Figure S1

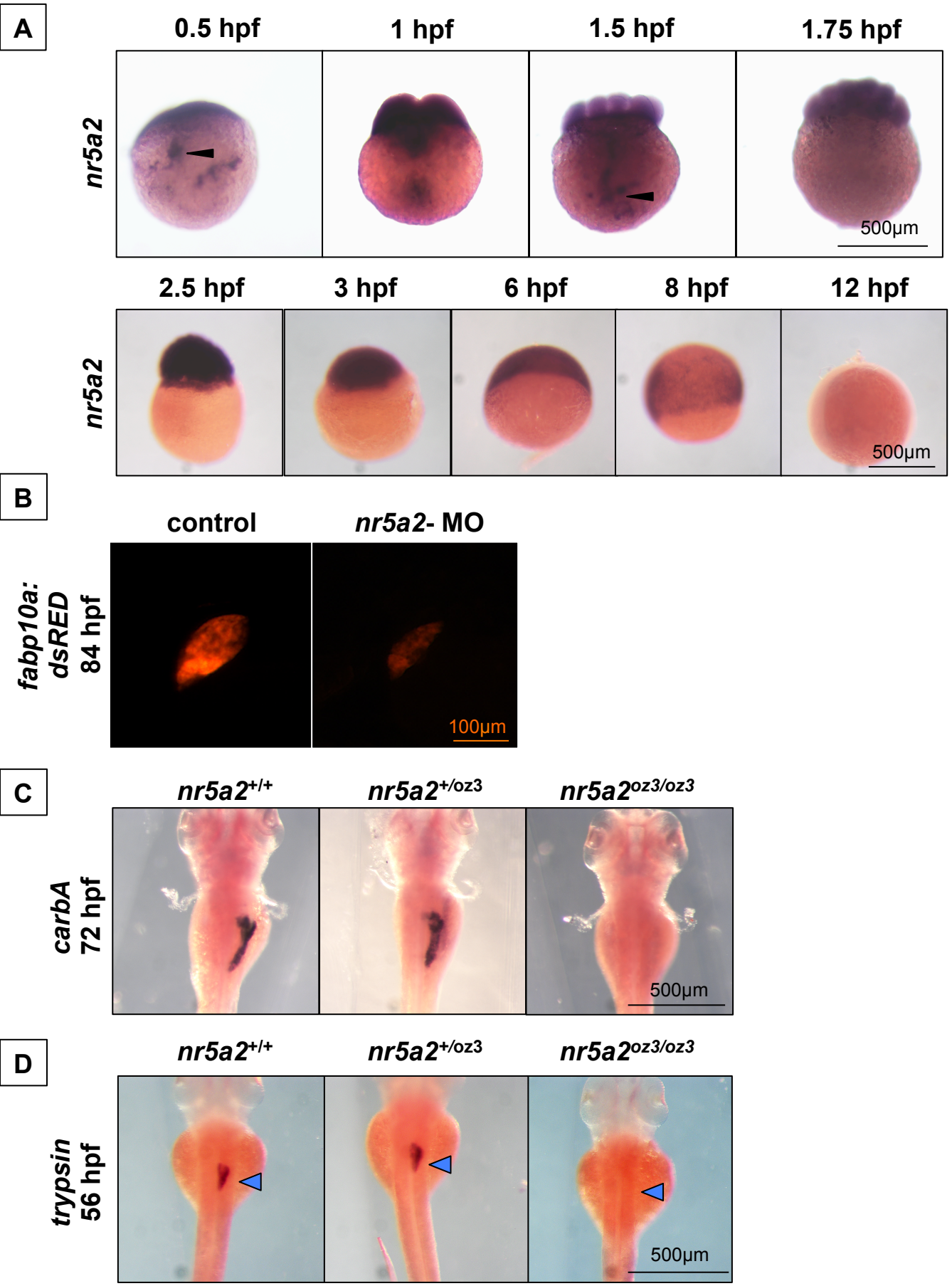

A
0.5 hpf
1 hpf
1.5 hpf
1.75 hpf
nr5a2
500µm
2.5 hpf
3 hpf
6 hpf
8 hpf
12 hpf
nr5a2
500µm
B
control
nr5a2- MO
fabp10a:
dsRED
84 hpf
100µm
C
nr5a2+/+
nr5a2+/oz3
nr5a2oz3/oz3
carbA
72 hpf
500µm
D
nr5a2+/+
nr5a2+/oz3
nr5a2oz3/oz3
trypsin
56 hpf
500µm

Figure S2

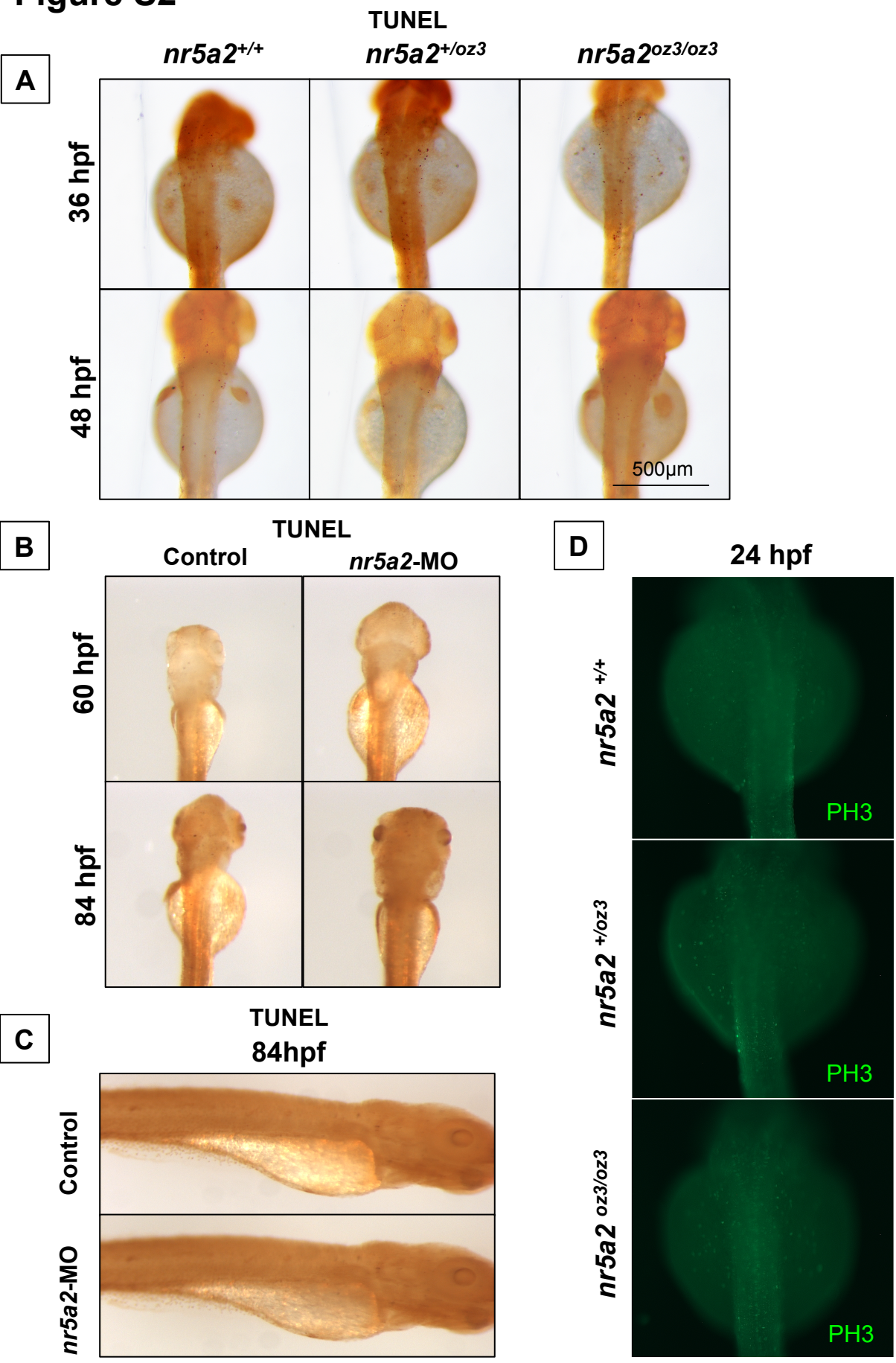

A
TUNEL
nr5a2+/+
nr5a2+/oz3
nr5a2oz3/oz3
36 hpf
48 hpf
500μm
B
TUNEL
Control
nr5a2-MO
60 hpf
84 hpf
C
TUNEL
84hpf
Control
nr5a2-MO
D
24 hpf
nr5a2 +/+
nr5a2 +/oz3
nr5a2 oz3/oz3
PH3
PH3
PH3

Figure S3

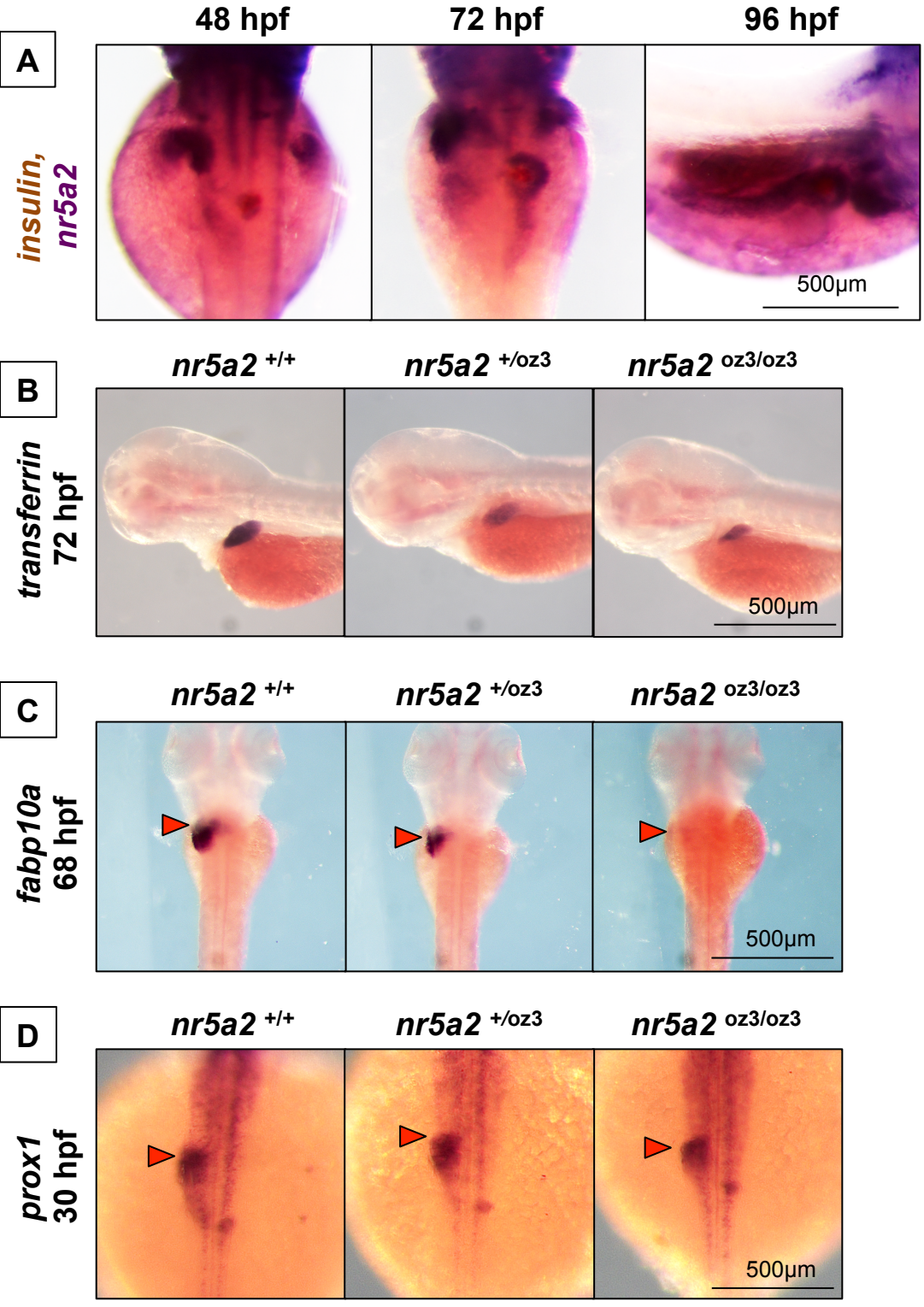

A
48 hpf
72 hpf
96 hpf
insulin,
nr5a2
500μm
B
nr5a2 +/+
nr5a2 +/oz3
nr5a2 oz3/oz3
transferrin
72 hpf
500μm
C
nr5a2 +/+
nr5a2 +/oz3
nr5a2 oz3/oz3
fabp10a
68 hpf
500μm
D
nr5a2 +/+
nr5a2 +/oz3
nr5a2 oz3/oz3
prox1
30 hpf
500μm

Figure S4

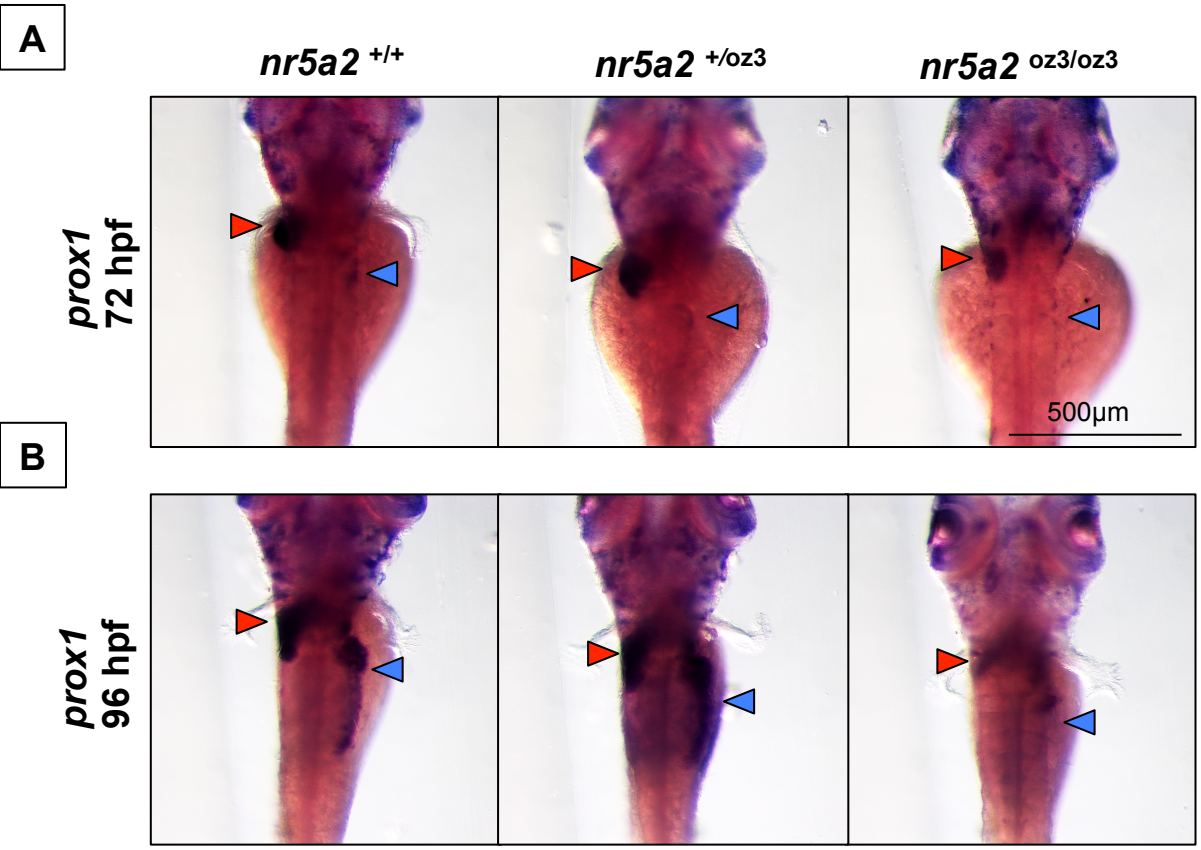
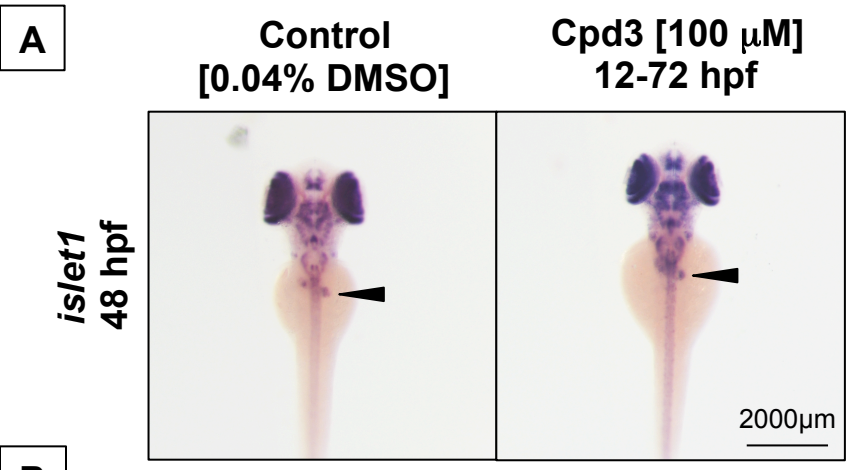
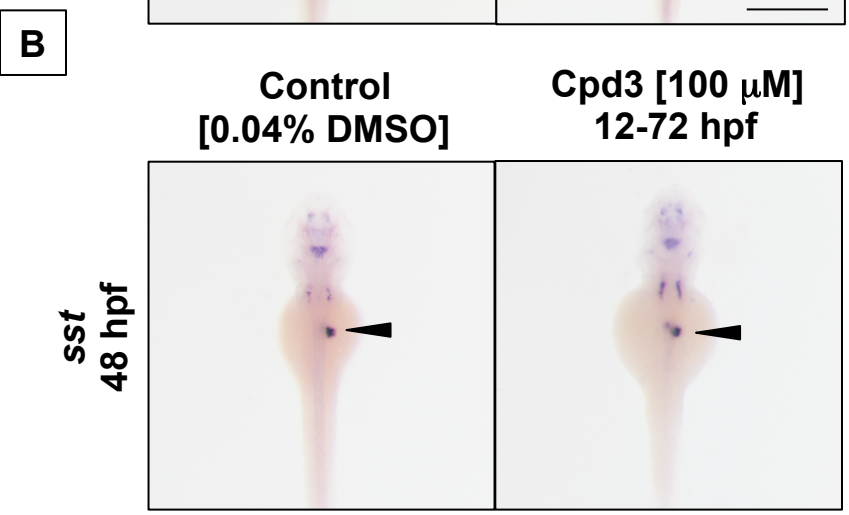

A
nr5a2 +/+
nr5a2 +/oz3
nr5a2 oz3/oz3
prox1
72 hpf
500µm
B
prox1
96 hpf

Figure S5


A
Control
[0.04% DMSO]
Cpd3 [100 μM]
12-72 hpf
islet1
48 hpf
2000μm


B
Control
[0.04% DMSO]
Cpd3 [100 μM]
12-72 hpf
sst
48 hpf

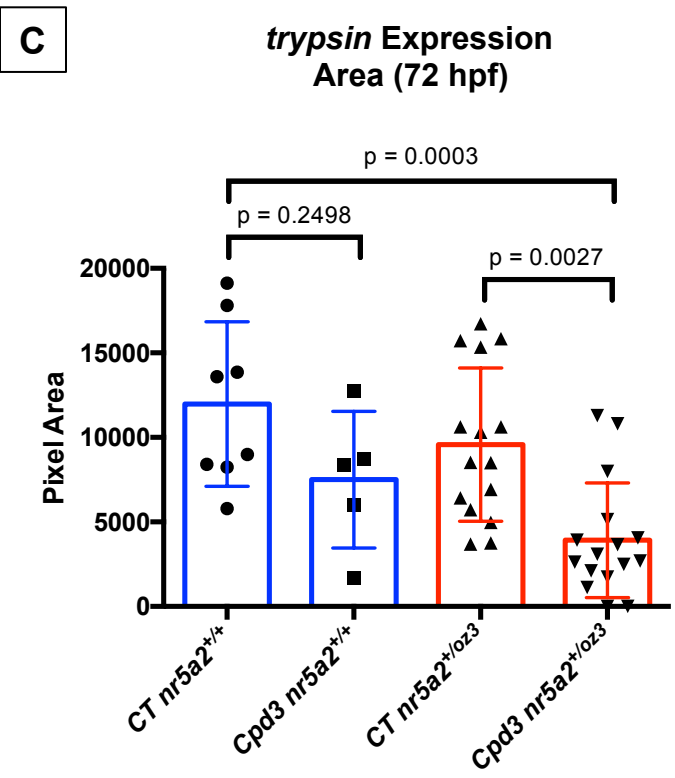

C
trypsin Expression
Area (72 hpf)
p = 0.0003
p = 0.2498
p = 0.0027
20000
15000
10000
5000
0
Pixel Area
CT nr5a2+/+
Cpd3 nr5a2+/+
CT nr5a2+/oz3
Cpd3 nr5a2+/oz3

Figure S6

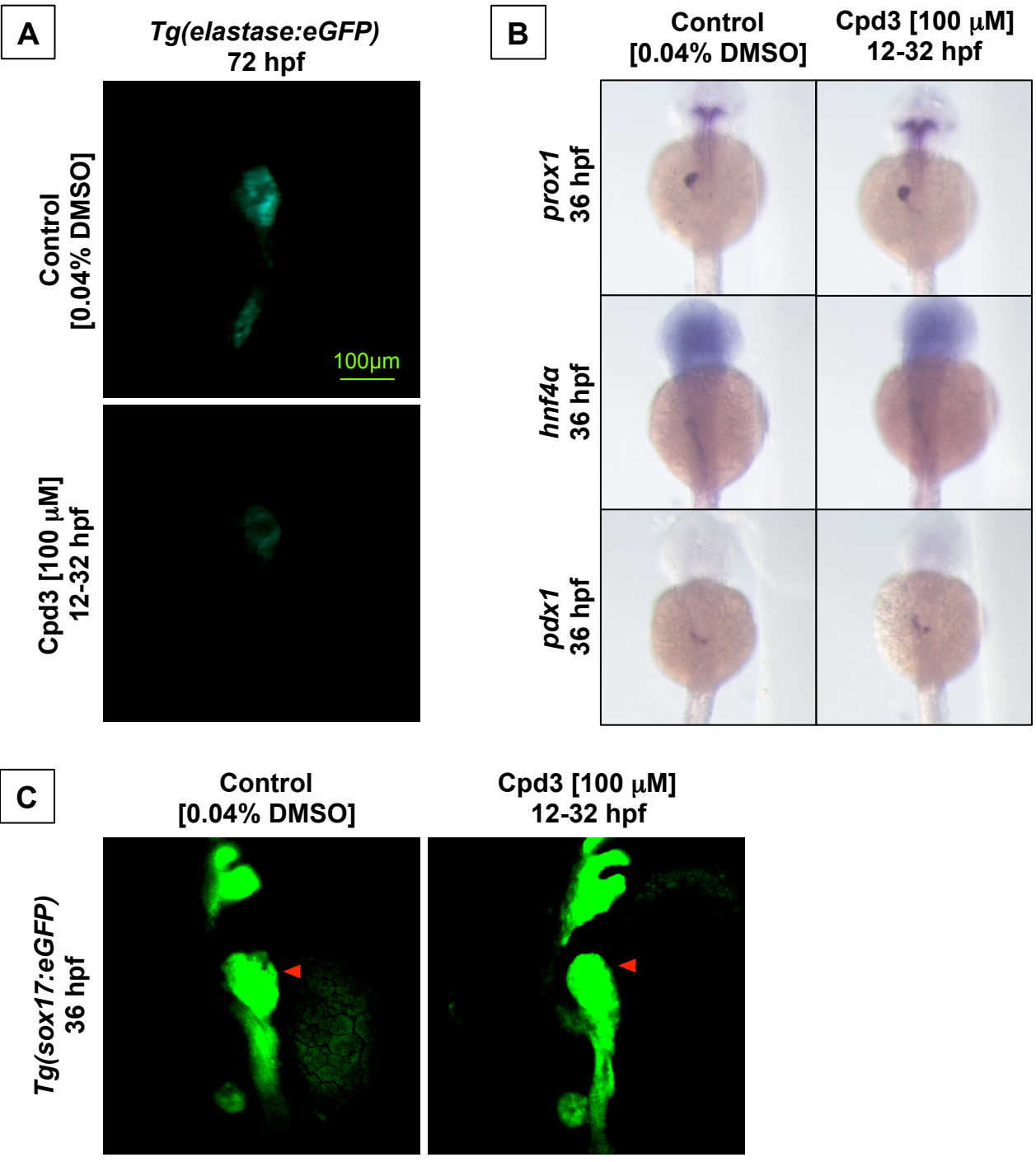
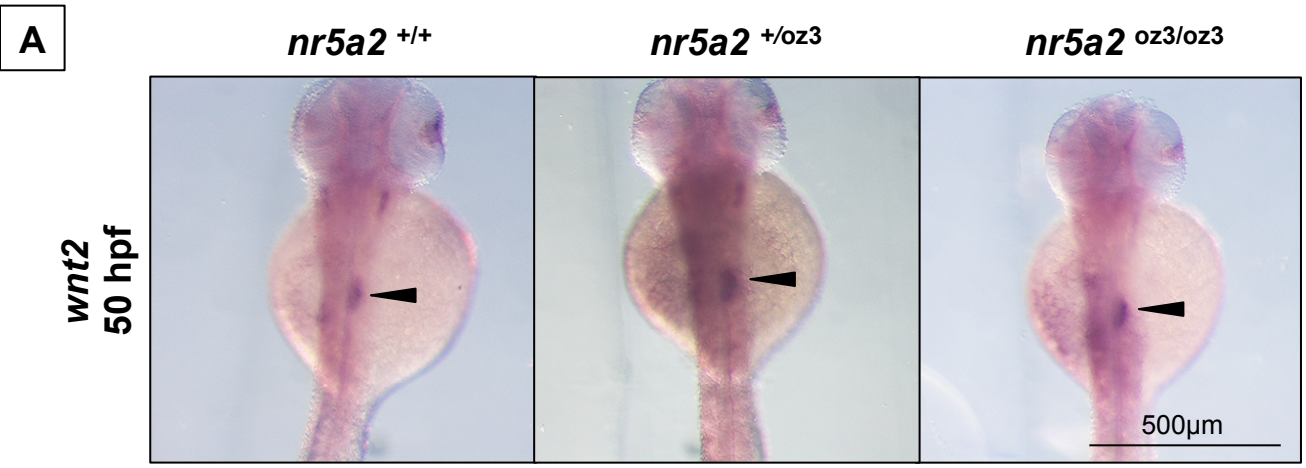
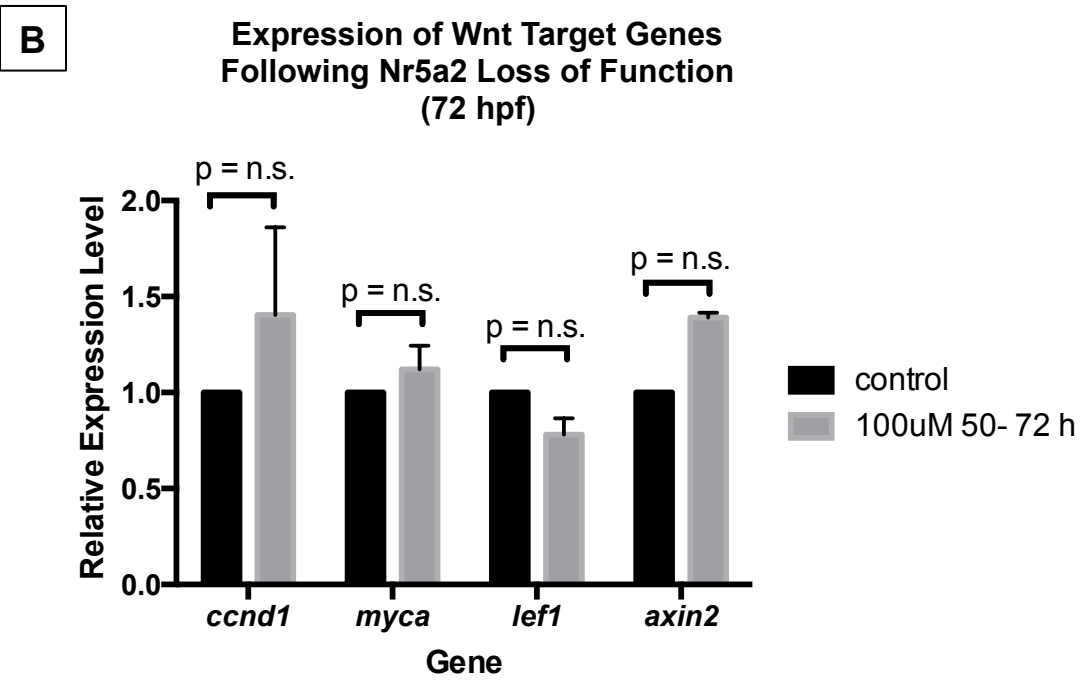

A
Tg(elastase:eGFP)
72 hpf
Control
[0.04% DMSO]
Cpd3 [100 μM]
12-32 hpf
100μm
B
Control
[0.04% DMSO]
Cpd3 [100 μM]
12-32 hpf
prox1
36 hpf
hnf4α
36 hpf
pdx1
36 hpf
C
Control
[0.04% DMSO]
Cpd3 [100 μM]
12-32 hpf
Tg(sox17:eGFP)
36 hpf

Figure S7


A
nr5a2 +/+
nr5a2 +/oz3
nr5a2 oz3/oz3
wnt2
50 hpf
500µm


B
Expression of Wnt Target Genes
Following Nr5a2 Loss of Function
(72 hpf)
p = n.s.
p = n.s.
p = n.s.
p = n.s.
Relative Expression Level
2.0
1.5
1.0
0.5
0.0
ccnd1
myca
lef1
axin2
Gene
control
100uM 50- 72 h

SUPPLEMENTARY FIGURES

Figure S1. Nr5a2 is required for the formation of the mature pancreas and liver

A) *nr5a2* expression in zygote through early segmentation stages. *nr5a2* is maternally deposited and expressed in the dividing blastomeres. *nr5a2* mRNA is actively shuttled to the dividing blastomeres in vesicles (black arrows) by the 1-cell to 2- cell stage. Expression of *nr5a2* persists to approximately 12 hpf, after which it is temporarily inactivated.

B) *nr5a2* morpholino injected *Tg(fabp10a:DsRed)* embryos have a reduced embryonic liver at 80 hpf.

C) *nr5a2*$^{oz3/oz3}$ embryos lack a mature exocrine pancreas by *in situ* hybridization for *carboxypeptidase A5 (carbA)*.

D) *trypsin*+ acinar cells fail to form in the pancreas region (blue arrows) of *nr5a2*$^{oz3/oz3}$ mutant embryos by 56 hpf.

Figure S2. *nr5a2* mutants and morphants display normal cell death and proliferation programs

A) TUNEL staining in *nr5a2*$^{+/+}$, *nr5a2*$^{+/oz3}$, and *nr5a2*$^{oz3/oz3}$ mutant embryos at 36 – 48 hpf. *nr5a2*$^{oz3/oz3}$ mutants do not show indications of excess cell death relative to *nr5a2*$^{+/+}$ sibling controls.

B) TUNEL staining in *nr5a2* morphant embryos at 60 - 84 hpf. *nr5a2* morphants do not show indications of excess cell death relative to controls, demonstrating that cell death is not a likely cause of liver or exocrine pancreas reduction.

C) TUNEL staining in *nr5a2* morphant embryos at 84 hpf. *nr5a2* morphants do not show indications of excess cell death relative to controls, demonstrating that cell death is not a likely cause of exocrine pancreas reduction.

D) Immunohistochemistry for phospho-histone 3 (PH3) at 24 hpf. *nr5a2*$^{oz3/oz3}$ embryos have no indication of reduced cellular proliferation in the endoderm region.

Figure S3. Additional *nr5a2*^{oz3} mutant phenotyping

A) Double *in situ* hybridization for *insulin* (brown) and *nr5a2* (purple) from 48 – 96 hpf. *nr5a2* and *insulin* are independently expressed from 72 – 96 hpf.

B) *In situ* hybridization for mature hepatocyte marker *transferrin A* demonstrates that *nr5a2*$^{oz3/oz3}$ embryos have a reduced liver size.

C) *In situ* hybridization for *fabp10a* at 68 hpf reveals that *fabp10a*+ hepatocytes fail to form in the liver region of *nr5a2*$^{oz3/oz3}$ embryos (red arrows).

D) *nr5a2*$^{oz3/oz3}$ embryos have normal *prox1* expression in the liver and pancreas regions at 30 hpf.

Figure S4. *Prox1* expression in the *nr5a2*^{oz3} mutant liver and pancreas regions

A) *prox1* expression is reduced in the liver bud (red arrows) and absent in the pancreas bud (blue arrows) of *nr5a2*$^{oz3/oz3}$ embryos at 72 hpf.

B) *prox1* expression is reduced in the liver bud (red arrows) and absent in the pancreas bud (blue arrows) of *nr5a2*$^{oz3/oz3}$ embryos at 96 hpf, demonstrating that *prox1* expression in the endoderm does not recover.

Figure S5. Effects of Cpd3 exposure on pancreas development

A) Endocrine pancreas cells (black arrows) expressing *islet1* are present by *in situ* hybridization in embryos treated with Cpd3 from 12 – 72 hpf.

B) δ-cells expressing *somatostatin* (*sst*) (black arrows) are present by *in situ* hybridization in embryos treated with Cpd3 from 12 – 72 hpf.

C) Quantification of exocrine pancreas size by area of *trypsin* expression at 72 hpf. 200 μM Cpd3 treatment elicits a robust reduction in pancreas size in heterozygote *nr5a2*$^{+/oz3}$ embryos relative to control *nr5a2*$^{+/oz3}$ siblings and control *nr5a2*$^{+/+}$ embryos. (n > 5 per group; for control *nr5a2*$^{+/+}$ vs. Cpd3 treated *nr5a2*$^{+/+}$, p = 0.2498; for control *nr5a2*$^{+/oz3}$ vs Cpd3 treated *nr5a2*$^{+/oz3}$, p = 0.0027; for control *nr5a2*$^{+/+}$ vs. Cpd3 treated *nr5a2*$^{+/oz3}$, p = 0.0003; ordinary one-way ANOVA with Tukey's multiple comparisons test).

Figure S6 Effects of 12 – 32 hpf Cpd3 exposure on pancreas, liver, and hepatopancreas progenitor development

A) Cpd3 treatment from 12 – 32 hpf reduces the size of the exocrine pancreas in *Tg(elastase:eGFP)* embryos at 72 hpf.

B) Cpd3 treatment from 12 – 32 hpf does not impact the size of the *prox1, hnf4α,* and *pdx1* liver and pancreas progenitor populations.

C) Cpd3 treatment from 12 – 32 hpf does not increase the size of the liver bud (red arrow) by confocal imaging of *Tg(sox17:eGFP)* embryos.

Figure S7: Wnt expression and signaling in Nr5a2 mutants and morphants

A) *wnt2* expression in the mesoderm region between the swim bladder and pancreas (arrows) is unaffected in *nr5a2*$^{+/oz3}$ and *nr5a2*$^{oz3/oz3}$ embryos relative to controls.

B) qPCR for Wnt target genes in whole embryos reveals that alterations in Nr5a2 activity by antagonist Cpd3 from 50 – 72 hpf does not significantly impact Wnt pathway activity.

Supplementary Table 1. qRT-PCR Primers

nr5a2 F	5’ TCA GCT TGG ACG TGA AGA AC 3’
nr5a2 R	5’ AAC TTG TCT GTC TGC TGA GG 3’
ef1α F	5’ GCG TCA TCA AGA GCG TTG AG 3’
ef1α R	5’ TTG GAA CGG TGT GAT TGA GG 3’
axin2 F	5’ GGACACTTCAAGGAACAACTAC 3’
axin2 R	5’ CCTCATACATTGGCAGAACTG 3’
lef1 F	5’ GAGGGAAAAGATCCAGGAAC 3’
lef1 R	5’ AGGTTGAGAAGTCTAGCAGG 3’
ccnd1 F	5’ GGAACTGCTGGCGCTAAATA 3’
ccnd1 R	5’ GACTTGCGAGAGGAAGTTGG 3’
myca F	5’ TGACTGTGGAAAAGCGACAG 3’
myca R	5’ GCTGCTGTTGATGCTGTGAT 3’