iScience, Volume *23*

Supplemental Information

Regulation of Gluconeogenesis by Aldo-keto-reductase 1a1b in Zebrafish

Xiaogang Li, Felix Schmöhl, Haozhe Qi, Katrin Bennewitz, Christoph T. Tabler, Gernot Poschet, Rüdiger Hell, Nadine Volk, Tanja Poth, Ingrid Hausser, Jakob Morgenstern, Thomas Fleming, Peter Paul Nawroth, and Jens Kroll

A

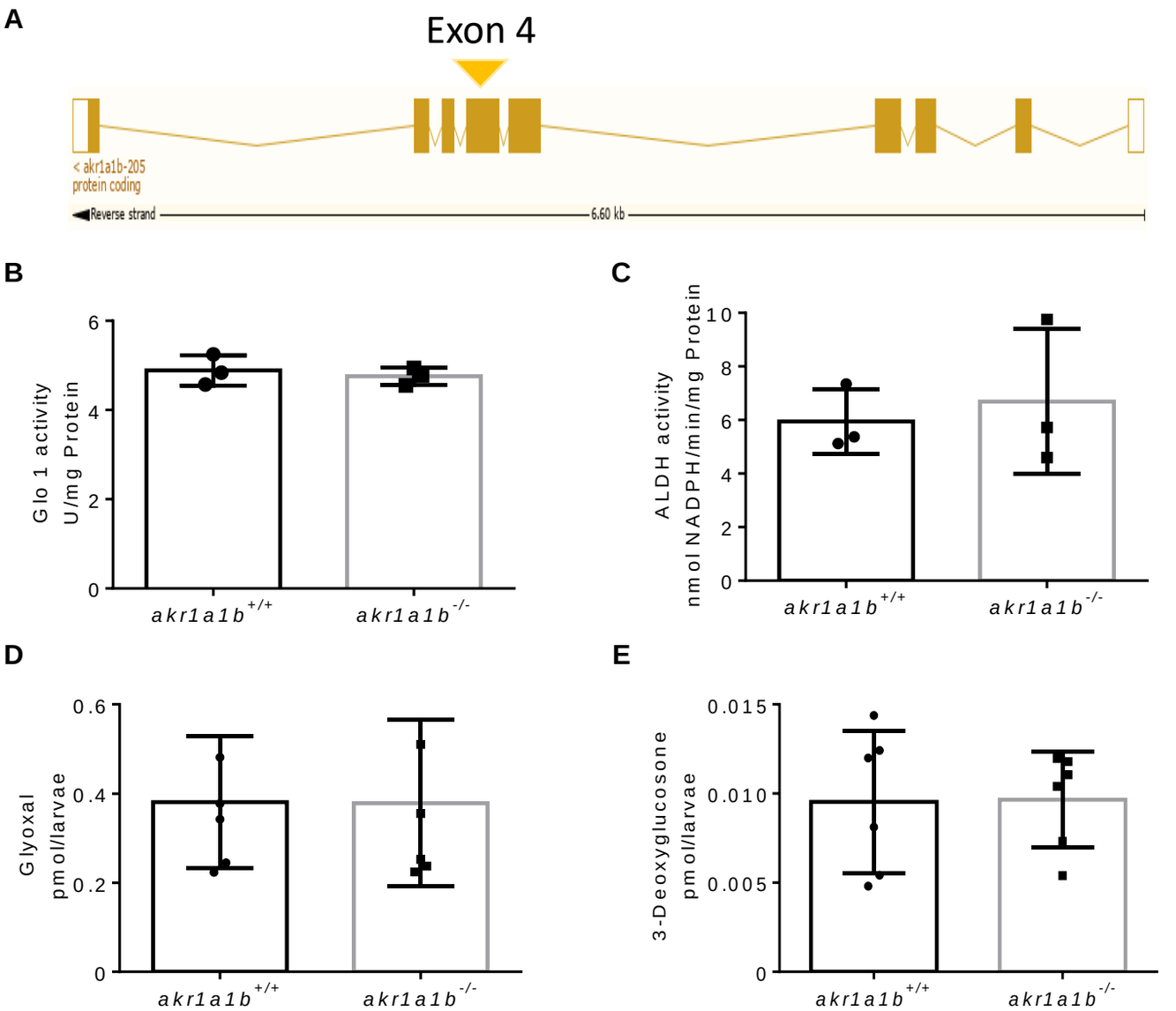
```
P14550 AK1A1_HUMAN          1   MAASCVLLHTGQKMPLIGLGTWKSEPGQVKAAVKYALSVGYRHIDCAAIYGNEPEIGEAL      60
Q9JII6 AK1A1_MOUSE          1   MTASSVLLHTGQKMPLIGLGTWKSEPGQVKAAIKHALSAGYRHIDCASVYGNETEIGEAL      60
Q6AZW2 A1A1A_DANRE          1   -MTATITLSTGQRMPTVGLGTWKSAPGQVKQAVLAALDCGYRHIDCAAAYSNEREVGEAL      59
                                  :: : * ***:** :******* ***** *:  **. ********: *.** *:****

P14550 AK1A1_HUMAN         61   KEDVGPGKAVPREELFVTSKLWNTKHHPEDVEPALRKTLADLQLEYLDLYLMHWPYAFER     120
Q9JII6 AK1A1_MOUSE         61   KESVGSGKAVPREELFVTSKLWNTKHHPEDVEPALRKTLADLQLEYLDLYLMHWPYAFER     120
Q6AZW2 A1A1A_DANRE         60   TERLGPGKSLRRDDIFVTSKLWNTKHHPDDVEEACRRSLSDLRLSYLDLYLIHWPMAFGR     119
                                .* :* **:: *:::**************:*** * *::*:**:*.******:*** ** *

P14550 AK1A1_HUMAN        121   GDNPFPKNADGTICYDSTHYKETWKALEALVAKGLVQALGLSNFNSRQIDDILSVASVRP     180
Q9JII6 AK1A1_MOUSE        121   GDNPFPKNADGTVRYDSTHYKETWKALEVLVAKGLVKALGLSNFNSRQIDDVLSVASVRP     180
Q6AZW2 A1A1A_DANRE        120   GDELIPRHPDGTIQYDDTHYRDIWAAMEKLVDQGLAKAIGLSNFNAKQIDDILSIAKHKP     179
                                **: :*:: ***: **.***:** *:* ** :**.:*:******::****:**:*. :*

P14550 AK1A1_HUMAN        181   AVLQVECHPYLAQNELIAHCQARGLEVTAYSPLGSSDRAWRDPDEPVLLEEPVVLALAEK     240
Q9JII6 AK1A1_MOUSE        181   AVLQVECHPYLAQNELIAHCHARGLEVTAYSPLGSSDRAWRHPDEPVLLEEPVVLALAEK     240
Q6AZW2 A1A1A_DANRE        180   VVNQVECHPYLVQAELVSHCWSRNLTVTAYSPLGSPDRPWVTPGEALLLDDPRVVGIAKS     239
                                .* ********.* **:** :*.* ********* ** *  *.* :**:** *:.:*:.

P14550 AK1A1_HUMAN        241   YGRSPAQILLRWQVQRKVICIPKSITPSRILQNIKVFDFTFSPEEMKQLNALNKNWRYIV     300
Q9JII6 AK1A1_MOUSE        241   HGRSPAQILLRWQVQRKVICIPKSINPSRILQNIQVFDFTFSPEEMKQLDALNKNWRYIV     300
Q6AZW2 A1A1A_DANRE        240   YNKTPAQVIIRWHIQRGVVCIPKSVTPSRIKQNIEVFDFKLSDEDMRLIESFNRNERFII     299
                                :.::***:::**::** *:*****:.**** ***:****.:* *:*: ::::*:* *:*:

P14550 AK1A1_HUMAN        301   PMLTVDGKRVPRDAGHPLYPFNDPY                                 325
Q9JII6 AK1A1_MOUSE        301   PMITVDGKRVPRDAGHPLYPFNDPY                                 325
Q6AZW2 A1A1A_DANRE        300   PTVIKDGQKIWRDAKHPHFPFIEPY                                 324
                                * :  **::: *** ** :** :**
```

B

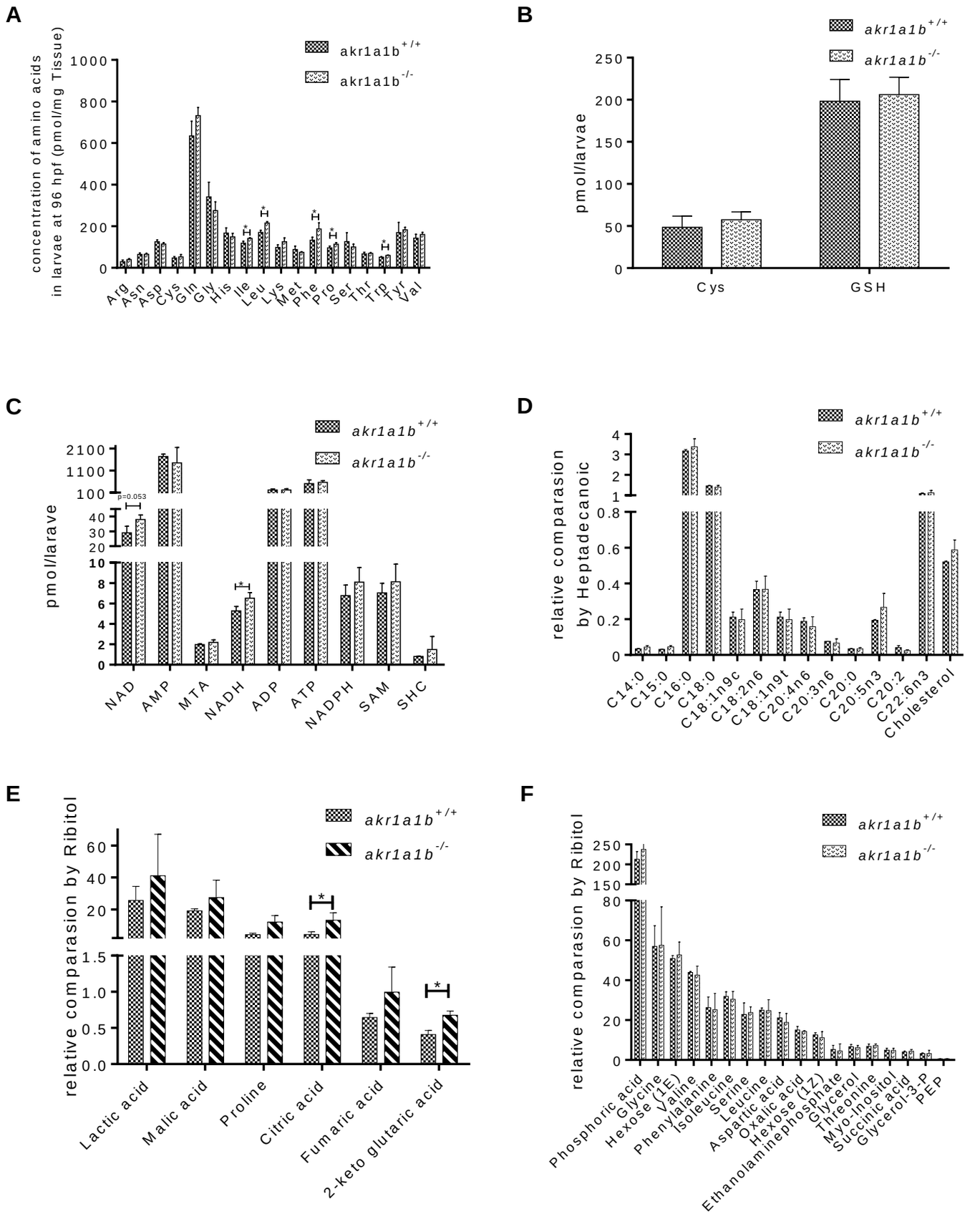
```
P14550      AK1A1_HUMAN       1   -MAASCVLLHTGQKMPLIGLGTWKSEPGQVKAAVKYALSVGYRHIDCAAIYGNEPEIGEA      59
Q9JII6      AK1A1_MOUSE       1   -MTASSVLLHTGQKMPLIGLGTWKSEPGQVKAAIKHALSAGYRHIDCASVYGNETEIGEA      59
A0A2R8QTH3 A0A2R8QTH3_DANRE   1   MSMNDFAVLSTGRKMPLLGLGTWKSEPGLVKQAVIWALESGYRHIDCAPIYANEPEIGEA      60
                                      . .:* **:****:********** ** *:  **. ******** :*.** *****

P14550      AK1A1_HUMAN      60   LKEDVGPGKAVPREELFVTSKLWNTKHHPEDVEPALRKTLADLQLEYLDLYLMHWPYAFE     119
Q9JII6      AK1A1_MOUSE      60   LKESVGSGKAVPREELFVTSKLWNTKHHPEDVEPALRKTLADLQLEYLDLYLMHWPYAFE     119
A0A2R8QTH3 A0A2R8QTH3_DANRE 61   FQETMGPDKGIRREDVFVTSKLWNTKHHPDDVEPSLLKTLKDLKLEYLDLYLIHWPYAFQ     120
                                 ::* :* .*.: **::**************:****:* *** **:********:******:

P14550      AK1A1_HUMAN      120   RGDNPFPKNADGTICYDSTHYKETWKALEALVAKGLVQALGLSNFNSRQIDDILSVASVR     179
Q9JII6      AK1A1_MOUSE      120   RGDNPFPKNADGTVRYDSTHYKETWKALEVLVAKGLVKALGLSNFNSRQIDDVLSVASVR     179
A0A2R8QTH3 A0A2R8QTH3_DANRE121   RGDTPFPRKEDGTLLYDDIDYKLIWAAMEKLVGKGLVRAIGLSNFNSRQIDDILSVASIK     180
                                 ***.***:: ***: **. .** ** *:* **.****:*:************:*****::

P14550      AK1A1_HUMAN      180   PAVLQVECHPYLAQNELIAHCQARGLEVTAYSPLGSSDRAWRDPDEPVLLEEPVVLALAE     239
Q9JII6      AK1A1_MOUSE      180   PAVLQVECHPYLAQNELIAHCHARGLEVTAYSPLGSSDRAWRHPDEPVLLEEPVVLALAE     239
A0A2R8QTH3 A0A2R8QTH3_DANRE181   PIVLQVESHPYLAQVELLSHCRDRGLVMTAYSPLGSPDRAWKHPDEPVLLEEPAIAALAK     240
                                 *:******.****** **::**: *** :********* ****:.**********.: ***:

P14550      AK1A1_HUMAN      240   KYGRSPAQILLRWQVQRKVICIPKSITPSRILQNIKVFDFTFSPEEMKQLNALNKNWRYI     299
Q9JII6      AK1A1_MOUSE      240   KHGRSPAQILLRWQVQRKVICIPKSINPSRILQNIQVFDFTFSPEEMKQLDALNKNWRYI     299
A0A2R8QTH3 A0A2R8QTH3_DANRE241   KYNKTPAQIIIRWQTQRGVVTIPKSITQSRIKENIQVFDFTLESEEMSQVTALHRGWRYI     300
                                 *:.::*****::***.** *: ******. *** :**:******:. ***.*: **::.****

P14550      AK1A1_HUMAN      300   VPMLTVDGKRVPRDAGHPLYPFNDPY                                325
Q9JII6      AK1A1_MOUSE      300   VPMITVDGKRVPRDAGHPLYPFNDPY                                325
A0A2R8QTH3 A0A2R8QTH3_DANRE301   VPTITVDGKSVPRDAGHPHYPFNDPY                                326
                                 ** :****** ******** *******
```

Supplement Figure 1: Sequence alignment of Akr1a1 across different species, related to Figure 1

Amino acid alignment shows a high similarity between the different species of the active site (red frame); first line: human Akr1a1; second line: mouse Akr1a1; third line:**(a)** zebrafish Akr1a1a, **(b)** zebrafish Akr1a1b (AOA2R8QTH3).

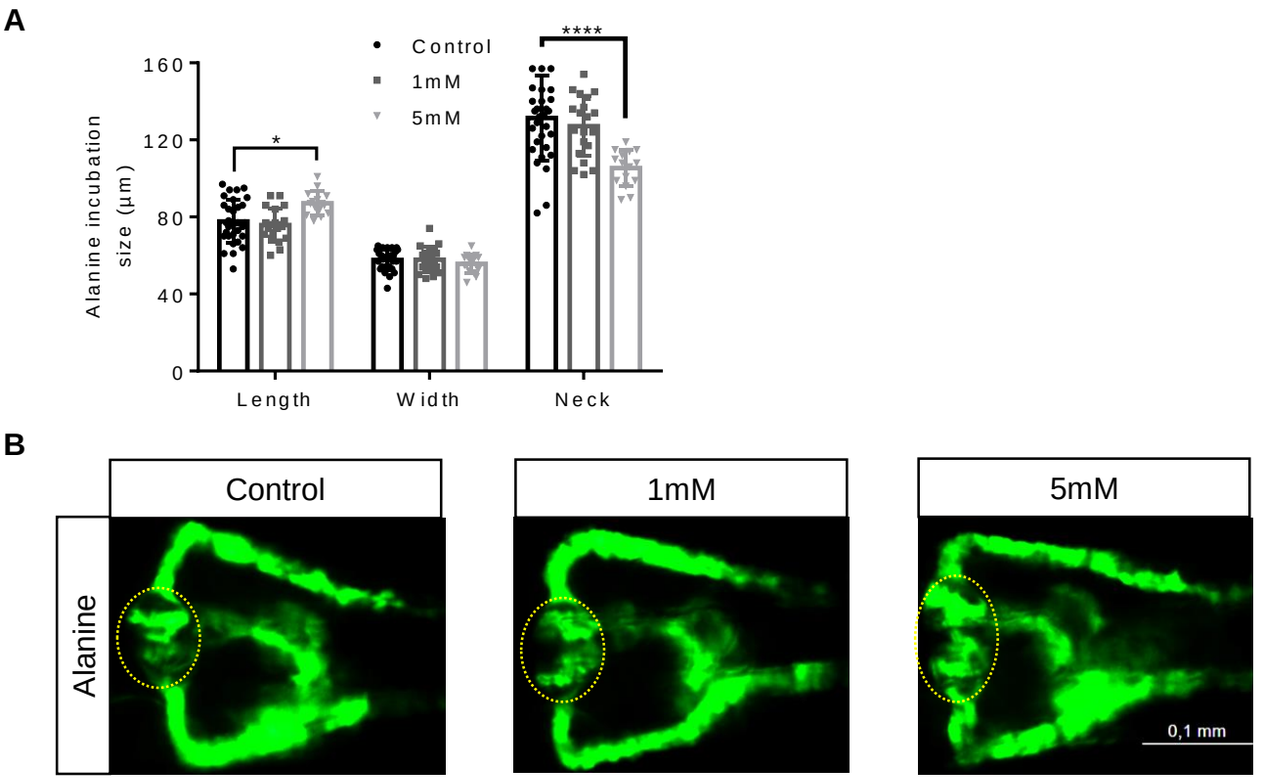


Supplement Figure 2: Characterization of *akr1a1b*$^{-/-}$ zebrafish, related to Figure 2
(a) Schematic depiction of the *akr1a1b* gene, which highlights exon 4 as CRISPR-Cas9 target site(https://www.ensembl.org/Danio_rerio/Transcript/Summary?db=core;g=ENSDARG00000052030;r=6:33918813-33925432;t=ENSDART00000145019). **(b,c)** Activity of compensatory enzymes, Glyoxalase 1 (Glo1) and Aldehyde Dehydrogenases (ALDH), were not altered as measured by spectrophotometric analysis in Δ-23 *akr1a1b*$^{-/-}$ zebrafish lysates at 96 hpf (n = 3 clutches with 50 larvae, mean ± SD). **(d,e)** Advanced Glycation End Products (AGEs) precursors Glyoxal and 3-Deoxyglucosone, were not altered in Δ-23 *akr1a1b*$^{-/-}$ 96 hpf old larvae, as determined by LC-MS/MS (D, E. n = 6 clutches with 50 larvae).



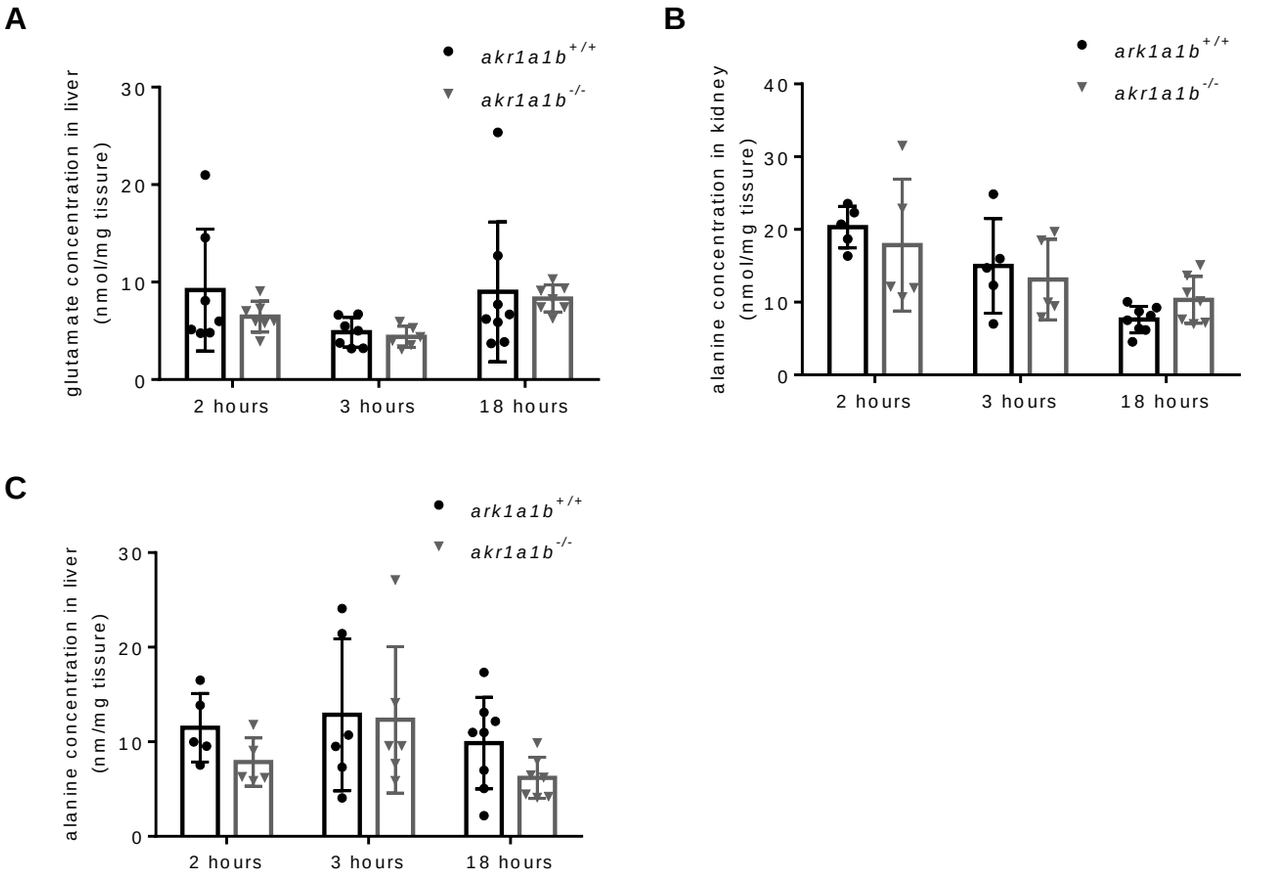
Supplement Figure 3: Metabolome profile in Δ-23 ***akr1a1b*$^{-/-}$ larvae, related to Figure 5**

(a) Amino acids, **(b)** thiols and **(c)** adenosine were slightly or not changed in *akr1a1b*$^{-/-}$ larvae as determined by using UPLC-FLR in zebrafish lysates at 96 hpf (n = 3 clutches with 50 larvae; mean ± SD). **(d)** Fatty acid and **(e,f)** primary metabolites were determined using GC/MS and only 2- keto glutaric acid and citric acid were increased in *akr1a1b*$^{-/-}$ larvae at 96hpf (n = 3 clutches with 50 larvae; mean ± SD). *p<0.05.



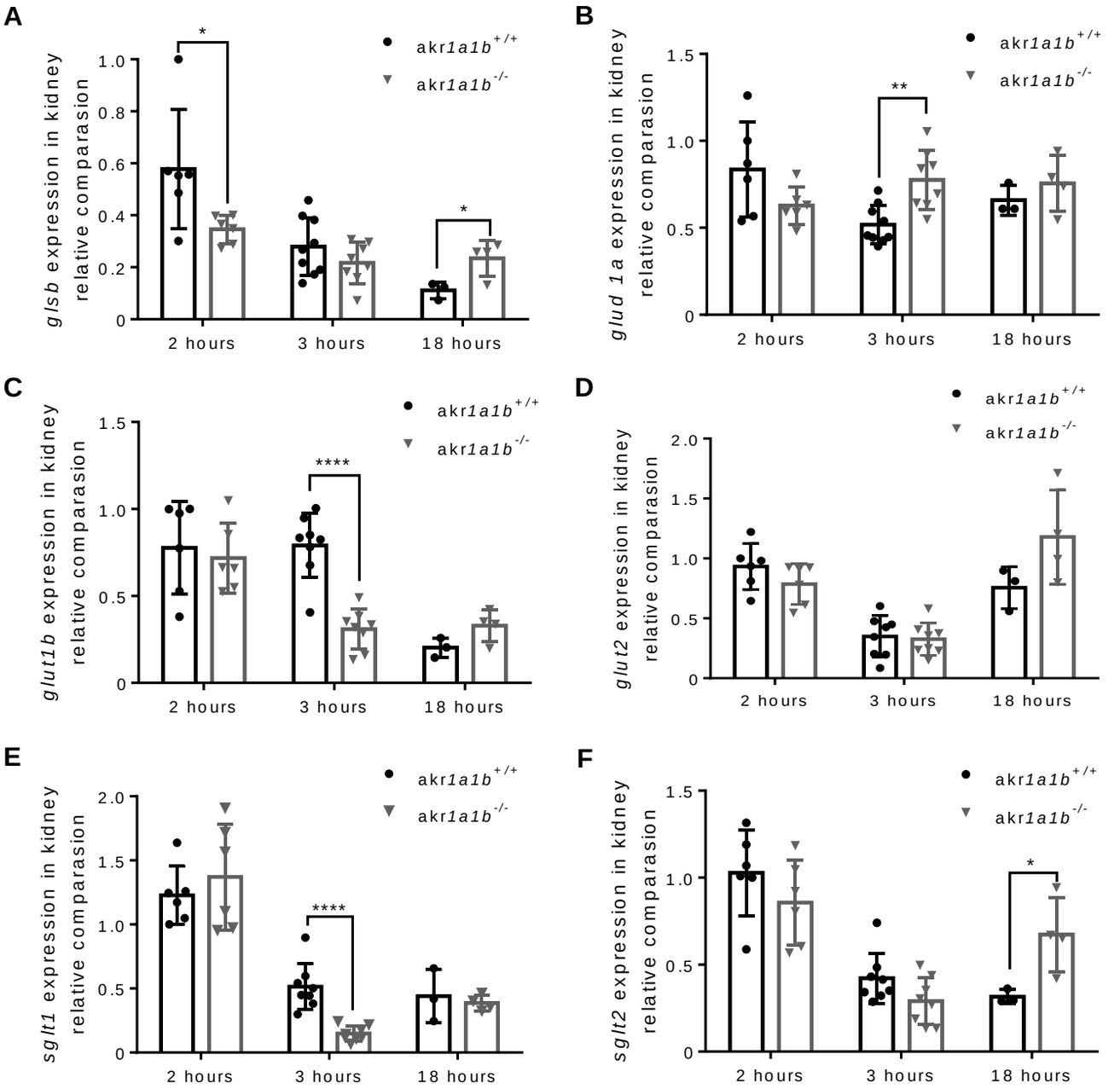
Supplement Figure 4: Alanine incubation of wild type embryos damaged the pronephros, related to Figure 5

(a,b) Enlarged glomerulus (encircled) and shortened tubular neck length in 48 hpf zebrafish embryos treated with alanine (n = 31 in control group; n = 20 in 0.1mM group; n = 15 in 1mM group , mean ± SD). * $p < 0.05$, **** $p < 0.0001$. Scale bar: 0.1 mm.



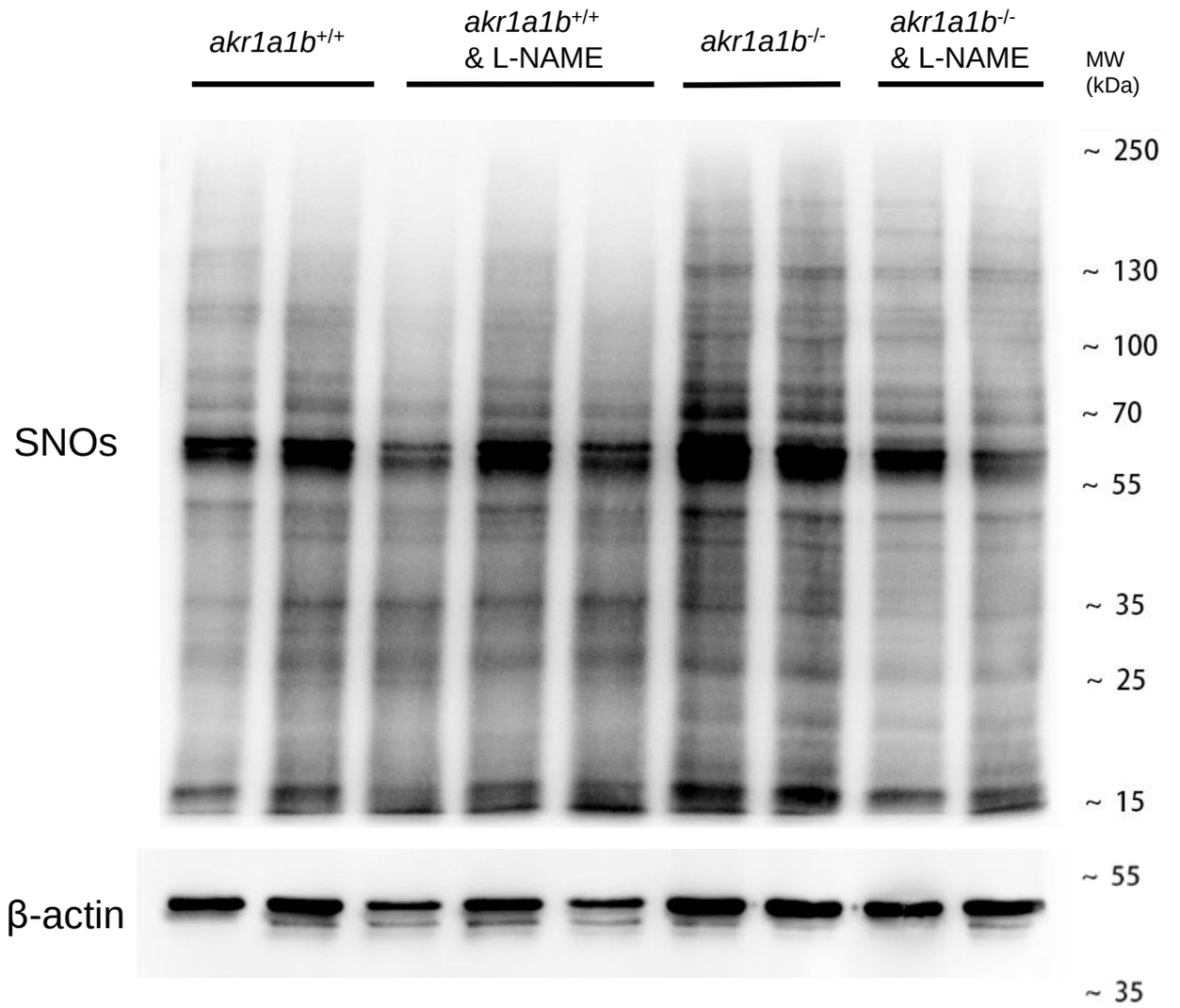
Supplement Figure 5: Unaltered concentrations of glutamate in Δ-23 *akr1a1b*$^{-/-}$ livers, and unaltered concentrations of alanine in Δ-23 *akr1a1b*$^{-/-}$ kidneys and livers, related to Figure 5

(a) Unaltered concentrations of glutamate in adult *akr1a1b*$^{-/-}$ livers (2 hours postprandial: n = 7 in *akr1a1b*$^{+/+}$, n = 7 in *akr1a1b*$^{-/-}$; 3 hours postprandial: n = 7 in *akr1a1b*$^{+/+}$, n = 6 in *akr1a1b*$^{-/-}$; 18 hours postprandial: n = 8 in *akr1a1b*$^{+/+}$, n = 7 in *akr1a1b*$^{-/-}$, mean ± SD). **(b,c)** Concentrations of alanine were not altered in adult *akr1a1b*$^{-/-}$ kidneys (2 hours postprandial: n = 5 in *akr1a1b*$^{+/+}$, n = 5 in *akr1a1b*$^{-/-}$; 3 hours postprandial: n = 5 in *akr1a1b*$^{+/+}$, n = 5 in *akr1a1b*$^{-/-}$; 18 hours postprandial: n = 8 in *akr1a1b*$^{+/+}$, n = 7 in *akr1a1b*$^{-/-}$,mean ± SD) and in adult *akr1a1b*$^{-/-}$ livers (2 hours postprandial: n = 5 in *akr1a1b*$^{+/+}$, n = 5 in *akr1a1b*$^{-/-}$; 3 hours postprandial: n = 5 in *akr1a1b*$^{+/+}$, n = 6 in *akr1a1b*$^{-/-}$; 18 hours postprandial: n = 8 in *akr1a1b*$^{+/+}$, n = 7 in *akr1a1b*$^{-/-}$, mean ± SD).



Supplement Figure 6: Expression of glutaminolytic enzymes and glucose transporters in Δ-23 *akr1a1b*$^{-/-}$ kidneys, related to Figure 6

Expression analysis of **(a)** *glutaminase* b (*glsb*), **(b)** *glutamate dehydrogenase 1 (glud1a)*, **(c)** *glucose transporter 1b* (*glut 1b)*, **(d)** glucose transporter 2 (*glut 2)*, **(e)** *sodium dependent glucose co-transporter 1 (sglt 1)* and **(f)** *sodium dependent glucose co-transporter 2 (sglt 2)* in adult *akr1a1b*$^{-/-}$ kidneys by RT-qPCR *(*2 hours postprandial: n = 6 in *akr1a1b*$^{+/+}$, n = 6 in *akr1a1b*$^{-/-}$; 3 hours postprandial: n = 9 in *akr1a1b*$^{+/+}$, n = 8 in *akr1a1b*$^{-/-}$; 18 hours postprandial: n = 3 in *akr1a1b*$^{+/+}$, n = 4 in *akr1a1b*$^{-/-}$, mean ± SD. * $p < 0.05$, ** $p < 0.01$, **** $p < 0.0001$.



Supplement Figure 7: L-NAME treatment reduced S-nitrosylated proteins in 96 hpf Δ-23 *akr1a1b*$^{-/-}$ larvae, related to Figure 7

Western blot shows increased S-nitrosylated proteins (SNOs) in 96 hpf *akr1a1b*$^{-/-}$ larvae, which was prevented by 20 µM L-NAME treatment.

Transparent Methods

Zebrafish lines and husbandry

All experimental procedures on animals were approved by Medical Faculty Mannheim (license no.: I-19/01) and Regierungspräsidium Karlsruhe (license no. G-98/15) and carried out following the approved guidelines. Embryos/larvae of the *Tg(wt1b:EGFP)* line, where the *wt1b* promotor drives expression of EGFP (Enhanced Green Fluorescent Protein) in pronephros [(Perner et al., 2007)]; *Tg(fli1:EGFP)* line, which expresses EGFP in the vasculature under the control of the *fli1* promoter, and thus enables the visualization of the vascular system in live zebrafish[(Lawson and Weinstein, 2002)], were raised and staged as described according to hours post-fertilization (hpf)[(Kimmel et al., 1995)]. Embryos/larvae were kept in egg water at 28.5°C with 0.003 % 1-phenyl-2-thiourea (PTU, Sigma) to suppress pigmentation. Adult zebrafish were held under a 13 hours light - 11 hours dark cycle and fed with live shrimps and fish flake food. For the study both sexes were used.

Mutant generation

The CRISPR target site for *akr1a1b* was identified and selected using ZiFiT Targeter 4.1. The *akr1a1b*-CRISPR oligonucleotide, forward: 5′-TAGGTCCAAGTACTCCAGCTTC -3′, reverse: 5′-AAACGAAGCTGGAGTACTTGGA-3′, were synthesized by Sigma Aldrich. The oligonucleotides were cloned into the pT7- gRNA plasmid (Addgene). BamHI-HF (Biolabs) was used for linearization. Cas9 mRNA was synthesized from pT3TS plasmid (Addgene) after linearizing with XbaI (Biolabs). Plasmids were purified with a PCR purification kit (Qiagen). CRISPR gRNA *in vitro* transcription was done by the T7 MEGAshortscript kit and mMESSAGE MACHINE kit for Cas9 mRNA (Invitrogen). Purification of RNA after TURBO DNAse treatment was done with the MiRNeasy Mini (gRNA) and RNeasy Mini (Cas9 mRNA) kits (Qiagen). *Akr1a1b* gRNA and Cas9 mRNA (150 pg/nl) was mixed with KCl (0.1 M). The RNA mixture (1 nL) was injected directly into one-cell embryos. For genotyping, PCR-products of genomic DNA were used for Sanger sequencing (primer: forward: 5’-GGCGAGAGGATGTGTTTGTG-3’; reverse: 5’- GGGGCTCTATTATGGTCTTTTCA- 3’).

Immunohistochemistry and antibody generation

Kidney tissue from wild-type zebrafish was used for the analysis of Akr1a1b protein expression. The experiment was performed on 4 µm paraffin-embedded tissue sections fixed in 4% paraformaldehyde (PFA). The sections were dewaxed, rehydrated, antigen retrieved by

boiling sections in sodium citrate buffer (0.01 M, pH 6.0) in 480 W microwave for 20 min, followed by removal of endogenous peroxidase activity by a solution of H_2O_2/methanol (3%) for 20 min, nonspecific antibody binding was blocked by incubation with 10% FBS/PBS for 1 hour. The primary antibody diluted in 10% FBS/PBS was applied overnight at 4°C. Afterward, sections were washed three times in PBS and then incubated in a 1:1000 diluted secondary antibody for 30min at room temperature. Diaminobenzidine (DAB, SK-4100, vector laboratories) was used for chromogens for HRP and counterstained by Hematoxylin. Then all sections were dehydrated and sealed for analysis. For Akr1a1b antibody generation, peptide AWKHPDEPVLLEEPAIAAL-C was synthesized and coupled to KLH (Keyhole Limpet Hemocyanin) by PSL GmbH, Heidelberg, Germany and subsequently injected into guinea pigs for immunization following standard procedures from CF Unit Antibodies, DKFZ Heidelberg, Germany.

Western blot analysis

For analysis of Akr1a1b expression, adult zebrafish livers were taken and lysed in NP40 lysis buffer (150 mmol/L NaCl, 50 mmol/L Tris-HCl, pH 7.4, 1% NP40, 10 mmol/L EDTA, 10 % glycerol, and protease inhibitors), followed by homogenization with a syringe and incubation on ice for 30 min on a shaker. The supernatant containing the protein lysate was diluted 5:1 with Laemmli sample buffer and boiled at 95°C for 5 min, separated via SDS-PAGE, and then transferred to a nitrocellulose membrane for antibody incubation (anti-Akr 1a1b antibody 1:1000, anti-Actin antibody A2228 from Sigma-Aldrich, 1:1000), secondary HRP-conjugated antibodies 1:1000 (for β-actin: rabbit anti-goat, P0160, Dako; for Akr1a1b: goat anti-guinea pig, ABIN101281, antibodies-online.com). Visualization by enhanced chemiluminescence (ECL) was acquired after incubation with HRP (Horseradish Peroxidase) substrate.

Determination of methylglyoxal (MG), 3-Deoxyglucosone (3-DG), and glyoxal

MG, 3-DG, and glyoxal levels of zebrafish larvae, whole body lysates, and eyes were determined by LC-MS/MS, as described previously[(Zhou et al., 2019)]. Tissue MG levels were determined by derivatization with 1,2-diaminobenzene (DB). Briefly, pre-weighed amounts of tissue (10 mg) were homogenized in ice-cold 20 % (wt/vol) trichloroacetic acid in 0.9 % (wt/vol) sodium chloride (20 µL) and water (80 µL). An aliquot (5 µL) of the internal standard (13C3-MG; 400 nM) was then added, and the samples mixed. Following centrifugation (20,817 g; 5 minutes at 4°C), 35 µL of the supernatant was transferred to an HPLC vial containing a 200-µL glass interest. An aliquot (5 µL) of 3% sodium azide (wt/vol) was then added to each sample, followed by 10 µL of 0.5 mM DB in 200 mM HCl containing 0.5 mM diethylenetriaminepentaacetic acid (DETAPAC) in water. The samples were then incubated for 4 hours at room temperature,

protected from the light. Samples were then analyzed by LC-MS/MS using an ACQUITY ultra-high-performance liquid chromatography system with a Xevo-TQS LC-MS/MS mass spectrometer (Waters). The columns were a Waters BEH C18 (100 × 2.1 mm) and a guard column (5 × 2.1 mm). The mobile phase was 0.1% formic acid in water with a linear gradient of 0% to 100% 0.1% formic acid in 50% acetonitrile/water over 0 to 10 minutes; the flow rate was 0.2 mL/min and the column temperature was 5°C. The capillary voltage was 0.5 kV, the cone voltage 20 V, the interscan delay time 100 ms, the source and desolvation gas temperatures 150°C and 350°C, respectively, and the cone gas and desolvation gas flows were 150 and 800 L/h, respectively. Mass transitions (parent ion → fragment ion; collision energy), retention time, the limit of detection, and recoveries were as follows: 145.0 → 77.1, 24eV, 5.93 minutes, 0.52 pmol, and 98%.

Determination of activity of Akr, ALDH and Glo1 enzymes

Akr activity was determined spectrophotometrically by measuring the rate of reduction of NADPH at 340 nm at pH 7.0 at 25°C. The assay mixture contained potassium phosphate (100 mM), methylglyoxal (MG, 0.1–2 mM) and NADPH (0.1 mM). One unit of enzyme activity is defined as the amount of the enzyme required to oxidize 1 μmol of NADPH/min. Glo1 activity measurement was based upon the formation of S-D-lactoylglutathione from the hemithioacetal substrate, prepared from the pre-incubation of sodium phosphate (50 mM, pH 6.6), methylglyoxal (2 mM) and reduced GSH (2 mM), and was determined spectrophotometrically by measuring the increase in absorbance at 235 nm at pH 6.6 at 25°C. ALDH activity was assayed at 25°C in 75 mM Tris-HCl (pH 7.6) containing BSA (10 mM), NADP (0.5 mM), and MG (0.1–2 mM).

Microscopy and analysis of pronephric alteration

For *in vivo* imaging of pronephric structures of embryos, 48 hpf *Tg(wt1b:EGFP)* embryos were anesthetized with tricaine (0.003%) and mounted in low melting point agarose (Promega, 1 %) dorsally. Images were taken by Leica DFC420 C camera, attached to a Leica MZ10 F modular stereo microscope. Alterations of the pronephros were quantified by measuring the size of glomerular length, width, and neck length using Leica LAS V4.8 software.

Determination of pronephros function

After 72 hpf, Texas-Red® tagged 70 kDa dextran (3 nl, 2 mg/ml in PBS) was injected into the sinus venous of larvae. Images of living fish were taken sequentially at approximately 1, 24, and 48 hours post-injection (hpi) using an inverted microscope (Leica DMI 6000B) with a camera

(Leica DFC420 C) and the Leica LAS application suite 3.8 software. NIH's ImageJ application was used to measure maximum fluorescence density in the heart area. The fluorescence values at 24 hpi and 48 hpi were divided by the fluorescence values of 1hpi; respectively, for each fish, the result was shown as a ratio of the fluorescence values[(Sharma et al., 2016)].

Analysis of kidney and liver morphology

For organ preparations, adult zebrafish were euthanized with tricaine (250 mg/L) until the operculum movement stopped entirely. The fish were decapitated behind the operculum then transferred into pre-cooled 1 × PBS. Livers and kidneys, which remained in the body, were fixed in 10 % buffered formalin, removed, routinely embedded in paraffin, and cut into 4 µm-thick sections for hematoxylin and eosin staining and for Periodic acid-Schiff reaction, partly in combination with diastase. All stainings were carried out according to standard protocols. Kidneys for EM study were fixed in 2 % glutaraldehyde/0.01 M Na-cacodylate buffer and further processed according to protocols of the Electron Microscopy Core Facility of Heidelberg University. For quantification of PAS-positive hyaline droplets on EM sections we have analyzed up to 20 images per genotype according to the following criteria: 0 = absent (a few small droplets in single tubulus sections were ignored and scored as "zero"), + = small and ++ = medium.

Detection of S-Nitrosylation

S-Nitrosylation was detected by using the Biotin Switch Assay Kit (Abcam, ab236207), with a modification of the "Biotin-Switch" method[(Jaffrey and Snyder, 2001)], according to the manufacturer's instructions to measure S-nitrosylated (S-NO) proteins in larval and adult lysates. Inhibition of S-Nitrosylation was achieved by L-NAME treatment (N5751-1G; Sigma-Aldrich).

Measurement of adult zebrafish blood glucose

Adult zebrafish were transferred to single boxes the day before. The next day, the fish were either directly euthanized for blood sugar measurements under overnight fasting conditions or fed with flake food (0.5 g) for postprandial conditions. After feeding, the zebrafish were euthanized in tricaine (250 mg/L), and blood glucose was measured[(Wiggenhauser et al., 2020)].

Metabolite analysis by GC/MS and UPLC-FLR

Detection was done in cooperation with the Metabolomics Core Technology Platform from the Centre of Organismal Studies Heidelberg. At 96 hpf, zebrafish larvae were anesthetized with tricaine (0.003%), collected, and snap-frozen in liquid nitrogen. Metabolites were

determined via semi-targeted gas chromatography-mass spectrometry (GC/MS) analysis and ultra-performance liquid chromatography with fluorescence detection (UPLC-FLR) analysis (67). Frozen, ground sample material from zebrafish was extracted in 100% MeOH (360 µL) for 15 minutes at 70°C with vigorous shaking. As internal standards, ribitol (20 µL, 0.2 mg/mL) and heptadecanoic acid (10 µL, 0.2 mg/mL) were added to each sample. After the addition of chloroform (200 µL), samples were shaken at 37°C for 5 minutes. To separate polar and organic phases, water (400 µL) was added, and samples were centrifuged for 10 min at 11,000 × g. For the derivatization, the polar (upper, 700 µL) phase was transferred to a fresh tube and dried in a speed-vac (vacuum concentrator) without heating. Pellets of the aqueous phase after extraction were re-dissolved in methoximation (20 µL) reagent containing methoxyamine hydrochloride (20 mg/mL, Sigma-Aldrich 226904) in pyridine (Sigma-Aldrich 270970) and incubated for 2 hours at 37°C with shaking. For silylation, N-methyl-N-(trimethylsilyl) trifluoroacetamide (MSTFA, 32.2 µL, Sigma M7891) and Alkane Standard Mixture (2.8 µL, 50 mg/mL C10–C40; Fluka 68281) were added to each sample. After incubation for 30 min at 37°C, samples were transferred to glass vials for GC/MS analysis. To analyze total fatty acids, the lower organic phase (chloroform, 150 µL) after extraction were transferred to a fresh reaction tube (1.5 mL) and dried in a speed-vac without heating. For transmethylation reactions, pellets were re-dissolved in TBME (tert-butyl methyl ether, 40 µL, Sigma-Aldrich) and TMSH (trimethylsulfoniumhydroxid, 20 µL, Sigma-Aldrich), incubated for 45 minutes at 50°C, and transferred to glass vials for GC/MS analysis of the fatty acid methyl esters (FAMEs). A GC/MS-QP2010 Plus (Shimadzu) fitted with a Zebron ZB 5MS column (Phenomenex; 30 meter × 0.25 mm × 0.25 µm) was used for GC/MS analysis. The GC was operated with an injection temperature of 230°C and a 1 µL sample was injected with split mode (1:10). The GC temperature program started with a 1 min hold at 40°C followed by a 6°C/min ramp to 210°C, a 20°C/min ramp to 330°C, and a bake-out for 5 min at 330°C using Helium as carrier gas with constant linear velocity. The MS was operated with ion source and interface temperatures of 250°C, a solvent cut time of 7 minutes, and a scan range (m/z) of 40 to 700 with an event time of 0.2 s. The “GCMS solution” software (Shimadzu) was used for data processing.

Incubation experiments with glutamate and alanine

Fertilized eggs were incubated at 28.5°C in 6 well plates with 5 ml solutions that was changed daily. The solution contained egg water, glutamate (0.1 and 1 mM), alanine (1 and 5 mM) and PTU (0.003%). Embryos were raised until 48 hpf and analyzed for pronephric structure.

Real time quantitative PCR analysis

Expression of *akr1a1b* gene and cytosolic phosphoenolpyruvate carboxykinase (cPEPCK) in embryos/larvae and in different organs of adult zebrafish were analyzed by RT-qPCR technology. All organs were dissected from adult zebrafish under either over-night fasting or postprandial conditions. Total RNA was isolated using the RNeasyMini-Kit (QiagenTM, 74104) following the manufacturer's protocol. First-strand cDNA was synthesized from the total RNA using the cDNA Synthesis Kit (ThermoFisher Scientific, K1671). *Akr1a1b* gene expression in embryos/larvae was normalized to *β-actin*, and cPEPCK expression was normalized to *Beta-2 microglobulin* (*b2m*). All primers for RT-qPCR are shown in table 1.

Measurement of glutamate and alanine in adult zebrafish kidneys and livers

Glutamate and alanine concentration was measured using the Glutamate Assay Kit (MAK004, Sigma-Aldrich) and Alanine Assay Kit (MAK001, Sigma-Aldrich) according to the manufacturer's instructions, and represented as ng/mg tissue.

Statistics

All data are presented as mean ± SD. Statistical significance between two groups was analyzed using two-tailed Student's *t*-test using GraphPad Prism, For comparisons among more than two groups, one-way or two-way ANOVA followed by appropriate multiple comparison tests was used. *P* value less than 0.05 was considered significant.

References for Supplements

Jaffrey, S.R., and Snyder, S.H. (2001). The biotin switch method for the detection of S-nitrosylated proteins. Sci STKE *2001*, pl1.

Kimmel, C.B., Ballard, W.W., Kimmel, S.R., Ullmann, B., and Schilling, T.F. (1995). Stages of embryonic development of the zebrafish. Dev Dyn *203*, 253-310.

Lawson, N.D., and Weinstein, B.M. (2002). In vivo imaging of embryonic vascular development using transgenic zebrafish. Dev Biol *248*, 307-318.

Perner, B., Englert, C., and Bollig, F. (2007). The Wilms tumor genes wt1a and wt1b control different steps during formation of the zebrafish pronephros. Dev Biol *309*, 87-96.

Sharma, K.R., Heckler, K., Stoll, S.J., Hillebrands, J.L., Kynast, K., Herpel, E., Porubsky, S., Elger, M., Hadaschik, B., Bieback, K.*, et al.* (2016). ELMO1 protects renal structure and ultrafiltration in kidney development and under diabetic conditions. Sci Rep *6*, 37172.

Wiggenhauser, L.M., Qi, H., Stoll, S.J., Metzger, L., Bennewitz, K., Poschet, G., Krenning, G., Hillebrands, J.L., Hammes, H.P., and Kroll, J. (2020). Activation of Retinal Angiogenesis in Hyperglycemic pdx1 (-/-) Zebrafish Mutants. Diabetes *69*, 1020-1031.

Zhou, H.L., Zhang, R., Anand, P., Stomberski, C.T., Qian, Z., Hausladen, A., Wang, L., Rhee, E.P., Parikh, S.M., Karumanchi, S.A.*, et al.* (2019). Metabolic reprogramming by the S-nitroso-CoA reductase system protects against kidney injury. Nature *565*, 96-100.