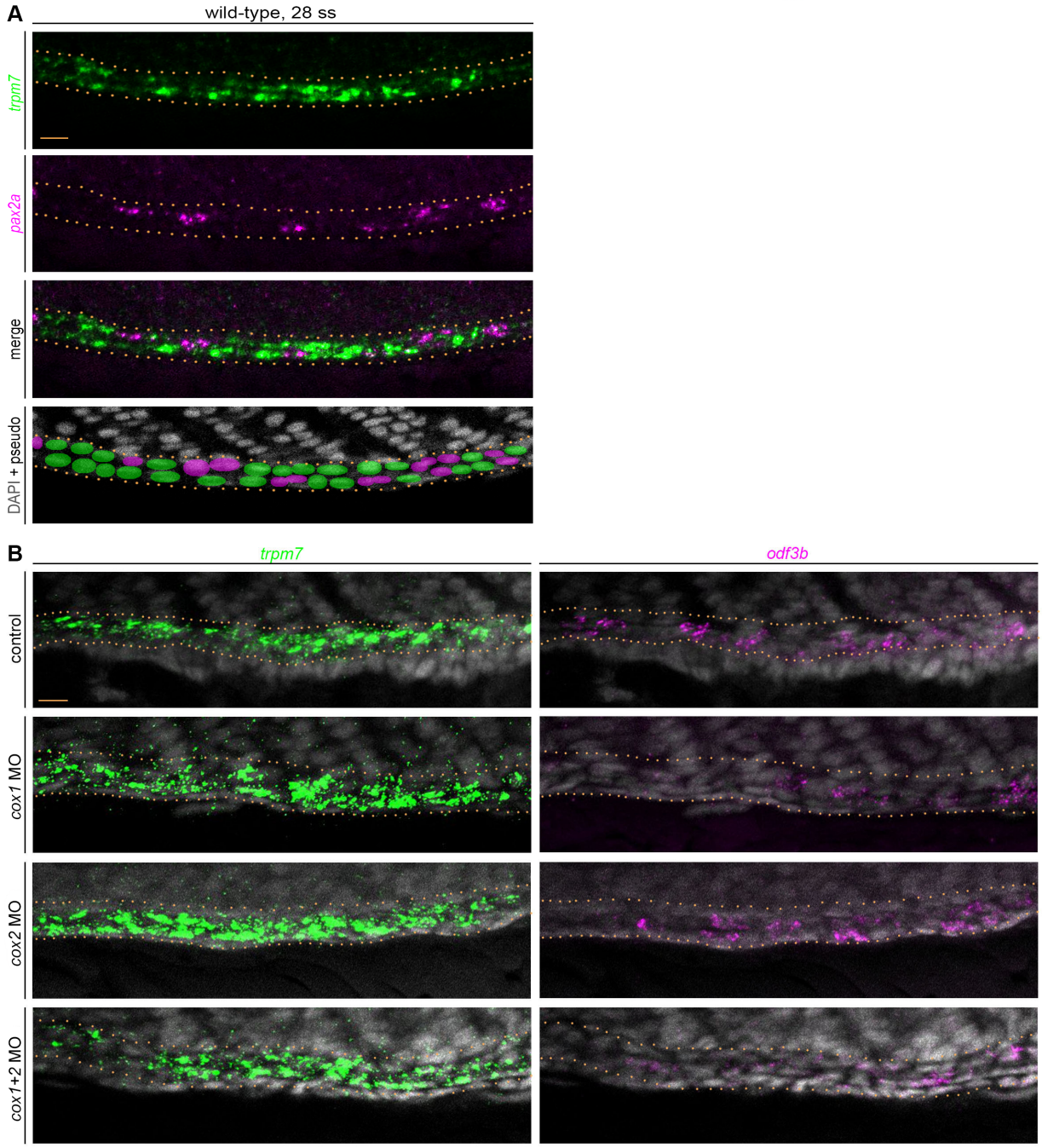

Fig. S6. FISH data corresponding to the representative embryos from Figure 3A to show different fluorescent channel combinations. Here, either *trpm7*/DAPI (green/grey) or *odf3b*/DAPI (magenta/grey) are provided. Orange dotted line demarcates the outline of the pronephros.

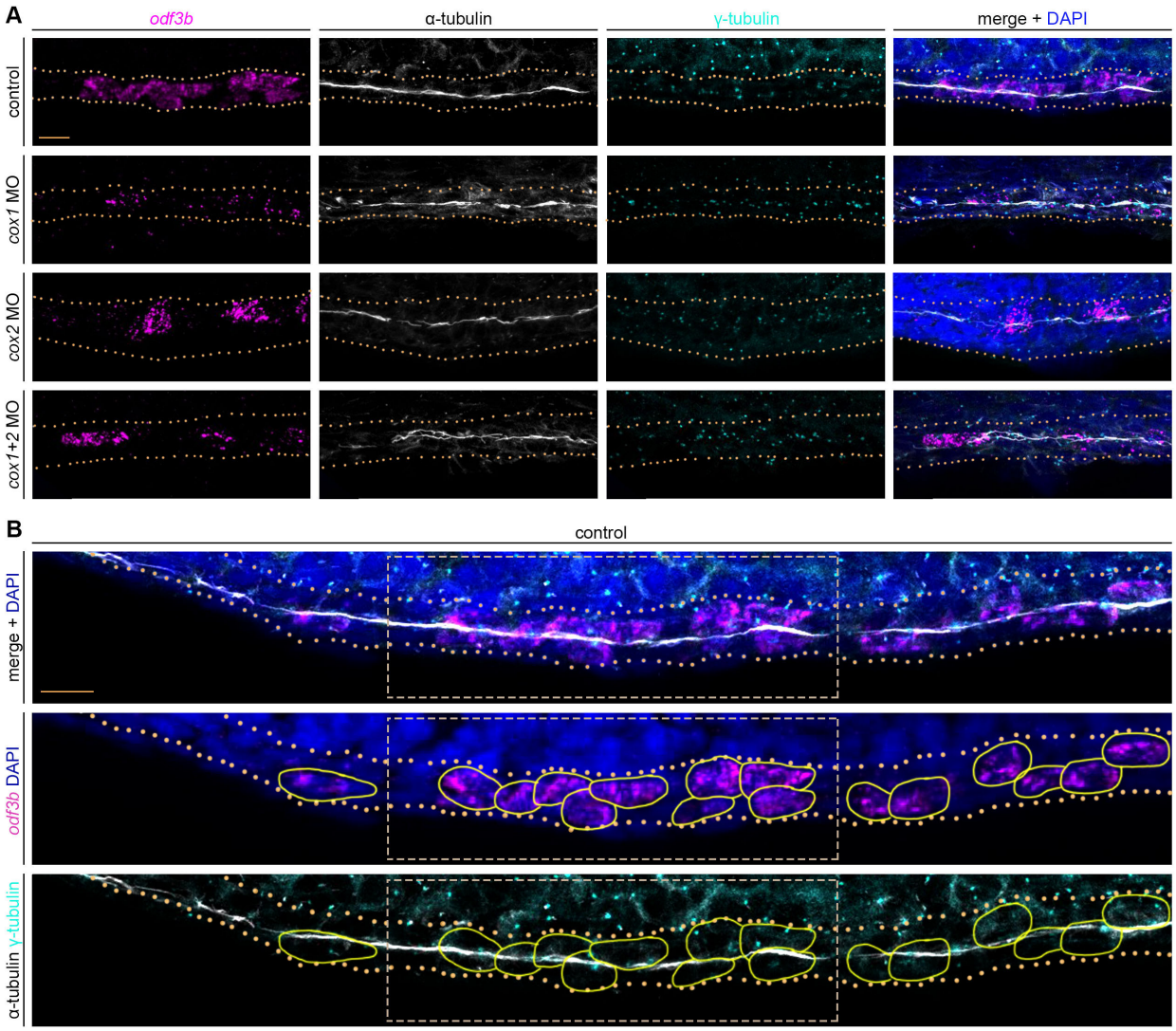

Fig. S7. FISH analysis of the representative embryos from Figure 4A and 4D to show different fluorescent channels and process of data analysis **(A)** Individual channels of 28 ss embryos that have undergone FISH combined with IF analysis to visualize maturing MCCs (*odf3b*), cilia (anti-acetylated α-tubulin), and basal bodies (γ-tubulin) in the pronephros. **(B)** Process of outlining *odf3b*$^{+}$ cells in yellow for basal body analysis. Using the composite of *odf3b* and DAPI, *odf3b*$^{+}$ cells (magenta) were outlined in yellow *in silico*. The yellow outlines were then duplicated to a composite of anti-acetylated α-tubulin and γ-tubulin to examine cilia outgrowth from basal bodies in the pronephros. The presented panels are from the control embryo in Figure 4A and 4D. The pink dotted box outlines the nephron region that is cropped in panel (A) and in Figure 4A and 4D. (A, B) Scale bar = 10 μm.

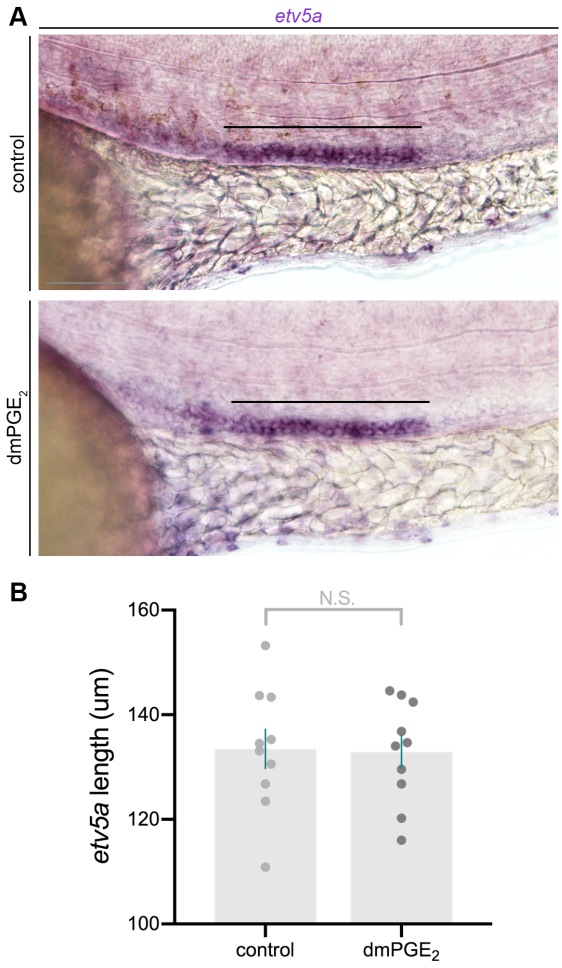

Fig. S8. (A) Representative images of 28 ss wild-type embryos treated with DMSO or 100 µM $dmPGE_2$, then analyzed via WISH for *etv5a* transcripts. The black lines denote *etv5a* transcript domain in the pronephros. Grey scale bar = 50 µm. (B) Quantification of *etv5a* domain length in the pronephros. Data is presented +/- SEM; student's T-test determined significance.

Etv4/5 binding site = **5'-GGAA/T-3'**

A 1kb upstream of zebrafish *cox1*-201

5'_ATGCACTCGAATTGATATGTTGG**GGAA**AAAACGTTAAATACAATTTTAACAAACA**GGAA**AATCCAAGATAA
ACATAAAAATATACAATTTAGTTAGAATTTTGTAGTCTGCATATATATTTACACAAGTCACTGTATAACAATGGC
TTGTTTTGTTTTGACTATC**GGAA**AAATATGTAAGGGGCTAAATAATTTTGTCCTTAAAATGATGTTTAAAAAATT
AAAAACTGCTTTCATTCTAGCCGAAATAAAACAAATAAGACTTTCTCCAGAAGAAAAAAATATTATCAGACATA
CTGTGAGAATTCCCTTACTGTTAAACATCATTTG**GGAA**ATATTTAAAAAAGAAAAAAAATTCAAAGGGGGACTA
ATAATTCTGACTTCAACTGTATATATATAGACGGTAGGTGAATAAACTCTTACTGTAAT**GGAT**ATAAAATTAAAT
GTACCAAAATTATTGCCCATATATATTTATGTCTAGTATTACCATATTATAAACGCCCAAAAATGTCAAAAAAGC
AACATCTAATGGAGATGAGCAAACTAATACTCTTGTAACTTAATGAGTTAATATGTTTCATTTA**GGAT**GTTCGT
ACAGCAGACCTTTTGACGTTTATTATATCACTCAGCAAACGCTTTCTAGACCAAACGCACTTACAAATGTTTAC
AATCTTGGTGCCATGTGGCTTTACGATCTTCACGAATGCTTGTTCATAAGATTATTGTTTTAAAGCATAAGAAT
TGCAGATGTTTTTGAAGTCCGAATACGTTGCGTCTTAGTAAAAGTGAGGTCTGTAGCAGACTCGAACACACG
GTGGCGCCCTGAAGGTTTACTTTTCTTTCTACTATCTTTTCAGTAACTTCAGAAACAAGTGTGACCCTCATGTC
TTTTTGTGTGTGCTTTGTTTTTAATCGTGAAGTTGTCCTGAAGC**GGAA**GGCTGGTGTTTCTTGTTTGCTTTCGC
TATAAAAGTTCCACTCGACTGCTTTTTTCCCCCTTCATCAATCTGCGAGAATC_3'

1 kb upstream of zebrafish *cox1*-202

5'_CTCAGATCACTCAGATCTTTA**GGAT**CATATAAACTAGAAACTTTGAGAGTTCAGTCAAAGCAGGGTGAATT
AGCTTTCAGCTACTACAATCCCCGCTGCT**GGAA**TCAGCTTCCAGAAACGATTAGATGTGCTGCAACATTAGG
CACATTGAAATCAAGACTGAAAACACATCTGTTTAGCTGTGCCTTTACTGAATGAGCACTGTGCTACGCCCCA
CAGATAGCACTATTGTGTCTTTCTTTTCTTTTTCATTCTTTTATAATCTGTTTTAACACATTTTCATCTGTTTTTAA
TCATTTTTATTATTTGTTTTTTATTTTCTTATACTTGTGTTTTTTTTTAATTCTTGTTTATGTAAAGCACTTTGATTT
GCCACTGAAATGTGGTATATAAATAAACTTGCCTTGCTTTGCCTTGATGAAAGATTTAGAAATTCGTTTAGTAA
ACAGACCTACTGTAA**GGAA**ACACCTGCTTGGCATAAATGCTTGAAAATGATTTAATAATTTTAATATTTAATAG
ACACTTAAAAGTAATCAAAAAGTAATCTGATGTATTCAGTTACTTTACTTTTACAAAGTAATTGAAAAGGTACCT
ATTTCATTTTAAATTGTGTAATTTGTAACCTGTAATCTATTAAATTTCCAAAGTAACCTCCCTAACACTGCAGAC
AGACAGAAAGATAGACAGACAGACAAACAGATAGATAGATAGACAGACAGACAGACAGACAGACAGACAAAC
AGATAGATAGATAGACAGACAGACAGACAGACAGACAGATAATTATATTATTGAAACATGCACTCCCAGTTTC
TCTCATAATTAAAAAATGCAAACTATTGATGACACTTTCTTAAACTTAAAGATAAACATTTTTGTCTTTTTTCTAA
GCATAAAATACCCCACGTGTTTACATGTTTTCAGTCATGTTTTATATGTTTTAGACAGCATAATATAATGTTTAT
TTACACTCAAAAGTGTTAATAAAACAATATTTAATATAATAGCCCTGCA_3'

B 1 kb upstream of zebrafish *cox2*-201

5'_TGTTTTTGCATGATTTGATAAATATGATCATTGTATTAGCCAAATATTTTGTTCATAAATGCAGATATTTCCA
AACACATCCACCACTCATTGTATGTATAAAAATACTTAAAATCTGTTT**GGAT**TCACTGGCTAAAATGAAAGCAA
AAATTTCCTTAAAATGCTTAAATTATAGCCGATTCTGTTTTATATTGATTTATTTAATGTTTTTTTCCACTCTTAAT
TTTATTTTAGTTTGAATGTTTTTTGTAAATCACATTTTTTTGTATTTTGGTATATTATTTTGAGTTGAGCCTTCCTT
GTGTGATACACAATTTGTAGAGTTGAATGCCATTTTGTTGTATTTAGATTTTGTATTTCCAATAATAAATAAATA
AATAAATAAATAAATAAATAAATAAATAAATAAATAAATAAATAAATAAATAAATAATAATAAAATAAA
GAAGCCTTAAATAAGTTTGACTTTATCAGTACATA**GGAT**TACGAATATCTAATAAAATGAGCCATTTGTATTCG
AAACGTTTTTAACTATATGATATATTATTACATATTAGCCTATATTGTCTTTATTGGTTTTCAAAAATGTCAGCAG
GTAATTAAAGACATTTTCTTTTCATAGATTCAATCCAGAAAGTTGTTTAAAACAATAATAGCCTACAAAAAATAA
ACGT**GGAA**TATTATTATTTCACCCATATTTATTTTTTACGTTATCTTTACAAAAAAAAAAAAAGCATTCTTCAATA
TGTTTTTTAGGCTAATCTGTGCAATATATAGGCCTGTATCATTTTTAAGTTTAATATCTCATACATTTAATCTTGT
CTTTTACACAAATACGCTAGTTGCGAAGAAAAACATAATTGCATGTAGTTTATTTATATTTATTTATATTTTGTTA
TATAGTTTTTTTTTTCAGGGTAAACATTTTAAGAGTGATTCGATTCATTTATACATACATTTCC**GGAT**TCATGAA
TATAAATCACTGTGT**GGAA**AAATGCAGTC**GGAT**GATGACG_3'

Fig. S9. *in silico* analysis of putative Etv4/5 binding sites (A) 1 kb upstream of *cox1*, where two distinct spliceoforms have slightly different upstream domains, and (B) 1 kb upstream of *cox2*. The sequence for the Etv4/5 binding site is in teal.

Additional Dataset S1 (separate file).

MCC ontogeny chemical screen data. MCC phenotypic data from WISH *odf3b* riboprobe assay are listed for each drug in the library collection. For each compound, the dosage tested is listed along with known biological function and molecule category. Green highlight indicates that the compound was scored as a hit, where at least 2/3 biological replicates were scored positively as a hit. Orange highlight indicates that the compound was scored as having no effect on MCC development. Red highlight indicates that the compound was lethal at the dosage tested. Red lines demarcate the beginning/end of chemicals located in plates 1 through 6 of the library.

Additional Dataset S2 (separate file).

Statistical comparisons of all quantifications presented in the study. The statistical comparisons are organized according to figure number and letter in order from the beginning to end of the figures and supplemental figures, as labeled.