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RESEARCH ARTICLE

Figures and figure supplements

Novel adverse outcome pathways revealed by chemical genetics in a developing marine fish

Elin Sørhus *et al*

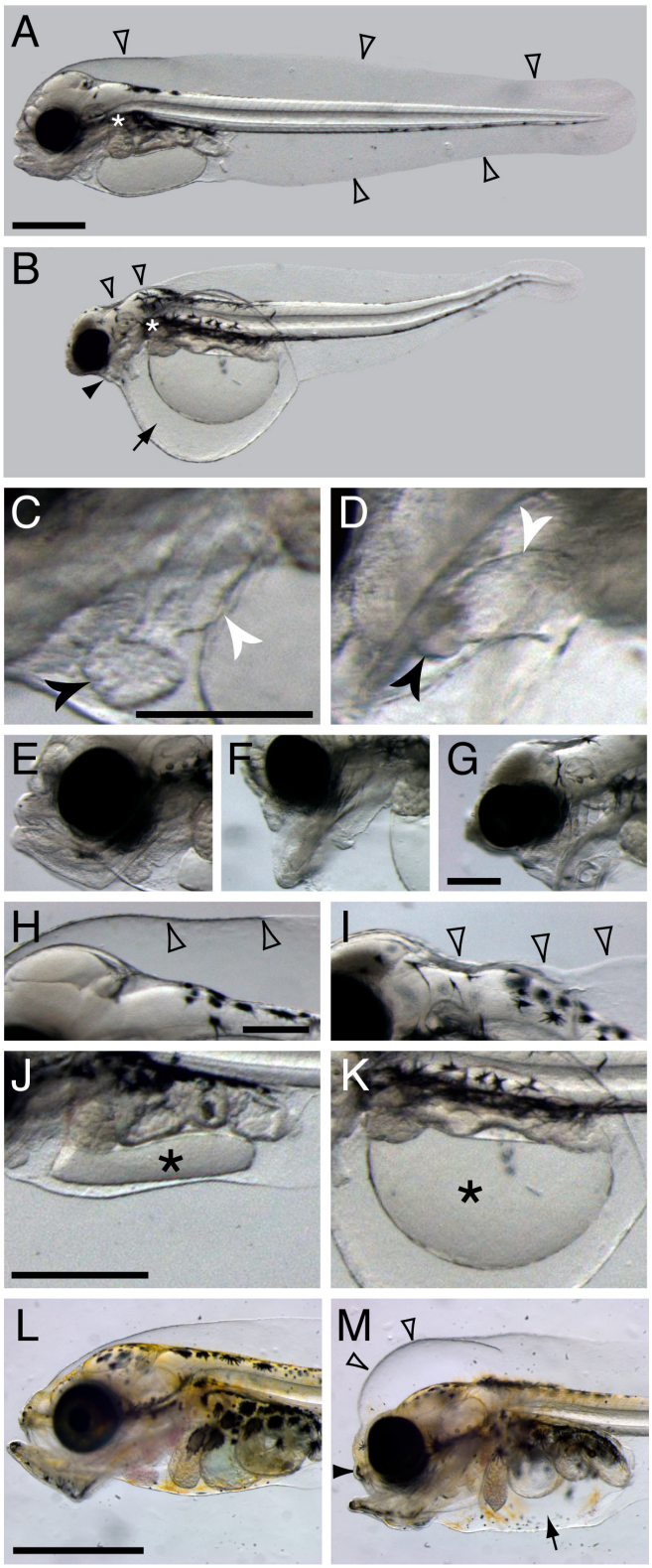

Figure 1. Terminal phenotypes after high dose exposure. Control (**A**) and exposed (**B**) three days post hatch (dph) larvae (6 days post embryonic exposure). Open arrowheads in (**A**) indicate the marginal finfold surrounding the

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Figure 1 continued

larvae and the white asterisk indicate the location of the connection between the dorsal space and the ventral yolk sac in the vicinity of the pectoral fin. In (**B**) the black arrowhead indicates severely reduced craniofacial outgrowth, while the black arrow indicates yolk sac edema. The ventricle and atrium in control (**C**) and embryonically exposed (**D**) animals are indicated by black and white arrows, respectively. (**E**) Normal craniofacial structure in control, and (**F**) moderate and (**G**) severe craniofacial defects in exposed animals. (**H**) Normal marginal finfold in control, (**I**) exposed animals with severe reduction of anterior marginal finfold (open arrowheads). Yolk mass (*) in control (**J**) and embryonically exposed larvae (**K**). (**L**) Control and (**M**) exposed 18 dph larvae. Open arrowheads indicate increased anterior marginal finfold, black arrowhead indicates reduced upper jaw outgrowth, and black arrow indicates edema formation in the peritoneal cavity in oil-exposed larvae (M). Scale bar: 0.2 mm (**C**,**D**; **E**–**G**; **H**–**K**) and 1 mm (**A**,**B** and **L**,**M**).

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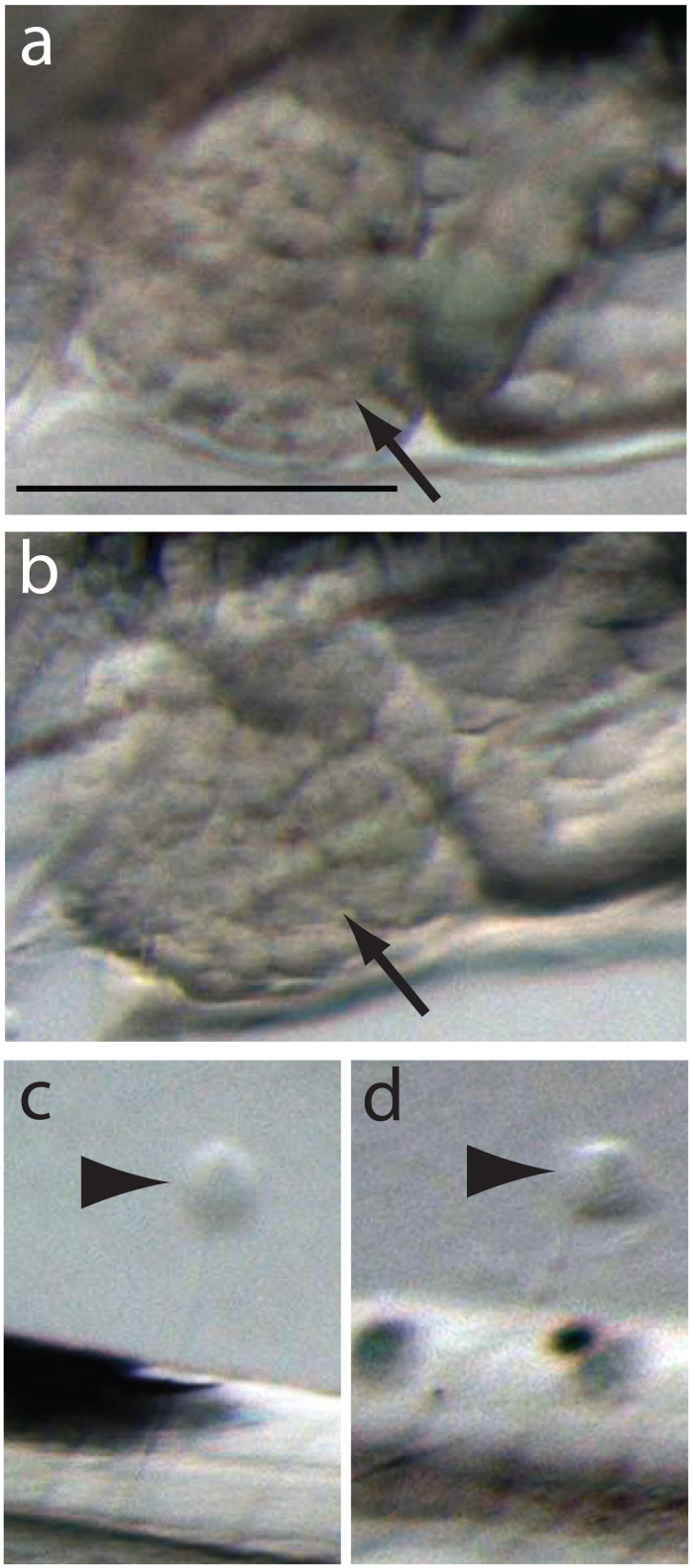
a
b
c
d

Figure 1—figure supplement 1. Normal development of liver and lateral line in the severe phenotypes. Normally developed livers and neuromast cells are indicated by black arrows and arrowheads in control (**A**, **C**) and severely affected hunchback phenotypes (**B**, **D**), respectively. Scale bar 0.2 mm.
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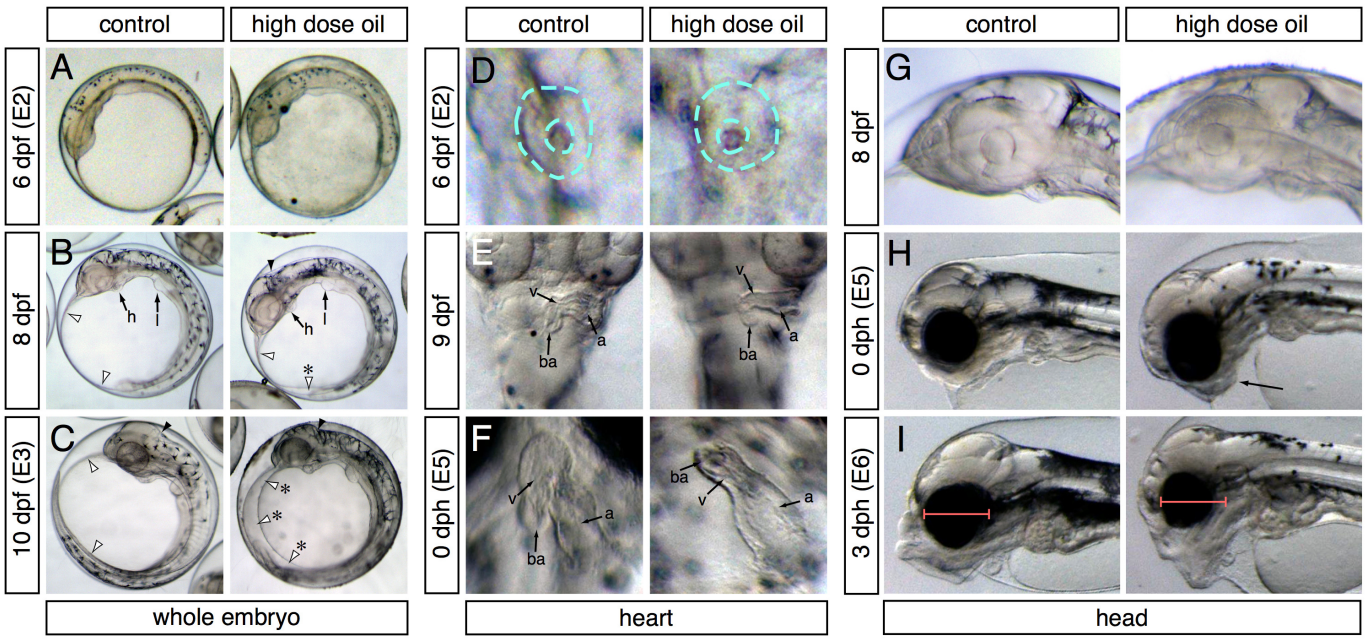

Figure 2. Appearance of phenotypes over time. In each panel control and high-dose-exposed embryos are shown on the left and right, respectively. (**A**–**C**) Lateral overview of whole embryos showing accumulation of edema (anterior to the left). (**A**) 6 dpf/E2 sampling point. (**B**) 8 dpf (between E2 and E3 sampling points). Heart (h) and liver bud (l) are indicated. White arrowheads indicate outer margins of the yolk sac membranes; asterisk indicates small pocket of edema. Black arrowheads indicate the hindbrain ventricle. (**C**) 10 dpf/E3 sampling point. Arrowheads same as (**B**); asterisks indicate expanded yolk sac edema. (**D**–**E**) High-magnification ventral views of the heart (anterior at top). (**D**) 6 dpf/E2. Dashed turquoise lines indicate outer border and lumen of midline cardiac cone. (**E**) 9 dpf (between E2 and E3). Arrows indicate the atrium (a), ventricle (v) and bulbus arteriosus (ba). (**F**) 0 dph (E5 sampling point). Chambers indicated as in (**E**). (**G**–**I**) Lateral views of the developing head (anterior to the left). (**G**) 8 dpf (between E2 and E3). (**H**) 0 dph (E5). Arrow indicates abnormal lower jaw cartilages in oil-exposed larva. (**I**) 3 dph (E6 sampling point). Red bars indicate difference in eye diameter between control and exposed larvae.

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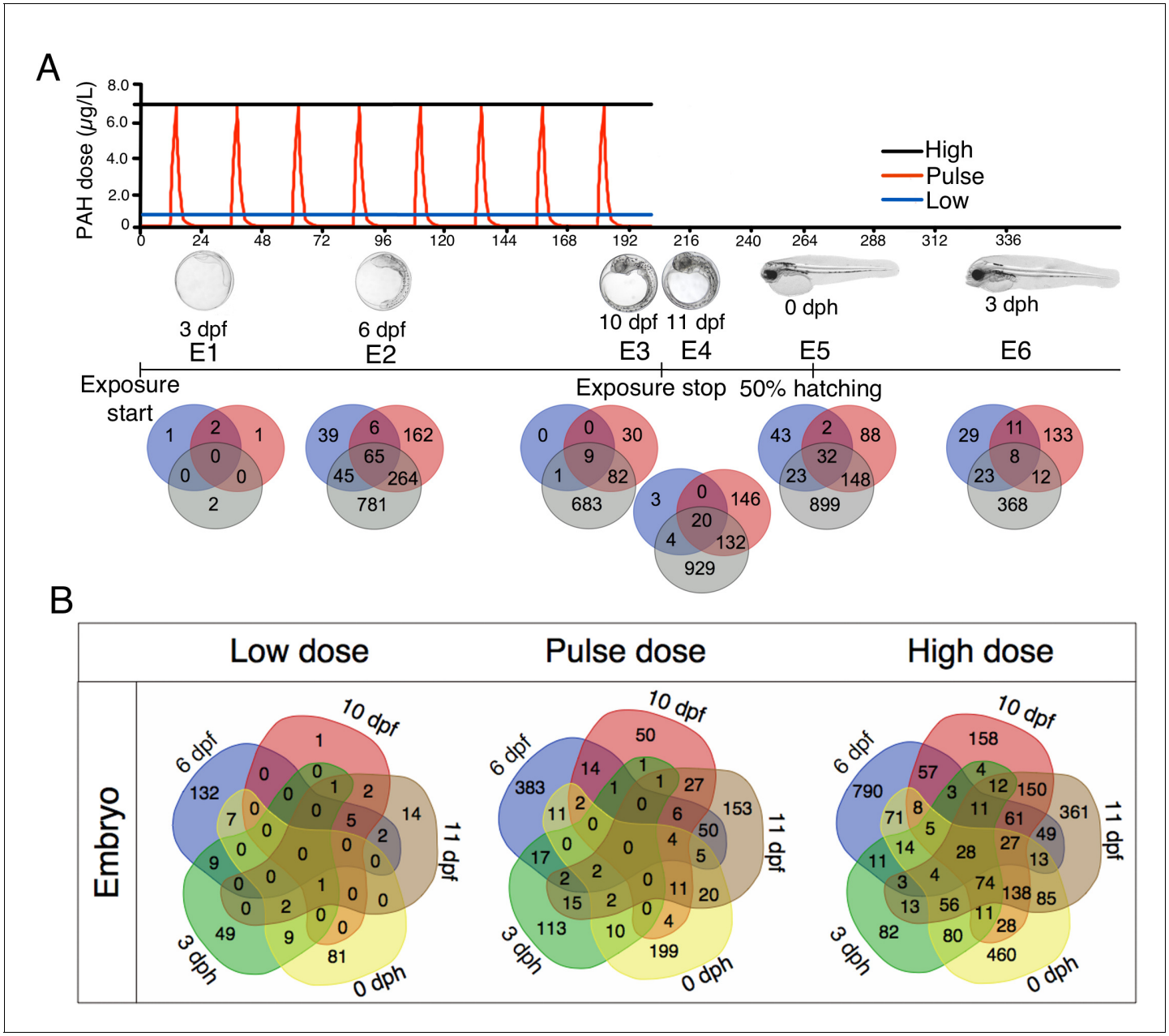

Figure 3. Exposure regimes and differentially expressed genes (DEGs) during embryonic development. (**A**) Embryos were exposed to a continuous high dose (black line; 6.7 ± 0.2 µg/L TPAH), a pulsed dose (red line; 0.09 ± 0.02–6.8 ± 1.0 µg/L TPAH) and a continuous low dose (blue line; 0.58 ± 0.05 µg/L TPAH) of crude oil. Photos indicate normal developmental progress at each of six sampling time points (E1–E6). Venn diagrams show shared and exclusive DEGs for each of the three oil exposures at E1–E6. (**B**) Venn diagrams illustrating the number of shared and exclusive DEGs at each stage in development up to hatching for the three exposure regimes.

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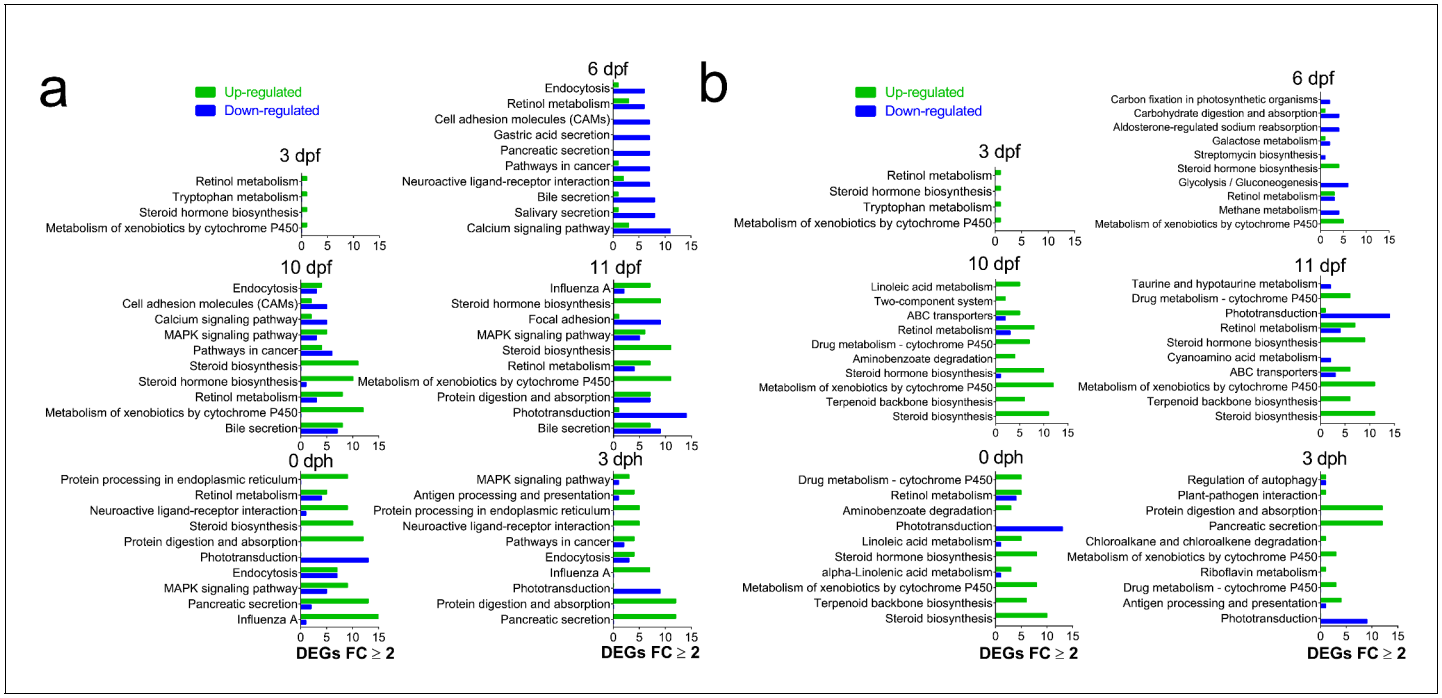

Figure 3—figure supplement 1. Most regulated KEGG pathways. (**A**) Pathways (Total) with highest number of DEGs ≥2 FC during and after embryonic exposure. (**B**) Pathways with the largest fraction of DEGs ≥2 FC/ Total number of genes in pathway (Normalized) during and after embryonic exposure.
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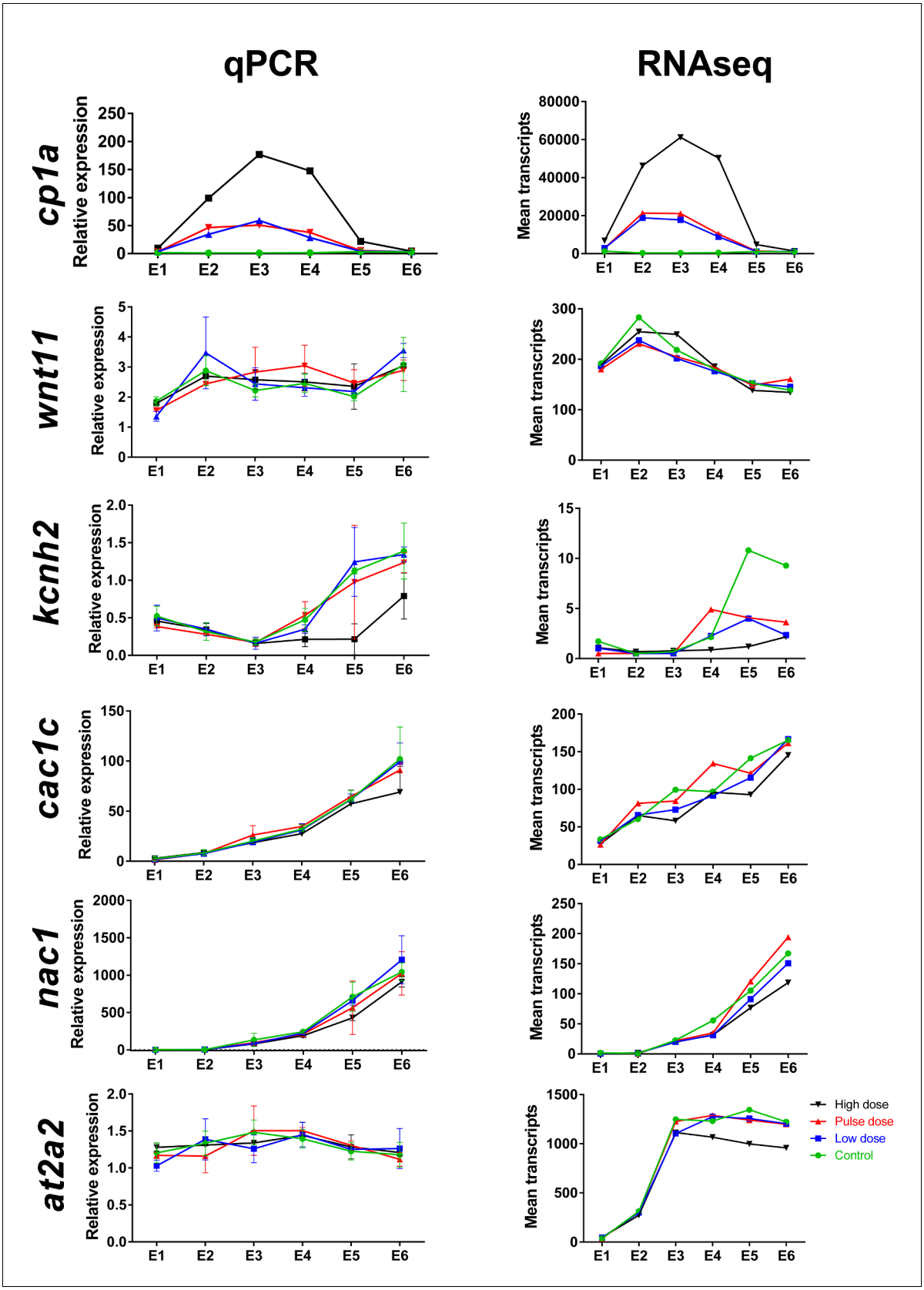

Figure 3—figure supplement 2. Comparison of mRNA read count data with real-time qPCR for selected genes during and after embryonic exposure. Genes include *cp1a* (cytochrome p450 1 a), *wnt11* (wingless-type MMTV integration site family member 11), *kcnh2* (potassium voltage-gated channel

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subfamily H member 2), *cac1c* (voltage-dependent L-type calcium channel), *nac1* (sodium/calcium exchanger 1), *at2a2* (sarcoplamsic-endoplasmic reticulum calcium ATPase). (**A**) Real-time qPCR. (**B**) Read count data from RNA sequencing. Data were normalized as described in Materials and methods.

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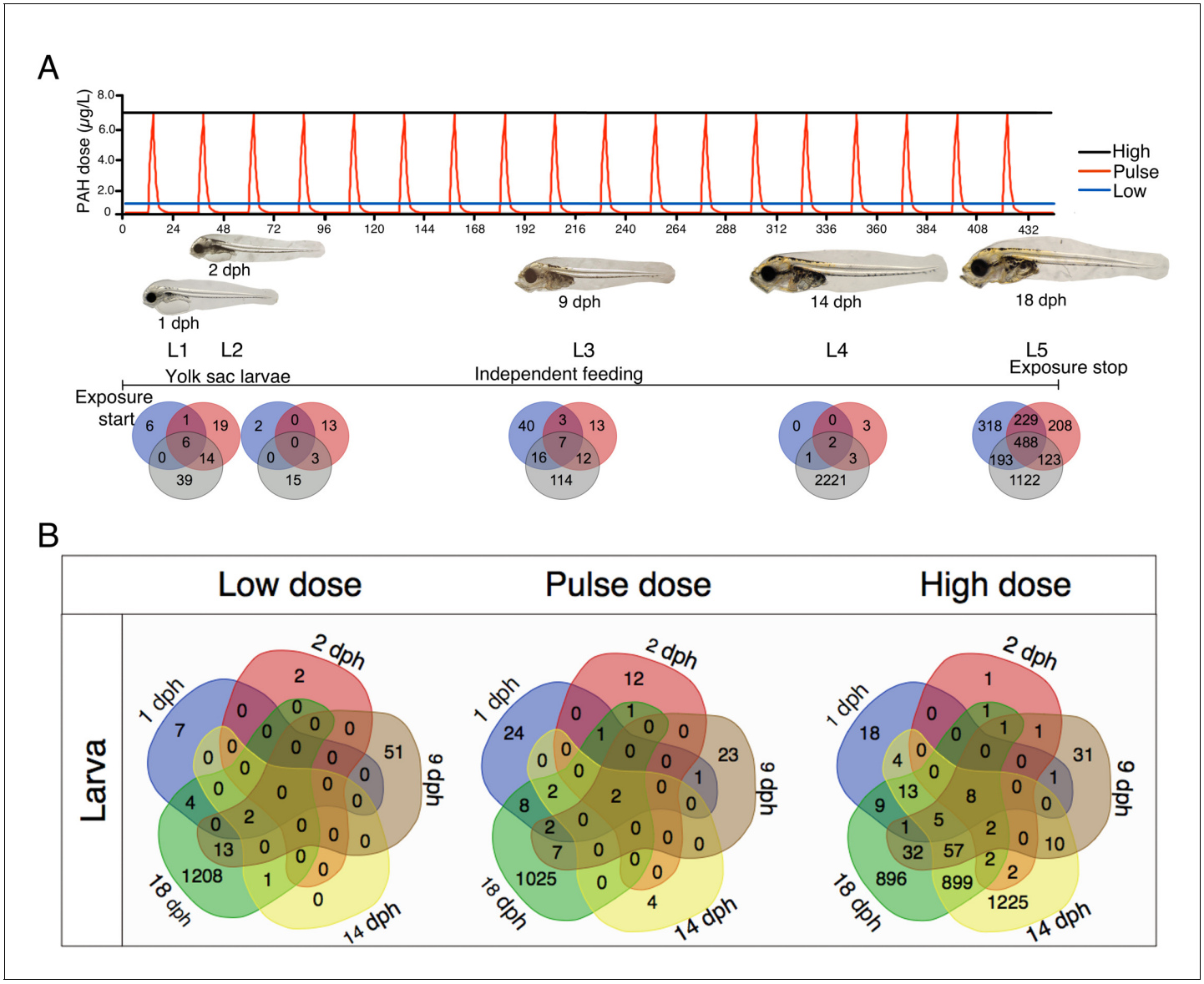

Figure 4. Exposure regimes and differentially expressed genes (DEGs) during larval development. (**A**) Larvae were exposed to a continuous high dose (black line; 7.6 ± 0.7 µg/L TPAH), a pulsed dose (red line; 0.3 ± 0.3–6.1 ± 0.5 µg/L TPAH), and a continuous low dose (blue line; 0.65 ± 0.08 µg/L TPAH) of crude oil. Photos indicate normal developmental progress at each of five sampling time points (L1–L5). Venn diagrams show shared and exclusive DEGs for each of the three oil exposures at L1–5. (**B**) Venn diagrams illustrating the number of shared and exclusive DEGs at each larval stage for the three exposure regimes.

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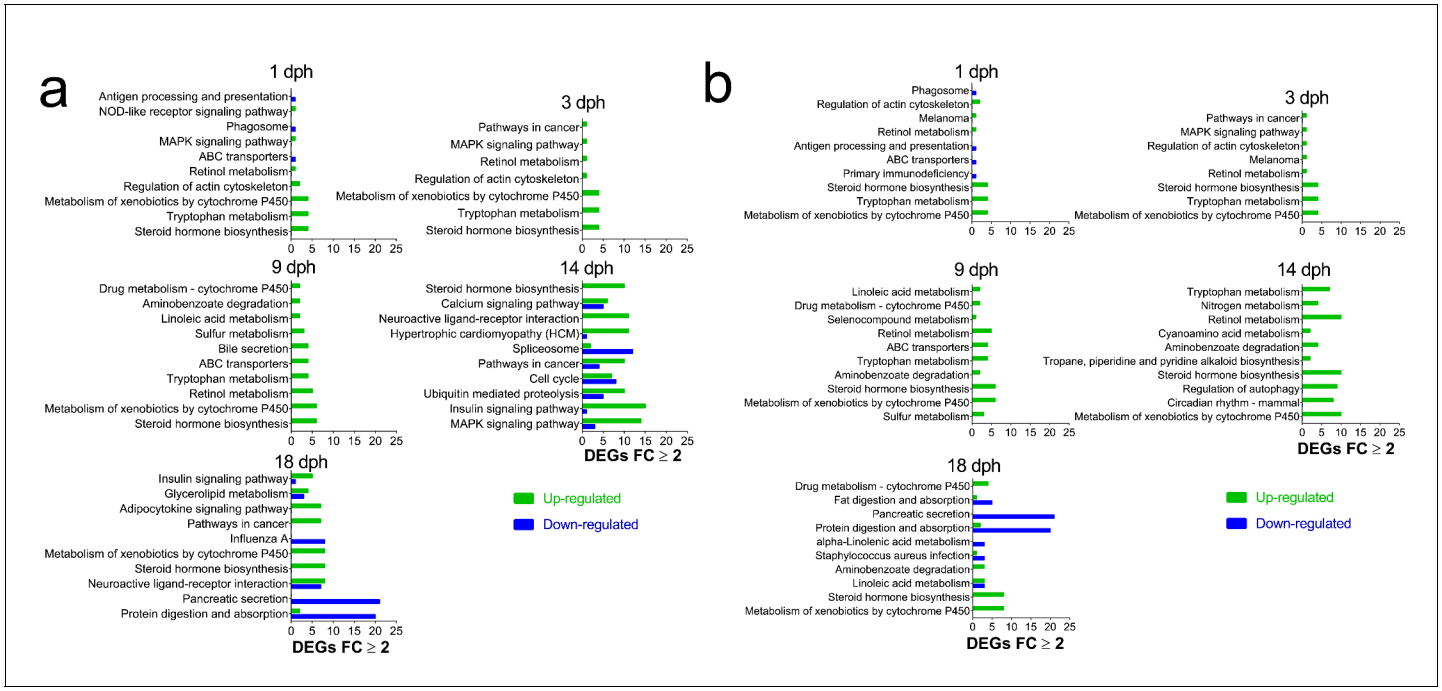

Figure 4—figure supplement 1. Most regulated KEGG pathways. (**A**) Pathways (Total) with highest number of DEGs ≥2 FC during larval exposure. (**B**) Pathways with the largest fraction of DEGs ≥2 FC/ Total number of genes in pathway (Normalized) during and after embryonic exposure.
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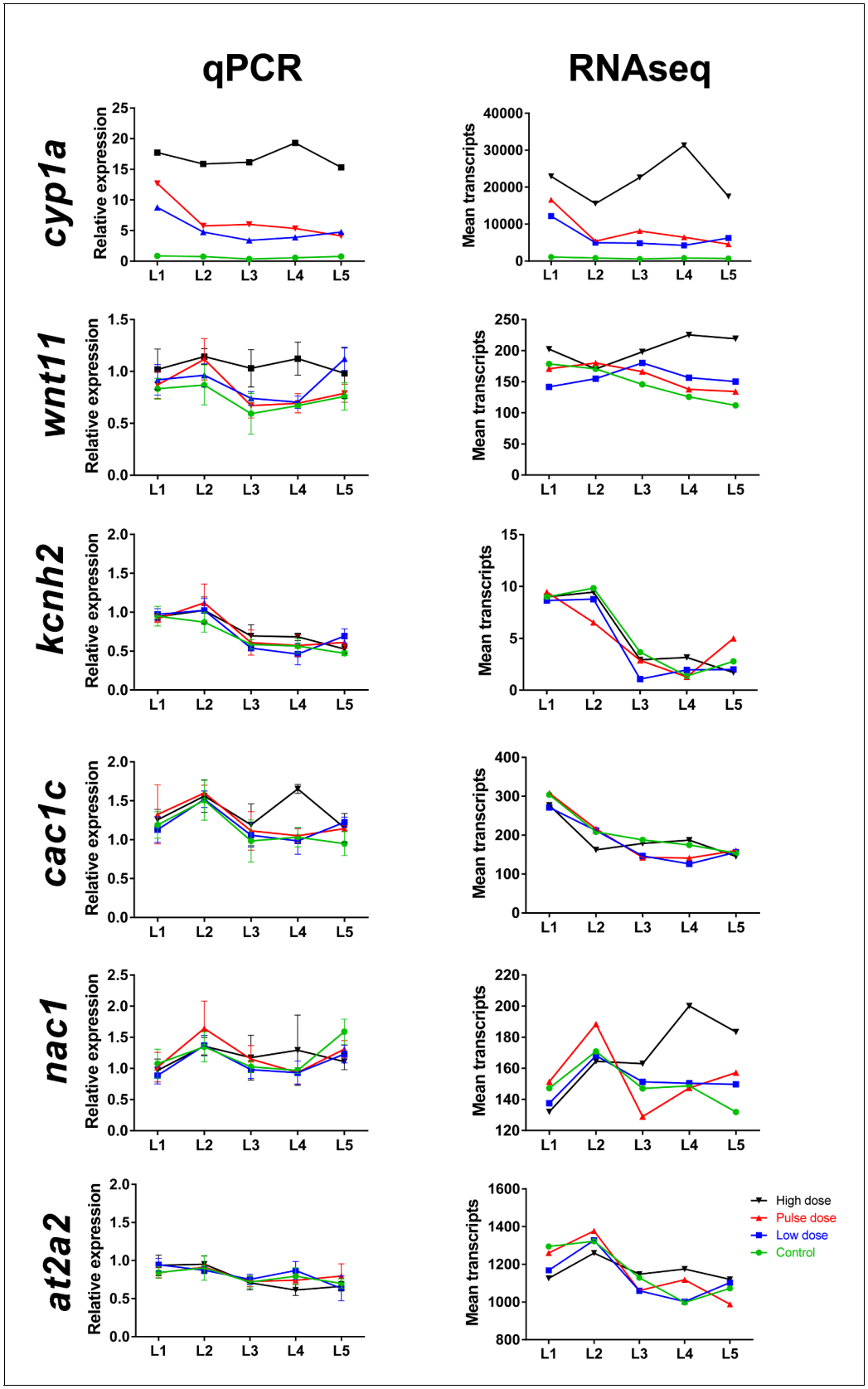

Figure 4—figure supplement 2. Comparison of mRNA read count data with real time qPCR for selected genes during larval exposure. Genes include *cp1a* (cytochrome p450 1 a), *wnt11*, *kcnh2* (potassium voltage-gated channel subfamily H member 2), *cac1c* (voltage-dependent L-type calcium

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Figure 4—figure supplement 2 continued

channel), *nac1* (sodium/calcium exchanger 1), and *at2a2* (sarcoplamsic-endoplasmic reticulum calcium ATPase). (**A**) Real-time qPCR. (**B**) Read count data from RNA sequencing. Data were normalized as described in Materials and methods.

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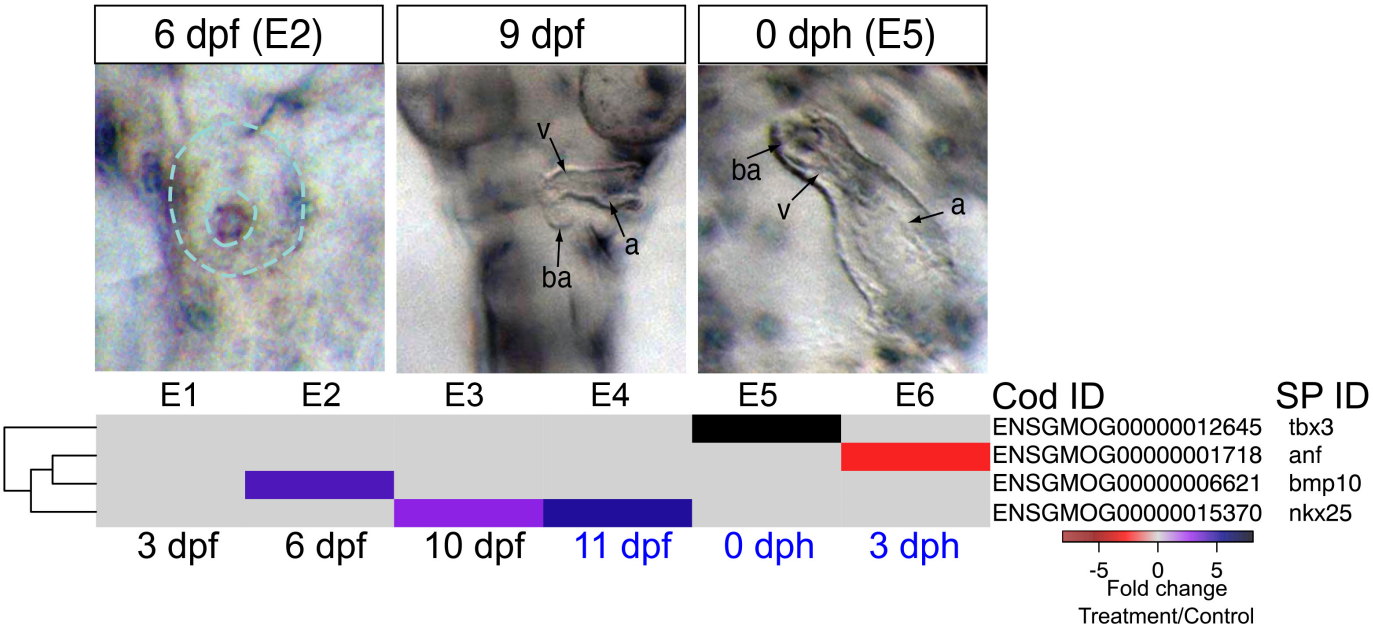

Figure 5. DEGs involved in cardiogenesis. Regulation of genes involved in cardiogenesis during and after embryonic exposure. Purple: increased expression, red: decreased expression in exposed group.

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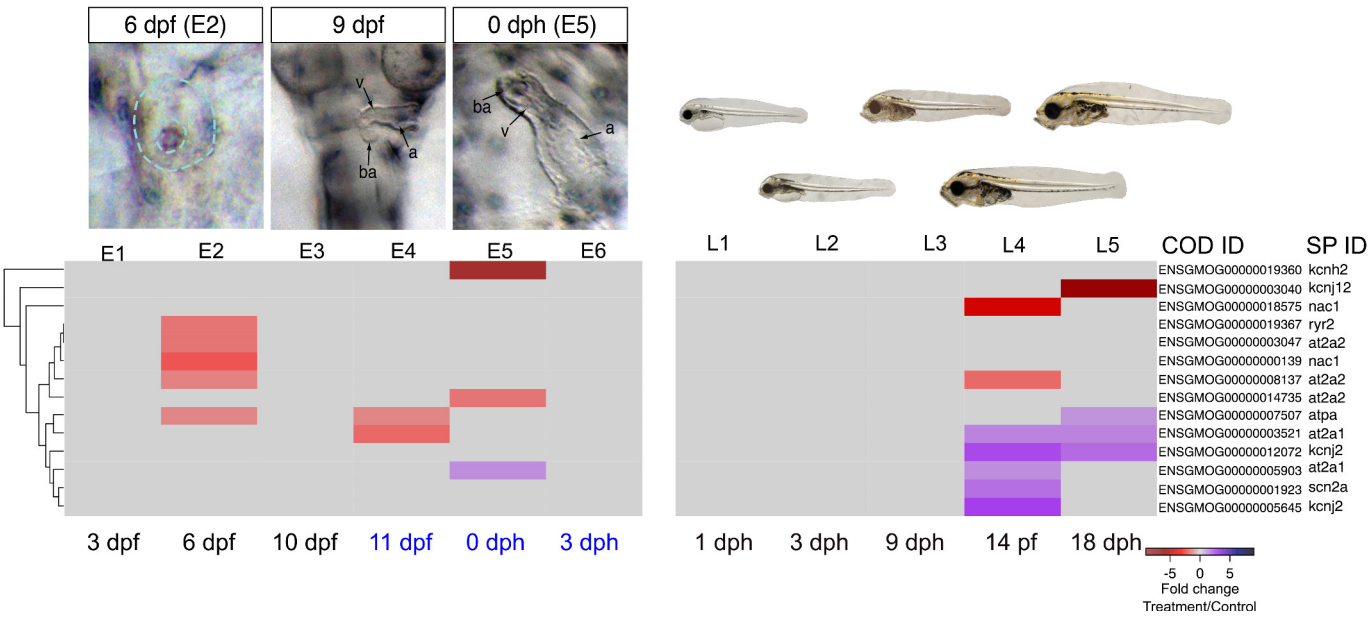

Figure 6. DEGs involved in E–C coupling. Embryonic developmental samples (E1–6) were collected during (black lettering) and after (blue lettering) crude oil exposure. Oil exposure was continuous across the larval sampling points (L1–5).

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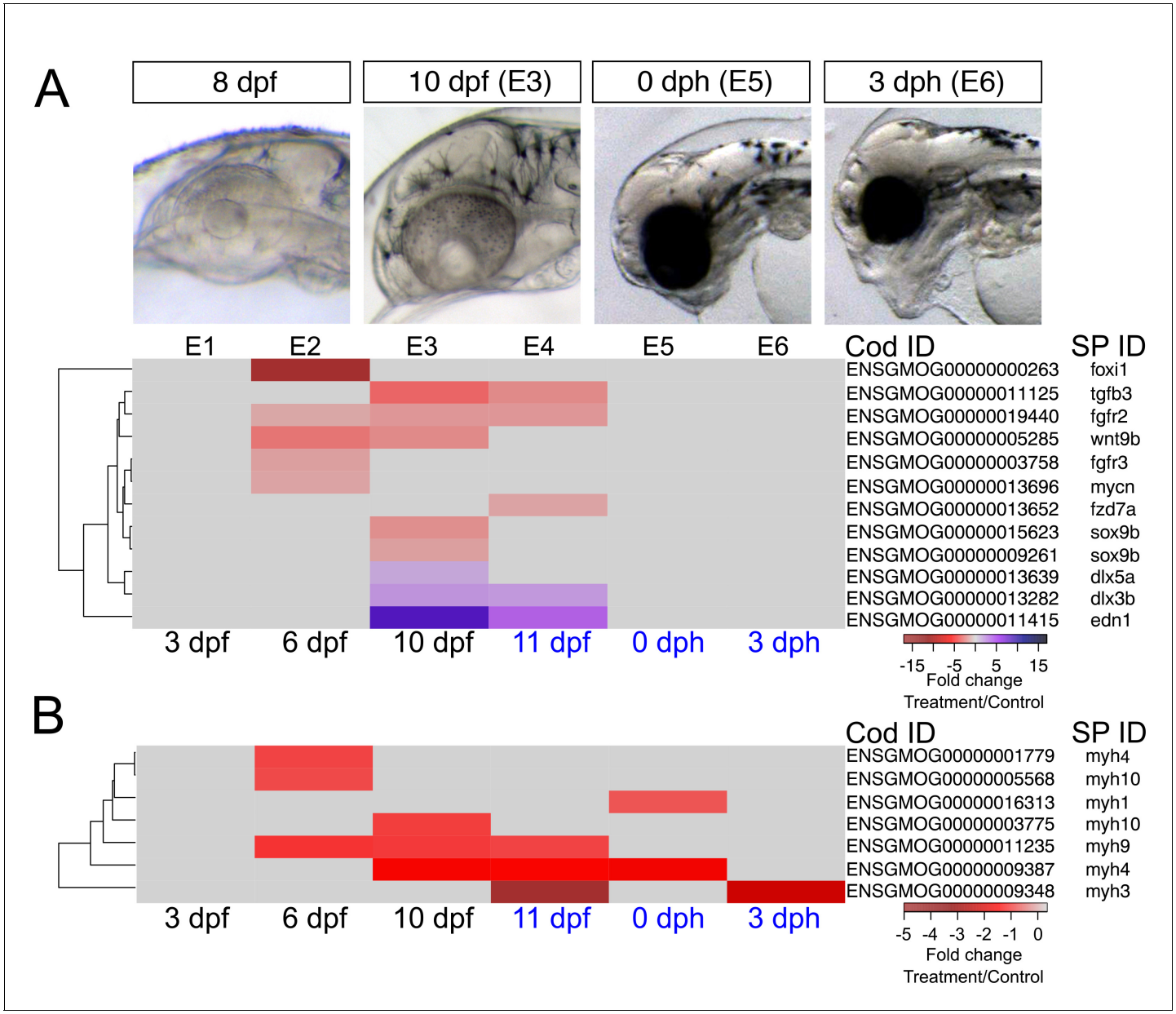

Figure 7. DEGs involved in craniofacial development. (**a**) Regulation of genes involved in craniofacial development during and after embryonic exposure. (**b**) Regulation of myosin heavy chain genes. Purple: increased expression, red: decreased expression in exposed group.
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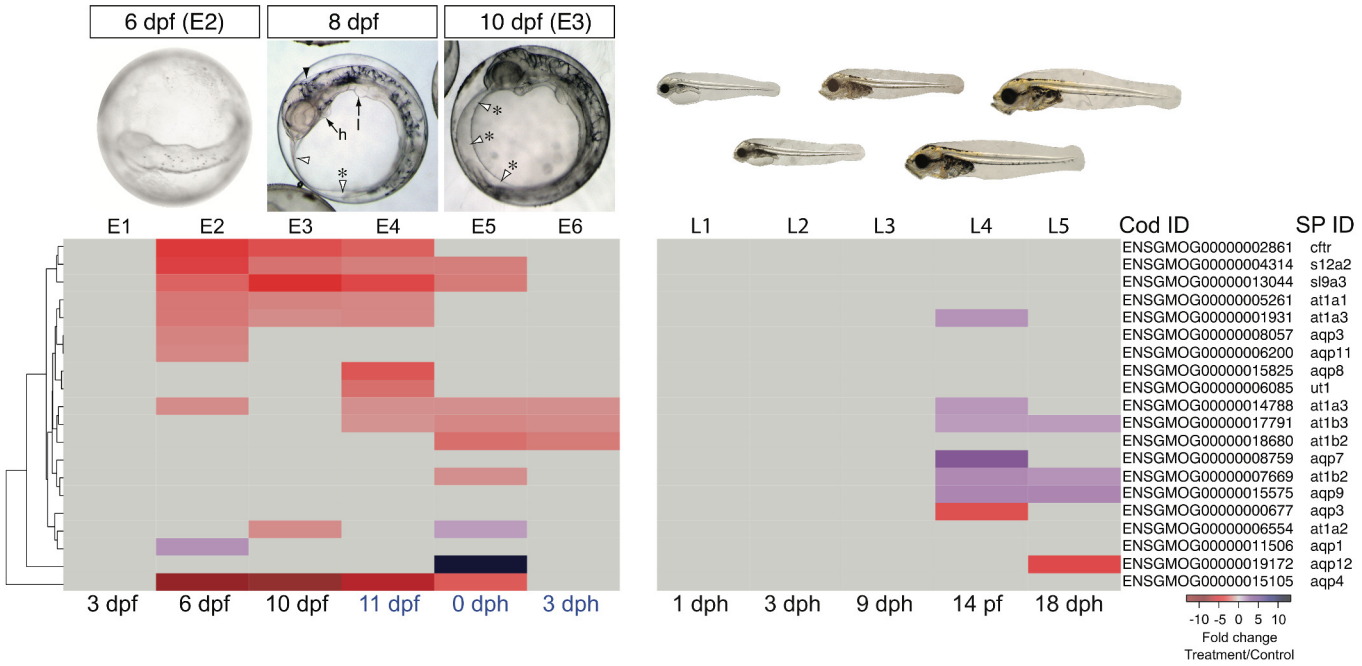

Figure 8. DEGs involved in osmoregulation. E1–E6: Embryonic exposure, L1–L5: Larval exposure. Black letters: during exposure, blue letters: after exposure.

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