

Supporting Figure Legends

Supporting Figure S1

A chemical genetic screen identifies E2 signaling as a negative regulator of embryonic zebrafish liver development

(A) Seven compounds were identified in the chemical genetic screen. Estrogenic compounds 17 β -estradiol (E2), estriol (E3), 17 α -ethynylestradiol (EE2), diethylstilbestrol, and quercetin decreased liver size, whereas the aromatase inhibitor chrysin and estrogen receptor antagonist tamoxifen increased liver size. (B) Representative images of *fabp10a* expression at 72 hpf (arrowheads) visualized by WISH in WT embryos exposed to 1% ethanol (control), EE2 10 μ M, EE2 25 μ M, or EE2 50 μ M from 24-72hpf. (C) Quantification of *fabp10a* area at 72 hpf. $n \geq 22$, **** $p < 0.0001$, one-way ANOVA. (D) Representative images of *fabp10a* expression at 72 hpf (arrowheads) visualized by WISH in WT embryos exposed to 1% ethanol (control), BPA 10 μ M, BPA 25 μ M, or BPA 50 μ M from 24-72hpf. (E) Quantification of *fabp10a* area at 72hpf. $n \geq 9$, *** $p < 0.001$, **** $p < 0.0001$, one-way ANOVA. (F) Representative images of *fabp10a* expression at 72 hpf visualized by WISH in WT embryos exposed to DMSO or E2 10 μ M in glass plates and in plastic plates. (G) Quantification of *fabp10a* expressing liver areas at 72hpf. ns=not significant, one-way ANOVA. All values represent mean \pm SEM, scale bars = 200 μ m.

Supporting Figure S2

Optimal E2 signaling is required for normal liver development

(A) Embryos exposed to DMSO and E2 from 18-72hpf developed smaller livers compared to controls. Progesterone and testosterone exposures did not impact liver development. (B) Quantification of GFP+ hepatocytes in Tg(*fabp10a:GFP*) embryos exposed to DMSO or E2 from 24-72hpf at 72hpf as analyzed by fluorescent activated cell sorting (FACS). *** $p < 0.001$, $n \geq 11$, two-tailed Student's *t*-test. Scale bar = 200 μ m. All values represent mean \pm SEM. (C) Analysis of *esr1*, *esr2a*, and *esr2b* expression in published microarray data from isolated gut:GFP (*foxA3*) cell populations at 2 and 4dpf reveals most significant expression of *esr2b* at 2dpf when hepatocyte differentiation begins. (D) Quantification of liver area as assayed by WISH for *fabp10a* of *esr2b* MO injected embryos at various indicated MO dosages. ns=not significant, * $p < 0.05$, ** $p < 0.01$, **** $p < 0.0001$, two-tailed Student's *t*-test. (E) Genomic structure of zebrafish *esr2b* with 5'UTR (white box) and coding exons (black boxes). Sequence alignment of *esr2b* in *esr2b*^{+/+} and *esr2b*^{-/-} embryos reveals 5-base pair deletion in the first exon. (F) Expression of *esr2b* in *esr2b*^{+/+} and *esr2b*^{-/-} embryos at 72hpf as assayed by WISH, showing *esr2b* expression in the liver (dotted line and an arrowhead) in WT but not in *esr2b*^{-/-} mutants. Scale bars = 200 μ m. (G) Histology sections with H&E staining of *esr2b*^{+/+} and *esr2b*^{-/-} embryos exposed to DMSO or E2 show no significant changes in liver architecture.

Supporting Figure S3 E2 signaling does not affect other endodermal or mesodermal lineages

(A) Embryos exposed to DMSO or E2 from 24-72hpf were WISH for *fabp10a*, *trypsin*, and *insulin*. E2 decreased liver size but did not affect exocrine or endocrine pancreas. Scale bars, 200 μ m. (B) Representative images of WISH for *transferrin* (*tfa*) at 72 hpf of *esr2b*^{-/-} mutants and WT siblings upon exposure to DMSO or E2 from 24-72 hpf. (C)

Quantification of liver size at 72 hpf. ns=not significant, **** $p < 0.0001$, one-way ANOVA. All values represent mean \pm SEM, all scale bars = 200 μm .

Supporting Figure S4

E2 signaling does not affect other endodermal or mesodermal lineages

(A) Bigenic endothelial cell and hepatocyte reporter embryos Tg(*fabp10a:GFP*:*flk1:mCherry*) exposed to DMSO or E2 from 24-72hpf reveal no differences in vasculature formation upon modulation of estrogen signaling. (B) Quantification of blood vessel surface area (μm^2) of DMSO control and E2 exposed embryos. (C) Quantification of blood vessel surface area/Liver area (%) of DMSO control and E2 exposed embryos. ns=not significant. Scale bars = 200 μm , Scale bars (insets) = 70 μm .

Supporting Figure S5

E2 signaling affects hepatobiliary fate decisions

(A) Liver size of WT and *cloche*^{-/-} embryos exposed to DMSO or E2 from 24-72 hpf as assessed by WISH for *fabp10a* at 72 hpf. E2 decreased liver size in WT at a similar extent to E2-induced decrease in liver size in *cloche*^{-/-} embryos. *cloche*^{-/-} mutants had smaller liver compared to WT. (B) Distribution graph of liver size showing % of embryos with large (L, dark grey), medium (M, light grey) or small (S, black) liver. $n \geq 8$. Scale bars, 200 μm . (C) Biliary tree marker *sox9b* in WT embryos exposed to DMSO, E2, or Ful from 24-72 hpf at 72 hpf. Ful decreased cholangiocyte formation compared to controls (red arrowheads), while enhancing *fabp10a* expression. (D) Biliary tree and liver size distribution of embryos as assessed by ISH for *sox9b* and *fabp10a* at 72hpf as % of embryos with large (L, dark gray), medium (M, light gray) or small (S, black) biliary tree

or liver. * $p < 0.05$, **** $p < 0.0001$, two-tailed Student's t -test. * indicates significant difference of % Ful-exposed embryos with small biliary tree compared to that of the controls. † shows significant difference of % Ful-exposed embryos with large liver compared to that of controls. (E) Bile duct marker 2F11 immunostaining of WT embryos and *esr2b* morphants at 72hpf. Scale bar = 70 μm . (F) Quantification of 2F11 staining surface area. $n \geq 10$, **** $p < 0.0001$, two-tailed Student's t -test. All values represent mean \pm SEM, scale bars = 200 μm .

Supporting Figure S6

E2 signaling affects hepatobiliary fate decisions

(A) Quantification of BEC surface areas (μm^2) of individual Tg(tp1blob:GFP) embryos exposed to DMSO or E2 from 24hpf. Each dotted line represents an individual embryo, each solid line represents average of all samples.

Supporting Figure S7

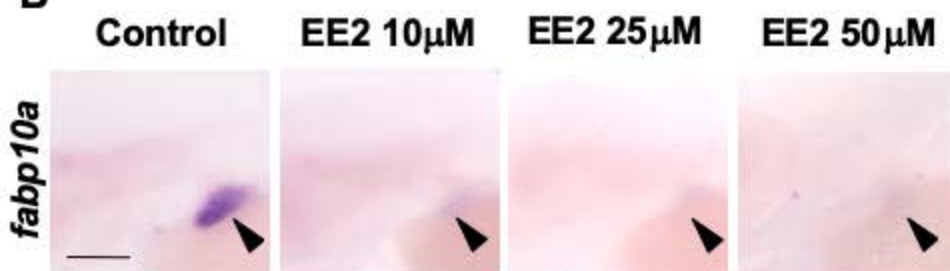
E2 signals through BMP pathway to impact hepatobiliary development

(A) Quantification of liver size in zebrafish embryos exposed to DMSO, E2 (10 μM), Dorsomorphin (7.5 μM , 10 μM , 12.5 μM), or E2 (10 μM) + Dorsomorphin (7.5 μM , 10 μM , 12.5 μM) from 24-72hpf at 72hpf. Liver size assessed by WISH for *fabp10a* at 72hpf. $n \geq 10$, * $p < 0.05$, ** $p < 0.01$, two-tailed Student's t -test. All values represent mean \pm SEM.

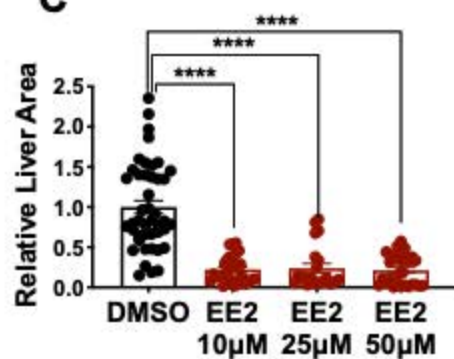
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Compound	Effect on liver size
Estradiol (E2)	↓
Estriol (E3)	↓
17 alpha-ethynylestradiol (EE)	↓
Diethylstilbestrol	↓
Quercetin	↓
Chrysin	↑
Tamoxifen	↑

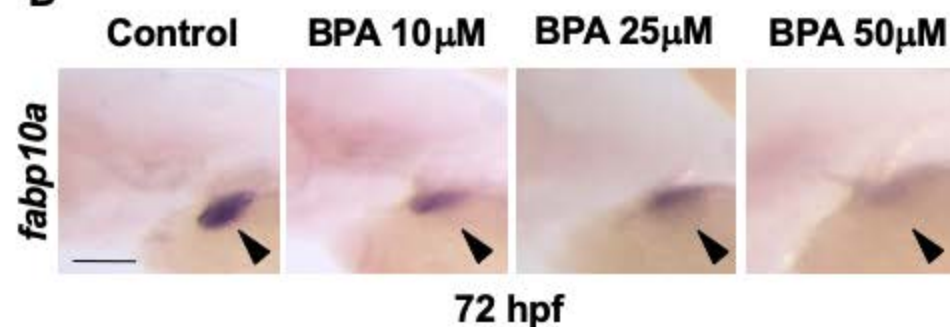
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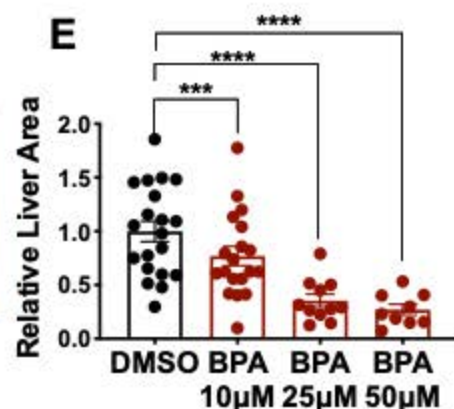
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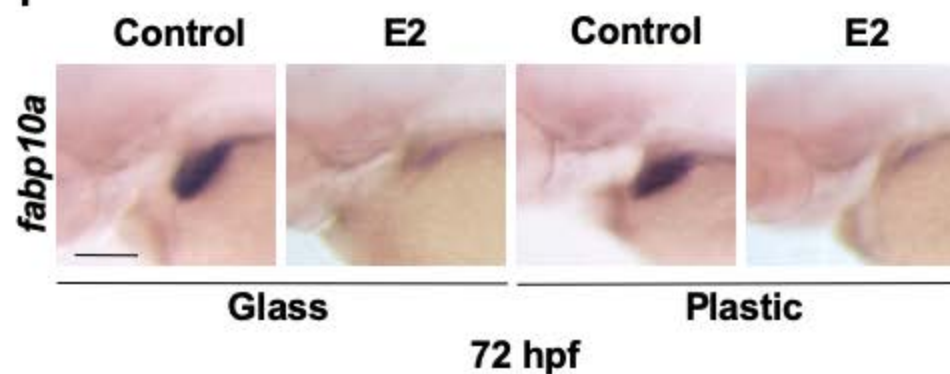
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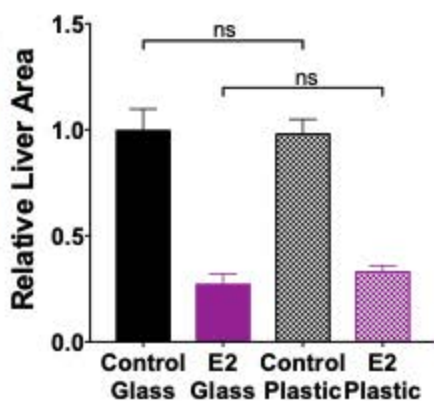
E



F



G



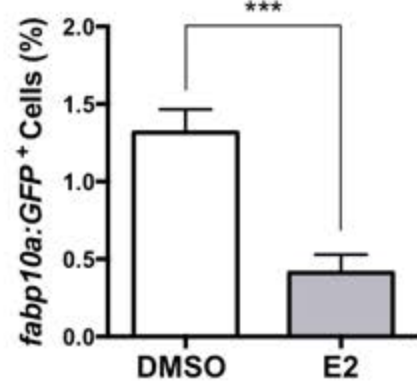
A

DMSO E2 Progesterone Testosterone

fabp10a

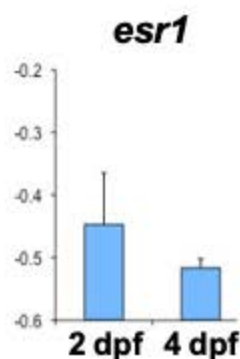
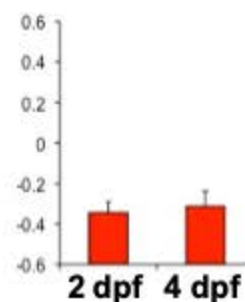
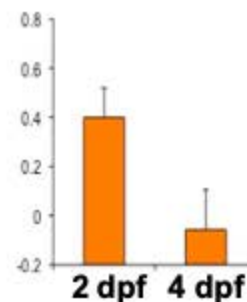
72 hpf

B

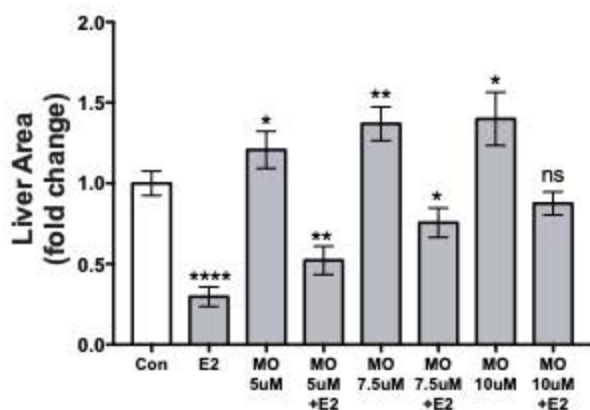


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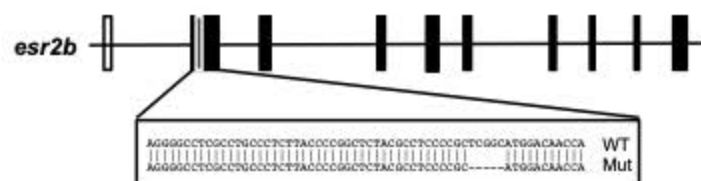
Relative expression

*esr2a**esr2b*

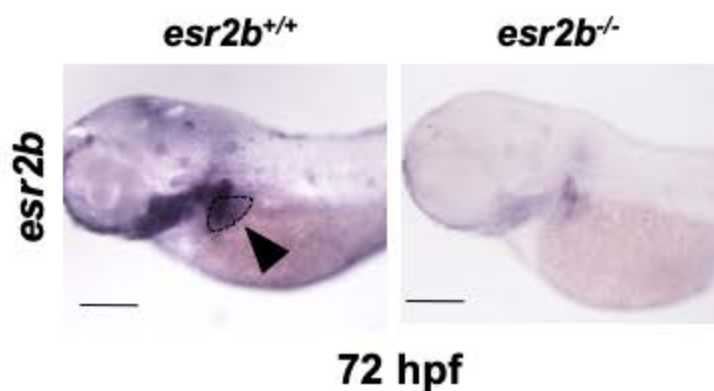
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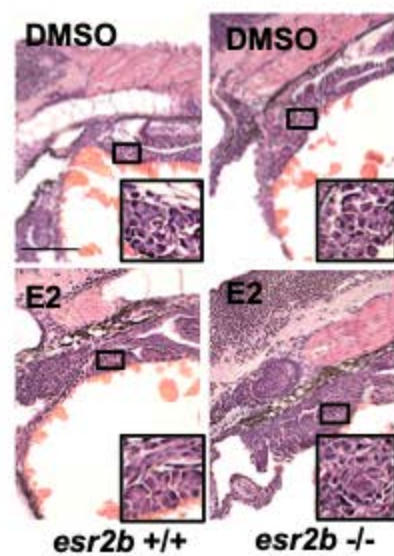
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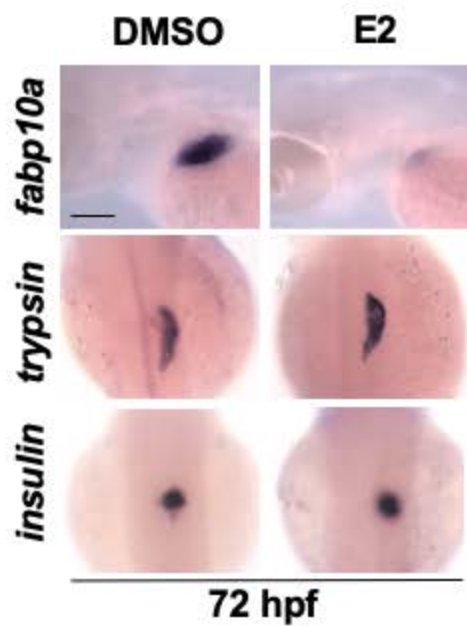
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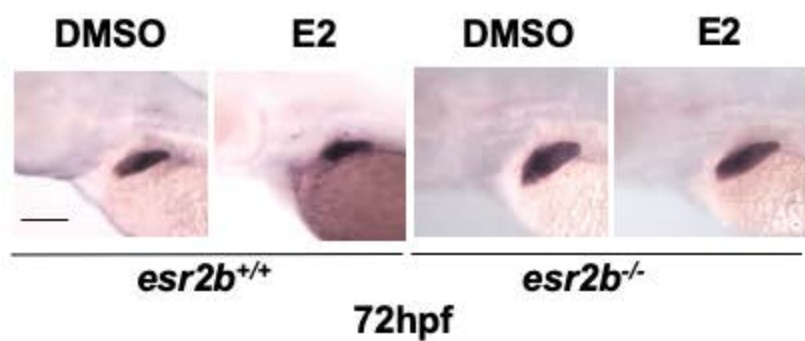
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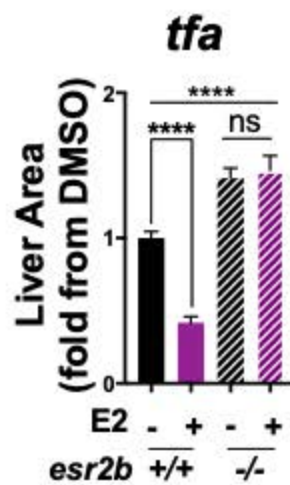
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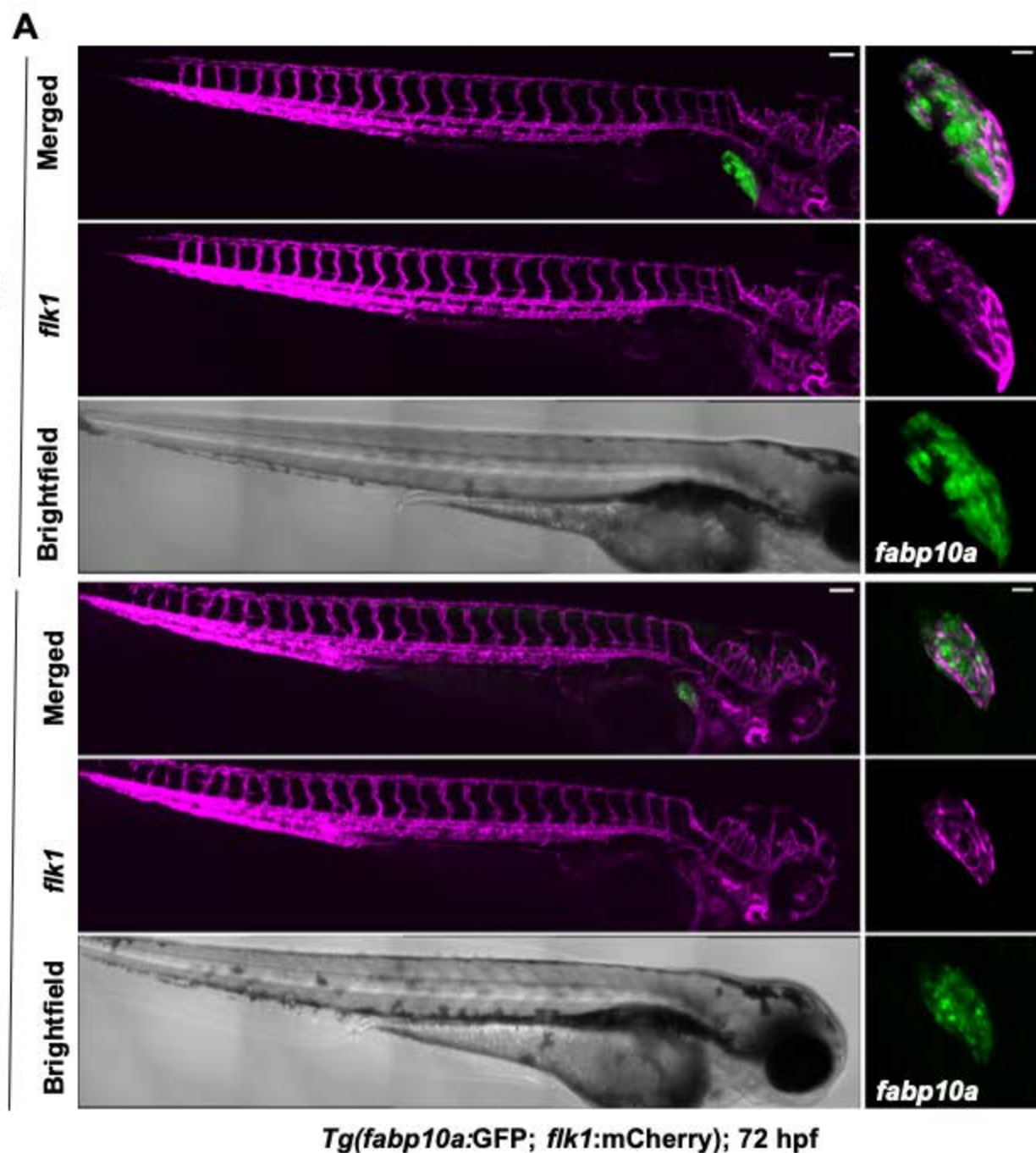
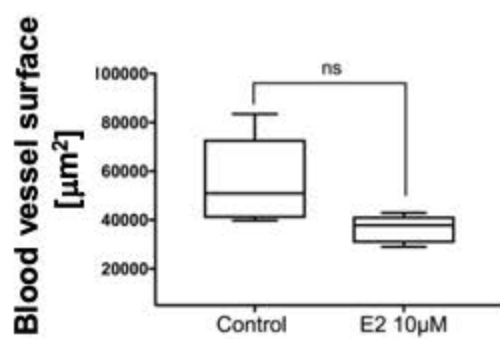
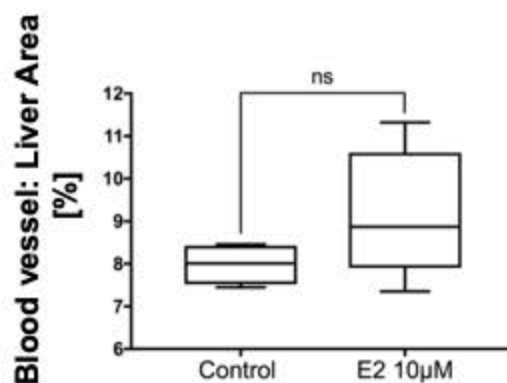


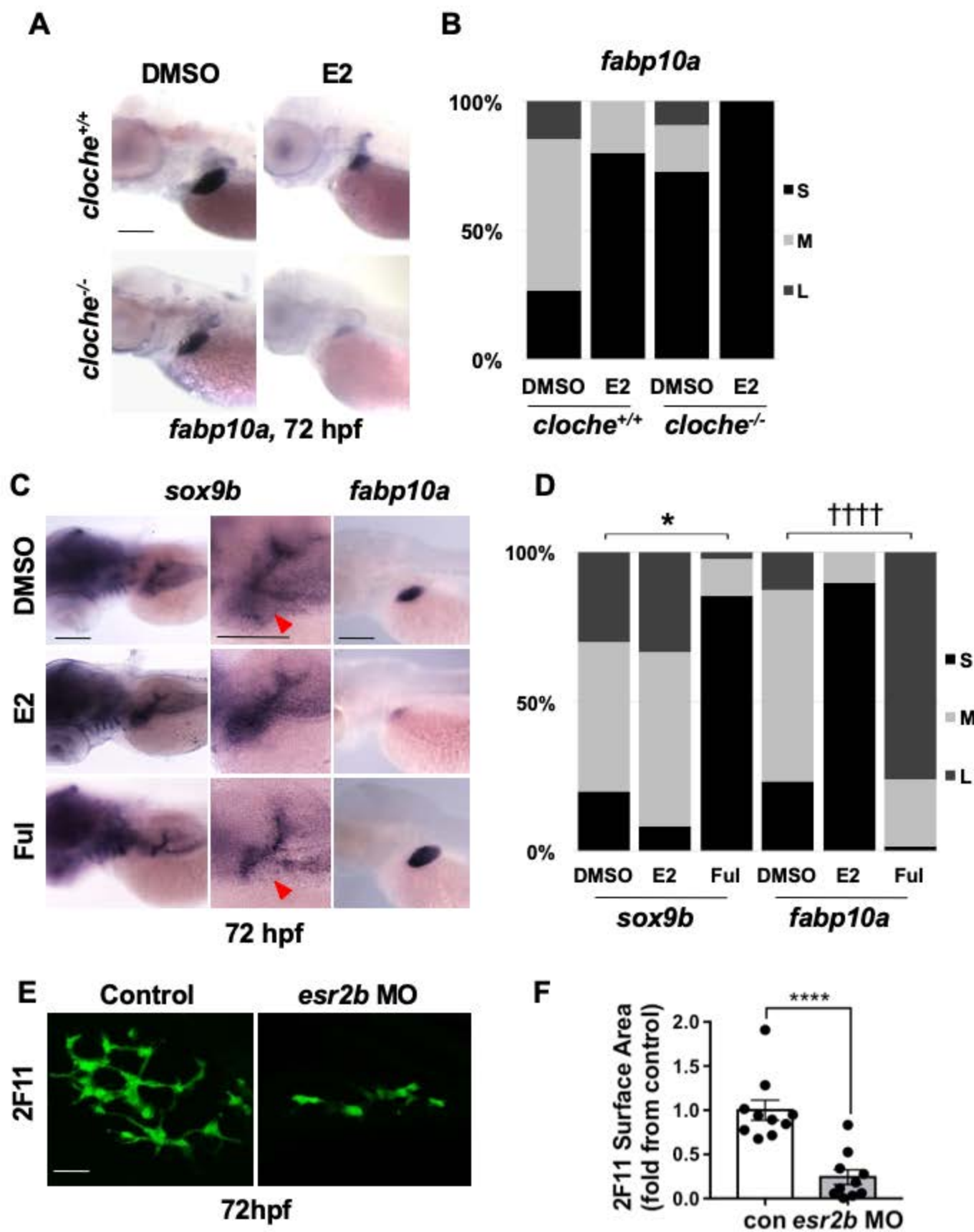
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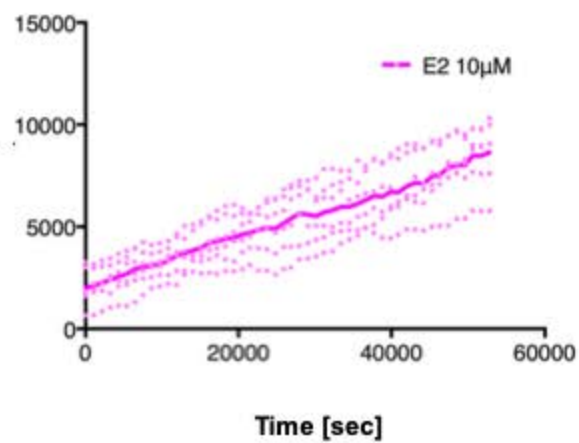
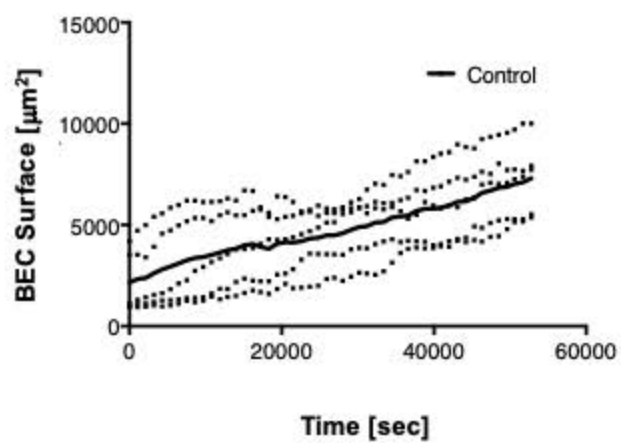
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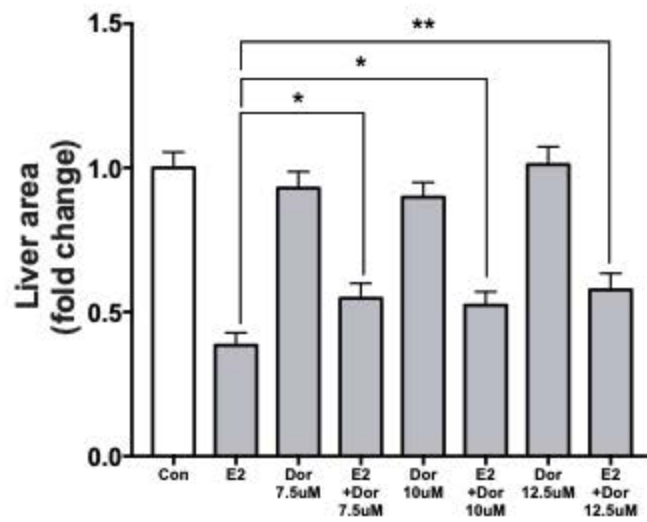
**B****C**



A



A



Supporting Table 1. Chemicals

Chemical	Concentration	Supplier, Catalog Number
β -Estradiol	10 μ M	Tocris, 2824
MPP dihydrochloride	80 μ M	Tocris, 1991
PHTPP	8 μ M	Tocris, 2662
Fulvestrant	10 μ M	Tocris 1047
Anastrozole	10 μ M	Tocris, 3388
Progesterone	10 μ M	Tocris 2835
Testosterone	10 μ M	Tocris 2822
Dorsomorphin	10 μ M	Tocris 3093
K02288	5 μ M	Tocris 4986

Supporting Table 2. Antibodies

Antibody	Application	Concentration	Supplier
CK7	IF	1:200	Abcam, Ab9021
Albumin	IF	1:200	Rockland,109-4133
2F11	Whole-mount IHC	1:500	abcam ab71286
FITC	Whole-mount IHC	1:100	abcam ab6724
p-Smad	WB	1:1000	Cell Signaling,13820
Smad	WB	1:1000	Cayman, 10822
β -actin	WB	1:5000	Cell Signaling,4970
α -Rabbit AlexaFlour 647-IgG1	IF	1:1000	Abcam, ab150075
α -Rabbit IgG-HRP	WB	1:1000	Santa Cruz, sc 2004