SUPPLEMENTARY FIGURES AND LEGENDS

'Polyamine biosynthesis is critical for growth and differentiation of the pancreas'

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Supplementary Fig. S1. Protein alignment of Odc1.

Comparison of human, mouse and zebrafish Odc1 protein sequence.

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human_ODC1  MNNFGNEEFDCHFLDEGFTAKDILDQKINEVSSSDDKDAFYVADLGDILKKHLRWLKALP
mouse_Odc1  MSSFTKDEFDCHILDEGFTAKDILDQKINEVSSSDDKDAFYVADLGDILKKHLRWLKALP
zfish_Odc1  MTACTGSDFDFAFLEEGFCARDIVEQKINESSLSDDKDAFYVADLGDVLKKHLRWLRVLP
            *.    .:**  :*:*** *:**::***** * **************:********:.**

human_ODC1  RVTPFYAVKCNDSKAIVKTLAATGTGFDCASKTEIQLVQSLGVPPERIIYANPCKQVSQI
mouse_Odc1  RVTPFYAVKCNDSRAIVSTLAAIGTGFDCASKTEIQLVQGLGVPAERVIYANPCKQVSQI
zfish_Odc1  RITPFYAVKCNDSRAVVTTLASLGAGFDCASKTEIQIVQSVGVDPSRIIYANPCKQVSQI
            *:************:*:*.***: *:************:**.:** ..*:*************

human_ODC1  KYAANNGVQMMTFDSEVELMKVARAHPKAKLVLRIATDDSKAVCRLSVKFGATLRTSRLL
mouse_Odc1  KYAASNGVQMMTFDSEIELMKVARAHPKAKLVLRIATDDSKAVCRLSVKFGATLKTSRLL
zfish_Odc1  KYASAHGVQMMTFDSEVELMKVARSHENAKLVLRIATDDSKAVCRLSVKFGATLKSSRLL
            ***: :**********:*******:* :****************************::****

human_ODC1  LERAKELNIDVVGVSFHVGSGCTDPETFVQAISDARCVFDMGAEVGFSMYLLDIGGGFPG
mouse_Odc1  LERAKELNIDVIGVSFHVGSGCTDPETFVQAVSDARCVFDMATEVGFSMHLLDIGGGFPG
zfish_Odc1  LERAKELGLDVIGVSFHVGSGCTDPETYSQAISDARYVFDIGAELGYNMSLLDIGGGFPG
            *******.:**:***************: **:**** ***:.:*:*:.* **********

human_ODC1  SEDVKLKFEEITGVINPALDKYFPSDSGVRIIAEPGRYYVASAFTLAVNIIAKKIVLKEQ
mouse_Odc1  SEDTKLKFEEITSVINPALDKYFPSDSGVRIIAEPGRYYVASAFTLAVNIIAKKTVWKEQ
zfish_Odc1  SEDTKLKFEEIAAVINPALDKYFPVDSGVRIIAEPGRYYVASAYTLAVNIIAKKVIMKEQ
            ***.*******:.*********** ******************:********** : ***

human_ODC1  TGSDDE-DESSEQTFMYYVNDGVYGSFNCILYDHAHVKPLLQKRPKPDEKYYSSSIWGPT
mouse_Odc1  PGSDDE-DESNEQTFMYYVNDGVYGSFNCILYDHAHVKALLQKRPKPDEKYYSSSIWGPT
zfish_Odc1  SASDEEEDVSNDRTLMYYVNDGVYGSFNCILYDHAHVLPTLHKKPKPDERMYPCSIWGPT
            ..**:* * *.::*:********************** . *:*:*****: *..******

human_ODC1  CDGLDRIVERCDLPEMHVGDWMLFENMGAYTVAAASTFNGFQRPTIYYVMSGPAWQLMQQ
mouse_Odc1  CDGLDRIVERCNLPEMHVGDWMLFENMGAYTVAAASTFNGFQRPNIYYVMSRPMWQLMKQ
zfish_Odc1  CDGLDRIVEQCSLPDMQVGDWLLFENMGAYTVAASSTFNGFQKPDIYYIMSRTAWQCMQQ
            *********:*.**:*:****:************:*******:* ***:** . ** *:*

human_ODC1  FQNPDFPP-EVEEQDASTLPVSCAWESGMKRHRAACASASINV
mouse_Odc1  IQSHGFPP-EVEEQDDGTLPMSCAQESGMDRHPAACASARINV
zfish_Odc1  IRAQGIPALPLEEPSAGNVPSHCGRESSLDVPAKPCPTQVL--
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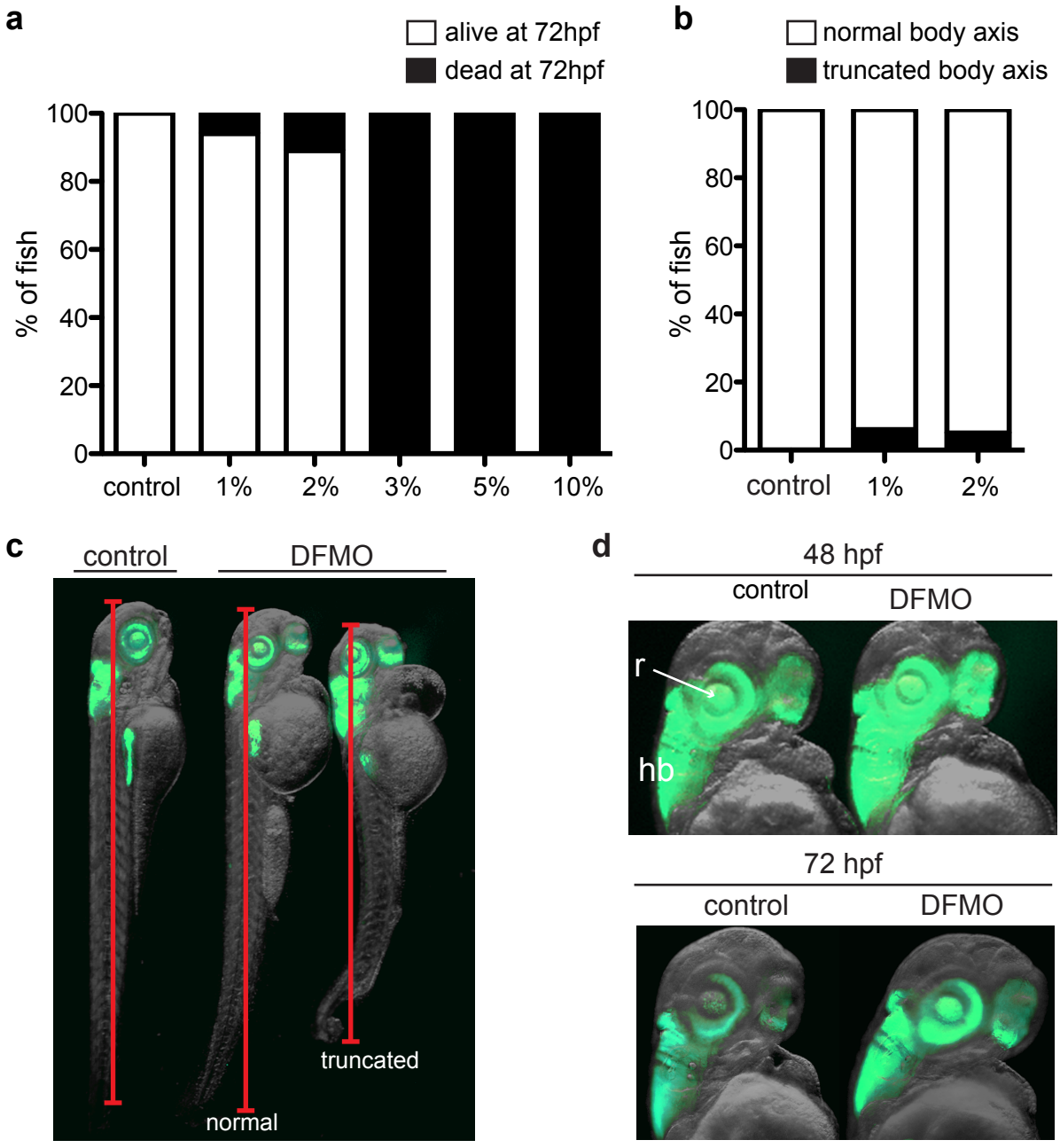
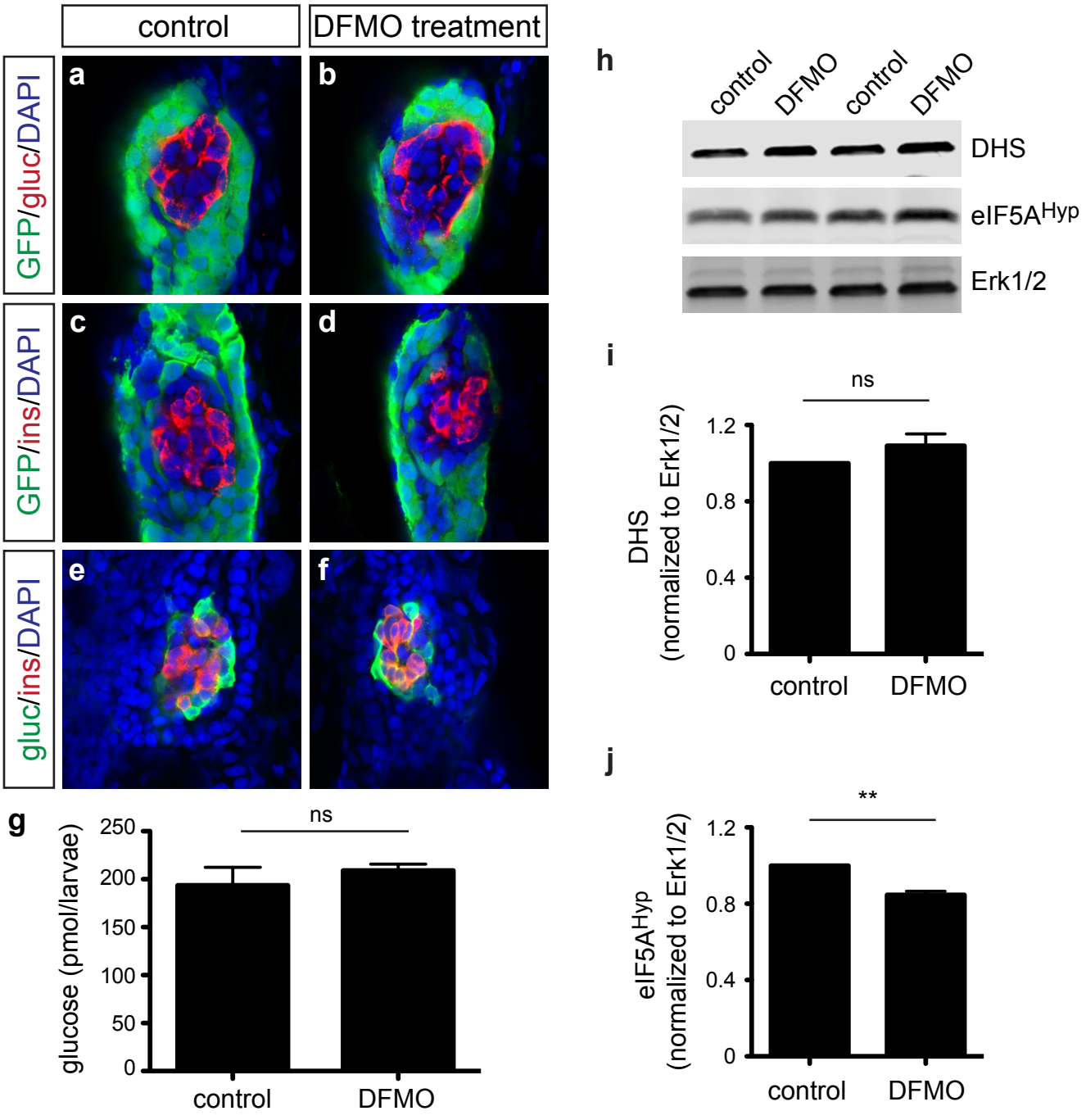
Supplementary Fig. S2. Titration of DFMO in zebrafish.

(a) Percentage of viable embryos in control (n = 180), 1% w/v DFMO-treated (n = 33), 2% w/v DFMO-treated (n = 45), 3% w/v DFMO-treated (n = 28), 5% w/v DFMO-treated (n = 24), and 10% w/v DFMO-treated (n = 15) embryos at 72 hpf. (b) Percentage of control (n = 32), 1% w/v DFMO-treated (n = 33), 2% w/v DFMO-treated (n = 40) embryos at 72 hpf with altered body axis due to DFMO treatment. (c) Representative images of control and DFMO-treated *Tg(ptf1a:gfp)* embryos displaying the phenotype of altered (truncated) body axis. (d) The *ptf1a*: GFP-expressing retina (r) and hindbrain (hb) were unaltered in DFMO-treated *Tg(ptf1a:gfp)* embryos compared with controls, at 48 hpf and 72 hpf.



Supplementary Fig. S3. Endocrine cell phenotype following inhibition of polyamine biosynthesis by DFMO treatment.

Representative images at 72 hps of glucagon-expressing cells in (a) control and (b) DFMO-treated *Tg*(*ptf1a:gfp*) embryos; insulin-expressing cells in (c) control and (d) DFMO-treated *Tg*(*ptf1a:gfp*) embryos; and insulin/glucagon co-expressing cells in (e) control and (f) DFMO-treated *Tg*(*ptf1a:gfp*) embryos. (g) Glucose measurement from control or DFMO-treated embryos at 72 hpf (n = 3 groups of 10 embryos/group) (p = 0.4821). (h) Western blot analysis for expression of DHS, eIF5A^{Hyp}, and Erk1/2 in control and DFMO-treated embryos at 72 hpf (n = 4 groups of 30 embryos/group); Erk1/2 was used as a loading control, as expression of this protein is not altered with DFMO treatment. Quantification showed no change in (i) DHS expression (p = 0.2370) but a significant decrease in (j) eIF5A^{Hyp} expression (p = 0.0039). gluc, glucagon; ins, insulin. * p < 0.05, ** p < 0.01, *** p < 0.001; ns, not significant.



Supplementary Fig. S4. Protein alignment of DHS and Eif5a.

Comparison of human, mouse and zebrafish (a) Dhps and (b) Eif5a protein sequence. The K50 residue of Eif5a is highlighted in red, and is the residue hypusinated by DHS.

a

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human_DHPS   MEGSLEREAPAGALAAVLKHSSTLPPESTQVRGYDFNRGVNYRALLEAFGTTGFQATNFG
mouse_Dhps   MEGTPPGAAPSSALAAVLKHSSALPPESAQVQGYDFNRGVDYHALLDAYRTTGFQATNFG
zfish_Dhps   ----MAGQAPPVARAAVLKESTPLPDNLPQIRGYDFNQGLNHRALLQSFITTGFQASSFG
                     **  * *****.*: ** :  *::*****:*::::***::: ******:.**

human_DHPS   RAVQQVNAMIEKKLEPLSQDEDQHADLTQSRRPLTSCTIFLGYTSNLISSGIRETIRYLV
mouse_Dhps   RAVQQVNAMIEKKLEPLAVDEDHHADLTQSRRPLTGCTIFLGYTSNLISSGIRETIRYLV
zfish_Dhps   LAVQEINKMIEKRLEPVQEECE----DSDSRQSALGCTIFLGYTSNLISSGVRESIRYLT
              ***::* ****:***:  : :     ::**:   .***************:**:****.

human_DHPS   QHNMVDVLVTTAGGVEEDLIKCLAPTYLGEFSLRGKELRENGINRIGNLLVPNENYCKFE
mouse_Dhps   QHNMVDVLVTTAGGVEEDLIKCLAPTYLGEFSLRGKELRESGINRIGNLLVPNDNYCKFE
zfish_Dhps   QHKMVDVIVTTAGGIEEDLIKCLAPTFLGEFSLKGKELRQQGINRIGNLLVPNENYCKFE
             **:****:******:**********:******:*****:.************:******

human_DHPS   DWLMPILDQMVMEQNTEGVKWTPSKMIARLGKEINNPESVYYWAQKNHIPVFSPALTDGS
mouse_Dhps   DWLMPILDQMVLEQNTEGVKWTPSKMISRLGKEINNPDSVYYWAHKNHIPVLSPALTDGS
zfish_Dhps   DWLMPILDQMVLEQKTEGTHWTPSKMIHRLGKEINNPDSVYYWAYKNDIPVFSPALTDGS
             ***********:**:***.:******* *********:****** **.***:********

human_DHPS   LGDMIFFHSYKNPGLVLDIVEDLRLINTQAIFAKCTGMIILGGGVVKHHIANANLMRNGA
mouse_Dhps   LGDMIFFHSYKNPGLVLDIVEDLRLINTQAIFAKRSGMIILGGGVVKHHIANANLMRNGA
zfish_Dhps   LGDMIYFHSYKNPGLVLDIVEDIRRLNSKAVFAKSTGMIILGGGLVKHHIANANLMRNGA
             *****:*****************:* :*::*:*** :********:***************

human_DHPS   DYAVYINTAQEFDGSDSGARPDEAVSWGKIRVDAQPVKVYADASLVFPLLVAETFAQKMD
mouse_Dhps   DYAVYINTAQEFDGSDSGARPDEAVSWGKIRMDAQPVKVYADASLVFPLLVAETFAQKAD
zfish_Dhps   DFAVFVNTGQEFDGSDSGARPDEAISWGKIRMDASPVKVYADASLVFPLLVAETFAHNAS
             *:**::**.***************:******:**.**********************:: .

human_DHPS   AFMHEKNED
mouse_Dhps   AFRAEKNED
zfish_Dhps   RLIQEKKSD
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b

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human_EIF5A  -MADDLDFETGDAGASATFPMQCSALRKNGFVVLKGRPCKIVEMSTSKTGKHGHAKVHLV
mouse_Eif5a  -MADDLDFETGDAGASATFPMQCSALRKNGFVVLKGRPCKIVEMSTSKTGKHGHAKVHLV
zfish_Eif5a  MADPDLDFASGDAGASATYPMQCSALRKNGFVVLKGRPCKIVEMSTSKTGKHGHAKVHLV
                 **** :********:*****************************************

human_EIF5A  GIDIFTGKKYEDICPSTHNMDVPNIKRNDFQLIGIQDGYLSLLQDSGEVREDLRLPEGDL
mouse_Eif5a  GIDIFTGKKYEDICPSTHNMDVPNIKRNDFQLIGIQDGYLSLLQDSGEVREDLRLPEGDL
zfish_Eif5a  GIDIFTNKKYEDICPSTHNMDVPNIKRNDYQLVDISEGFLSLMMDNGDVREDLRVPDGDL
             ****** **********************:**: *.:*:***: *.*:******:*:***

human_EIF5A  GKEIEQKYDCGEEILITVLSAMTEEAAVAIKAMAK
mouse_Eif5a  GKEIEQKYDCGEEILITVLSAMTEEAAVAIKAMAK
zfish_Eif5a  GKEIENKFAAGEEMLVTVLSAMGEESAVALKPMTK
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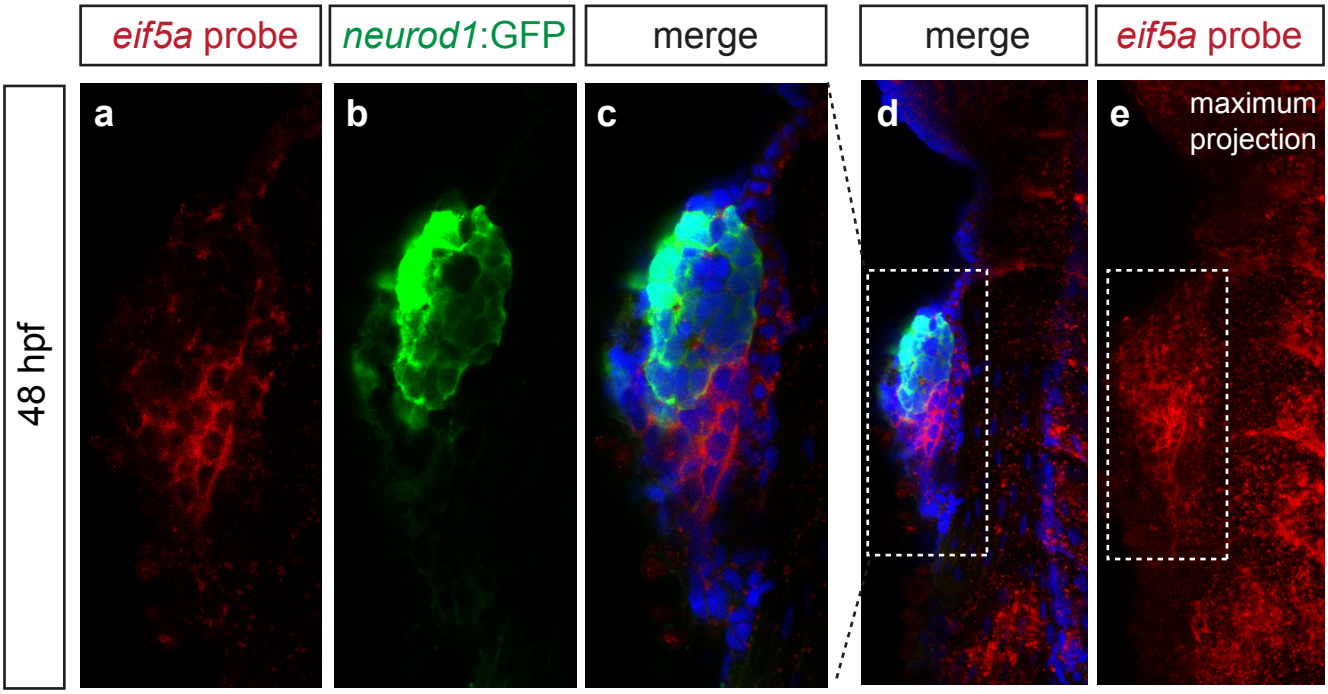
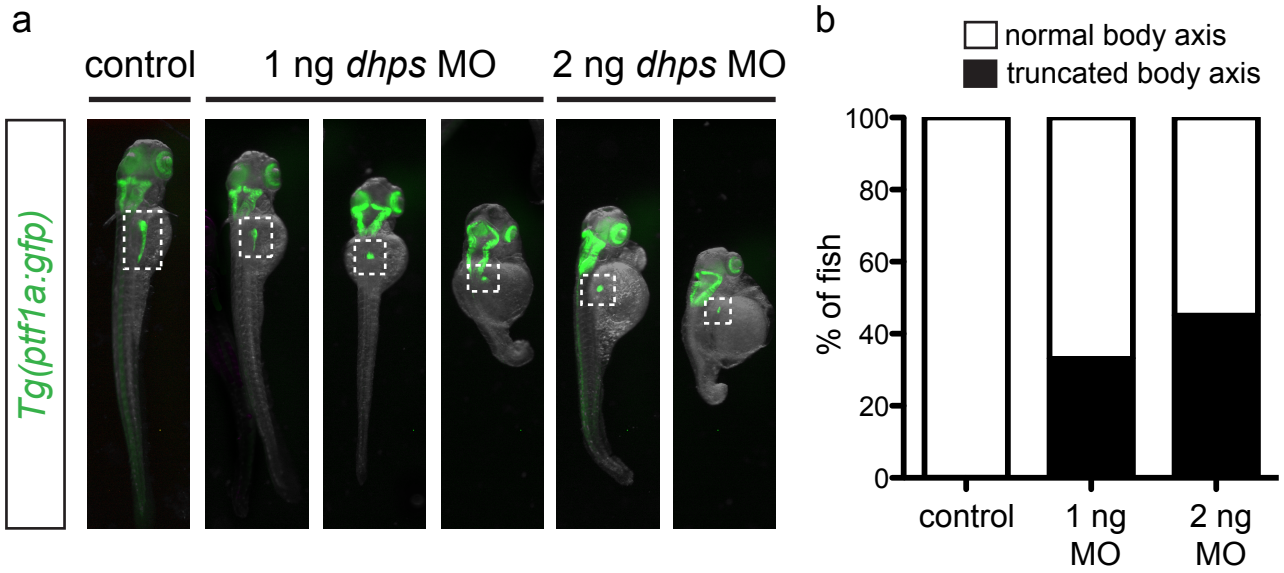
Supplementary Fig. S5. Expression patterns of *eif5a* in zebrafish at 48 hpf.

At 48 hpf, (a) *eif5a* has strong expression in the exocrine compartment compared with the (b) *neurod1*:GFP+ endocrine cell compartment; (c) shows the merged image. (d) Image showing the pancreas (dotted box) and adjacent neural tube. (e) The corresponding maximum intensity projection of *eif5a* expression in the pancreas at 48 hpf; dotted box denotes pancreas domain.



Supplementary Fig. S6. Phenotypic penetrance and expressivity in *dhps* MO-injected embryos.

(a) Control, 1 ng MO-injected, and 2 ng MO-injected *Tg(ptf1a:gfp)* embryos displaying the phenotype of normal or altered (truncated) body axis. White dotted box demarks pancreas.
(b) Percentage of control (n = 20), 1 ng MO-injected (n = 46), and 2 ng MO-injected (n = 23) embryos with altered body axis following implementation of the *dhps* MO.
(c) Sequencing data showing the deletion of exon 2 as a result of *dhps* MO knockdown. Primers used for PCR amplification are highlighted in green; the deleted region is indicated by dashed lines.



c

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zf dhps cDNA GCGCTGTGAAATGTGAGTGAAACCAACGAGAAACTACACCTAAAAAGCCGG
zf dhps MO  GCGCTGTGAAATGTGAGTGAAACCAACGAGAAACTACACCTAAAAAGCCGG
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zf dhps cDNA ATTTACATCCATAACCCCCGTGCGGATCGGTGAGCTCCTCATGGCGGGTCA
zf dhps MO  ATTTACATCCATAACCCCCGTG----------------------------
            **********************----------------------------

zf dhps cDNA GGCTCCGCCGGTGGCCCGGGCCGCGGTGCTGAAGGAGAGCACGCCTCTGCC
zf dhps MO  ------------------------------------------------
            ------------------------------------------------

zf dhps cDNA GGACAATCTGCCTCAGATCAGAGGATACGACTTTAACCAGGGCCTGAACCA
zf dhps MO  ------------------------------------------------
            ------------------------------------------------

zf dhps cDNA CAGAGCGCTGCTGCAGTCCTTCATCACTACCGGCTTCCAGGCCTCCAGCTT
zf dhps MO  ------------------------------------------------
            ------------------------------------------------

zf dhps cDNA CGGCCTGGCAGTGCAGGAGATCAATAAGATGATAGAGAAGAGGCTGGAGCC
zf dhps MO  ------------------------------ATAGAGAAGAGGCTGGAGCC
            ------------------------------**********************

zf dhps cDNA GGTGCAGGAGGAGTGTGAGGACAGTGATTCTCGTCAGTCTGCTTTGGGATG
zf dhps MO  GGTGCAGGAGGAGTGTGAGGACAGTGATTCTCGTCAGTCTGCTTCGGGATG
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zf dhps cDNA CACTATATTCCTGGGCTACACGTCAAAC
zf dhps MO  CACTATATTCCTGGGCTACACGTCAAAC
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