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Supporting Information

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Reduced Acrolein Detoxification in *akr1a1a* Zebrafish Mutants Causes Impaired Insulin Receptor Signaling and Microvascular Alterations

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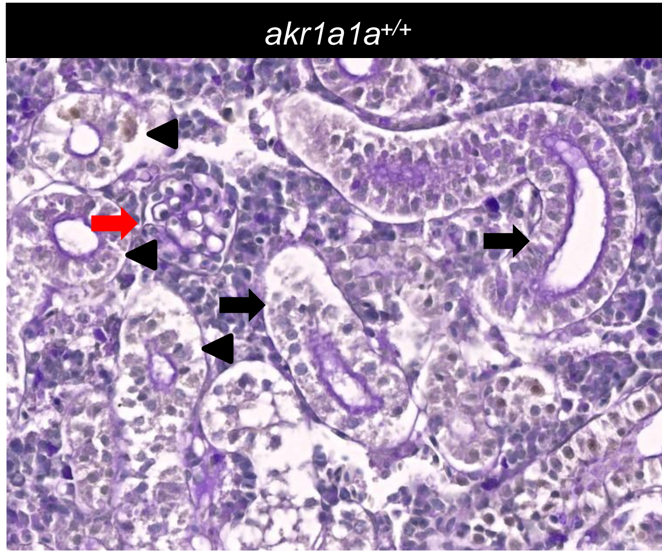
A



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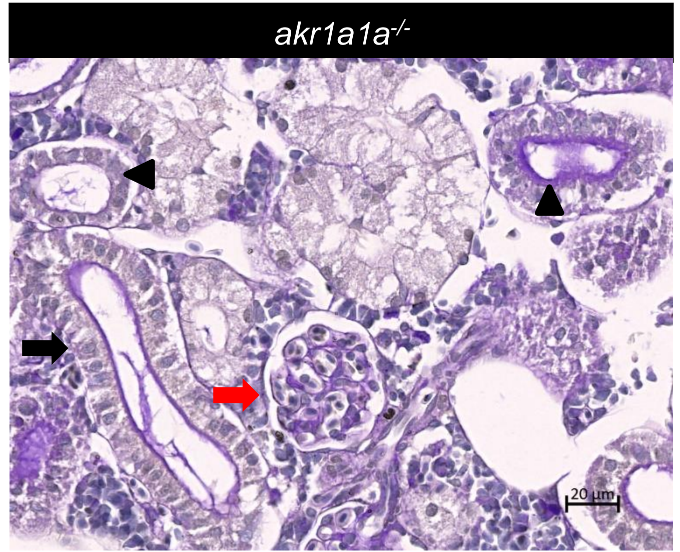


Figure.S1 Unaltered renal morphology in adult *akr1a1a$^{-/-}$* mutants.
(A,B). Representative Periodic acid–Schiff (PAS) staining showed normal gross structure of *akr1a1a$^{+/+}$* *and akr1a1a$^{-/-}$* kidneys. Red arrow: glomeruli. Black arrow: proximal tubule. Black triangle: distal tubule. Black scale bar: 20 μm.

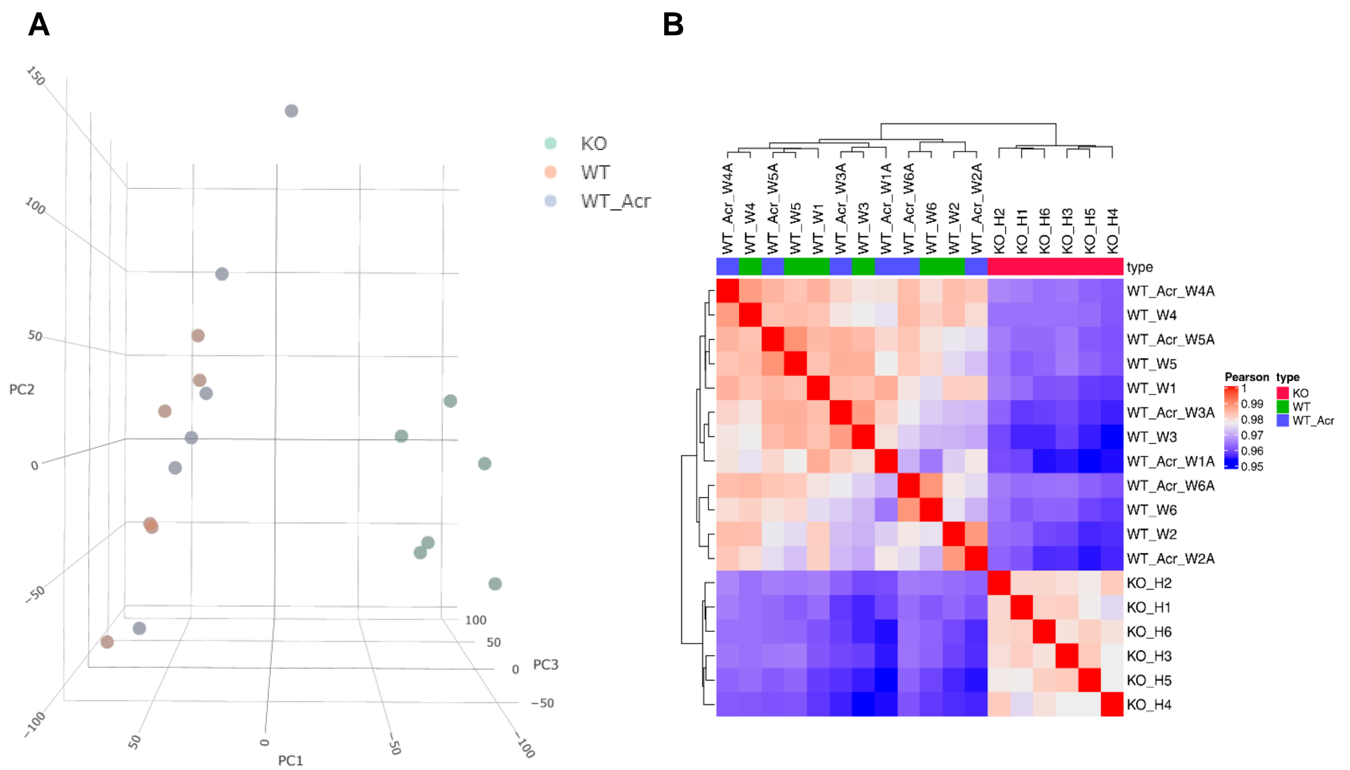


Figure.S2 An overview of RNA Sequencing Results.

(A). Results of the quality control in gene expression analysis between *akr1a1a*$^{+/+}$, *akr1a1a*$^{-/-}$ and *akr1a1a*$^{+/+}$ with ACR treatment zebrafish larvae at 120 hpf. Principal component 1, 2 and 3 are on the axis. The plots showed the *akr1a1a*$^{-/-}$ (n = 6) in green, *akr1a1a*$^{+/+}$ (n = 5) in red and *akr1a1a*$^{+/+}$ with ACR treatment in blue. (B). Heatmaps of each samples showed comparable property between *akr1a1a*$^{-/-}$, *akr1a1a*$^{+/+}$ and *akr1a1a*$^{+/+}$ with ACR treatment zebrafish larvae.

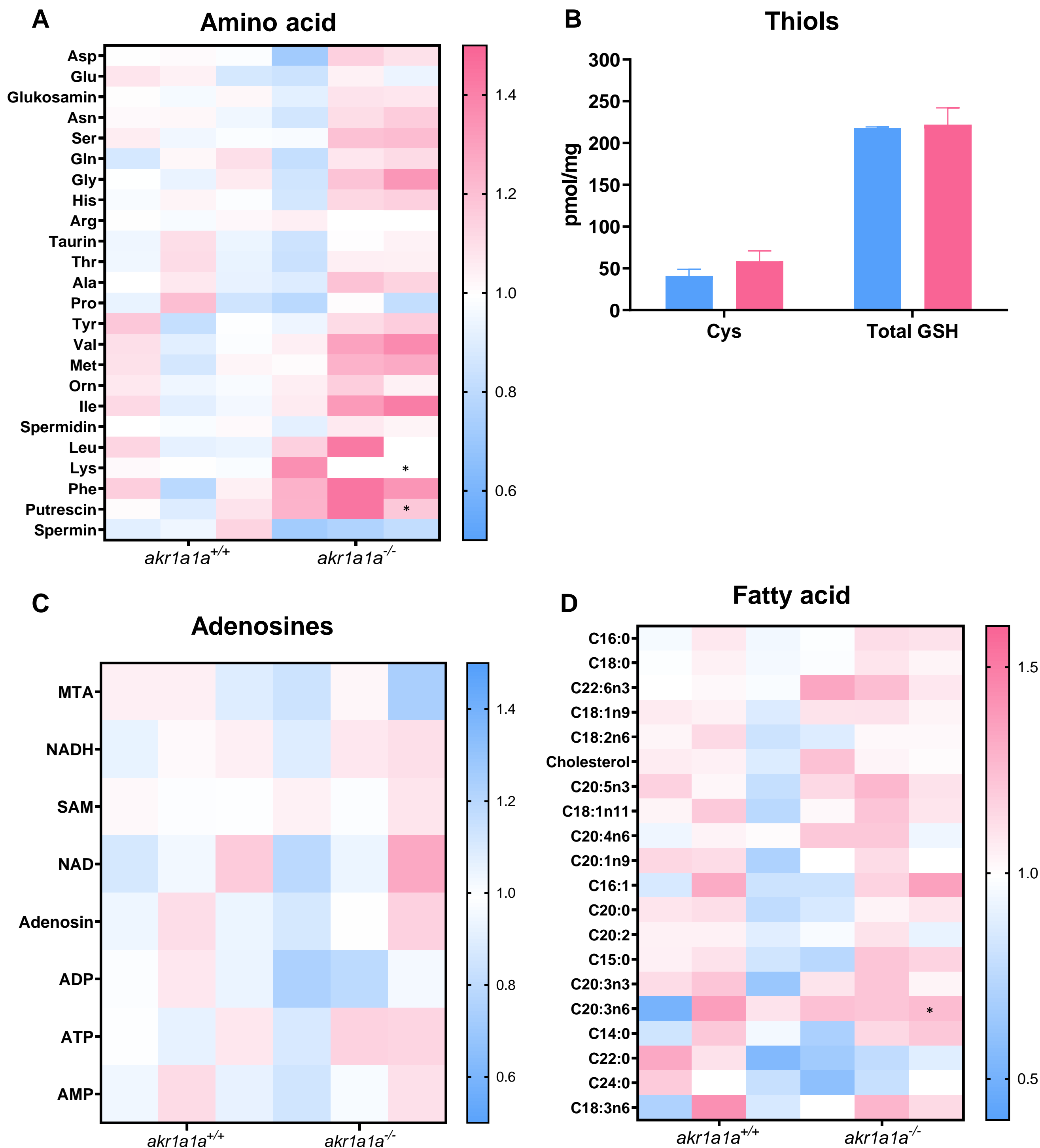
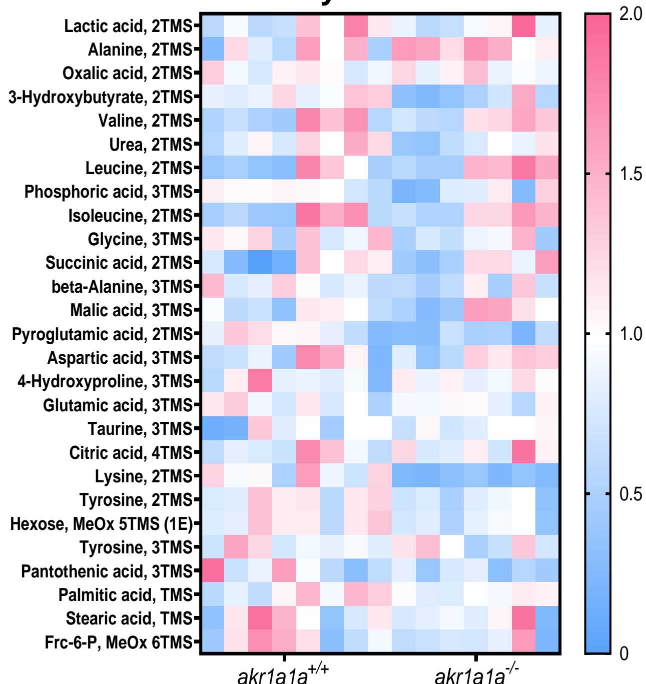


Figure.S3 Metabolomic profiling displayed minor alterations between *akr1a1a*$^{+/+}$ and *akr1a1a*$^{-/-}$ zebrafish larvae at 96 hpf. (A). Lysine and putrescin were significantly increased in *akr1a1a*$^{-/-}$ mutants. Leucine and phenylalanine displayed increasing tendency while spermin displayed a decreasing tendency in *akr1a1a*$^{-/-}$ mutants. n = 3 clutches with 50 larvae. (B). Cysteine and GSH were unaltered in *akr1a1a*$^{-/-}$ mutants, n = 3 clutches with 50 larvae. (C). Adenosines were unaltered in *akr1a1a*$^{-/-}$ mutants , n = 3 clutches with 50 larvae. (D). C20:3n6 was increased in *akr1a1a*$^{-/-}$ mutants, n = 3 clutches with 50 larvae. For statistical analysis Student's t-test was applied; *p<0.05.



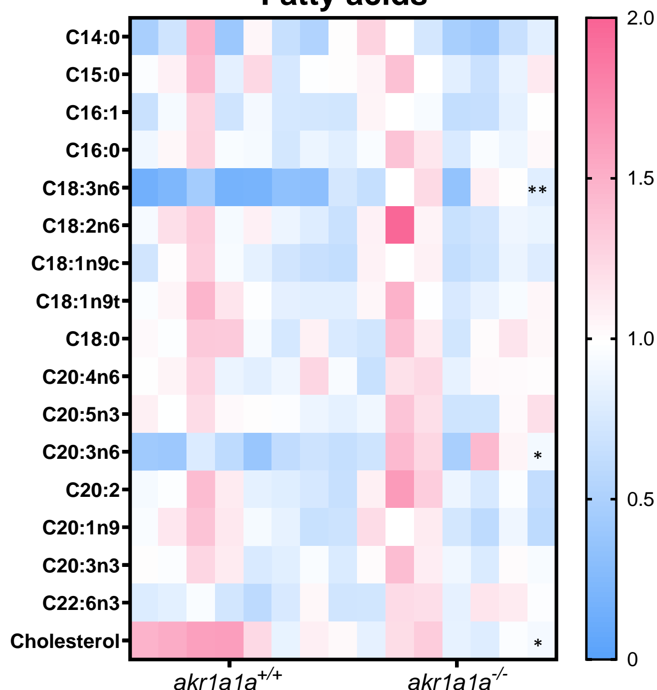


Figure.S4 Metabolomic profiling displayed minor alterations between adult *akr1a1a*$^{+/+}$ and *akr1a1a*$^{-/-}$ zebrafish livers.
(A). Primary metabolites displayed non-significant alterations in *akr1a1a*$^{-/-}$ mutants, n = 7-8. (B). C20:3n6 and C18:3n6 were increased while cholesterol was decreased significantly in *akr1a1a*$^{-/-}$ mutants, n = 7-8. For statistical analysis Student's t-test was applied; *p<0.05. ** p<0.01.

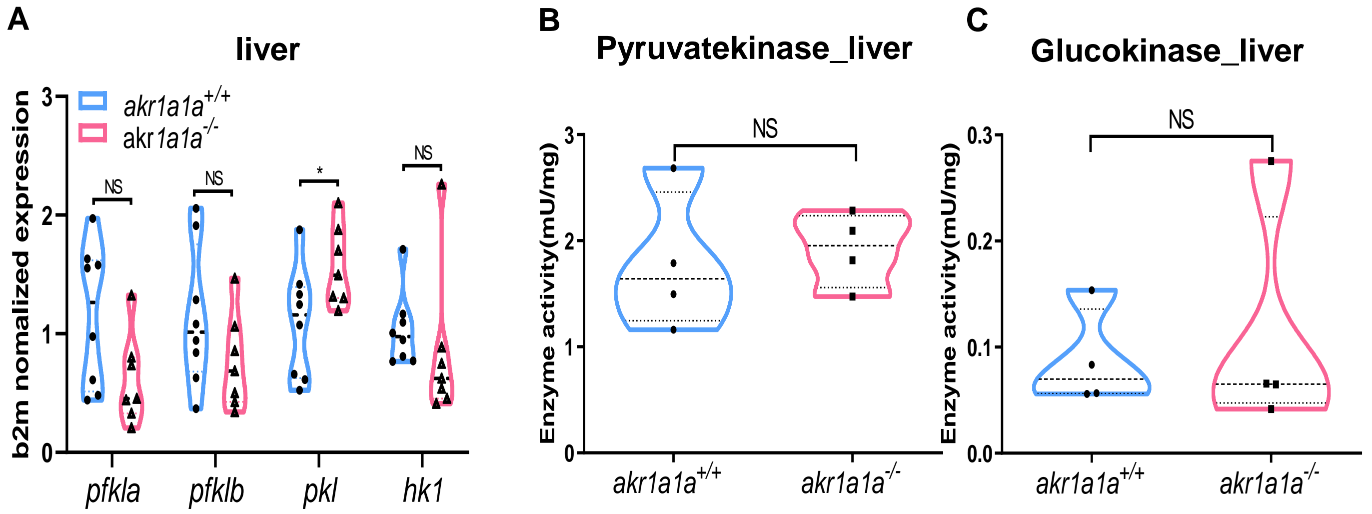


Figure.S5 Glycolytic enzyme expression and activity in adult *akr1a1a$^{-/-}$* livers.
(A). *Pfk* and *hk1* mRNA expression showed decreasing tendency while *pk* expression increased in *akr1a1a$^{-/-}$* livers. n = 6. (B-C). PK and GK enzyme activity were unaltered in *akr1a1a$^{-/-}$* livers. n = 4. For statistical analysis Student's t-test was applied. *p<0.05. NS, not significant. Pfk, Phosphofructokinase. Pk, Pyruvate kinase. Hk, Hexokinase. GK, Glucokinase.

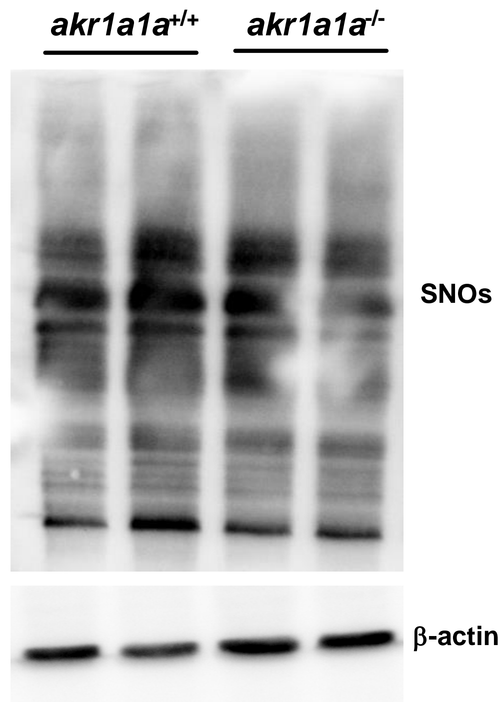


Figure.S6 S-nitrosylated proteins were unaltered in *akr1a1a*$^{-/-}$ livers
Western blot showed unchanged S-nitrosylated proteins (SNOs) in *akr1a1a*$^{-/-}$ livers. β-actin served as loading control.

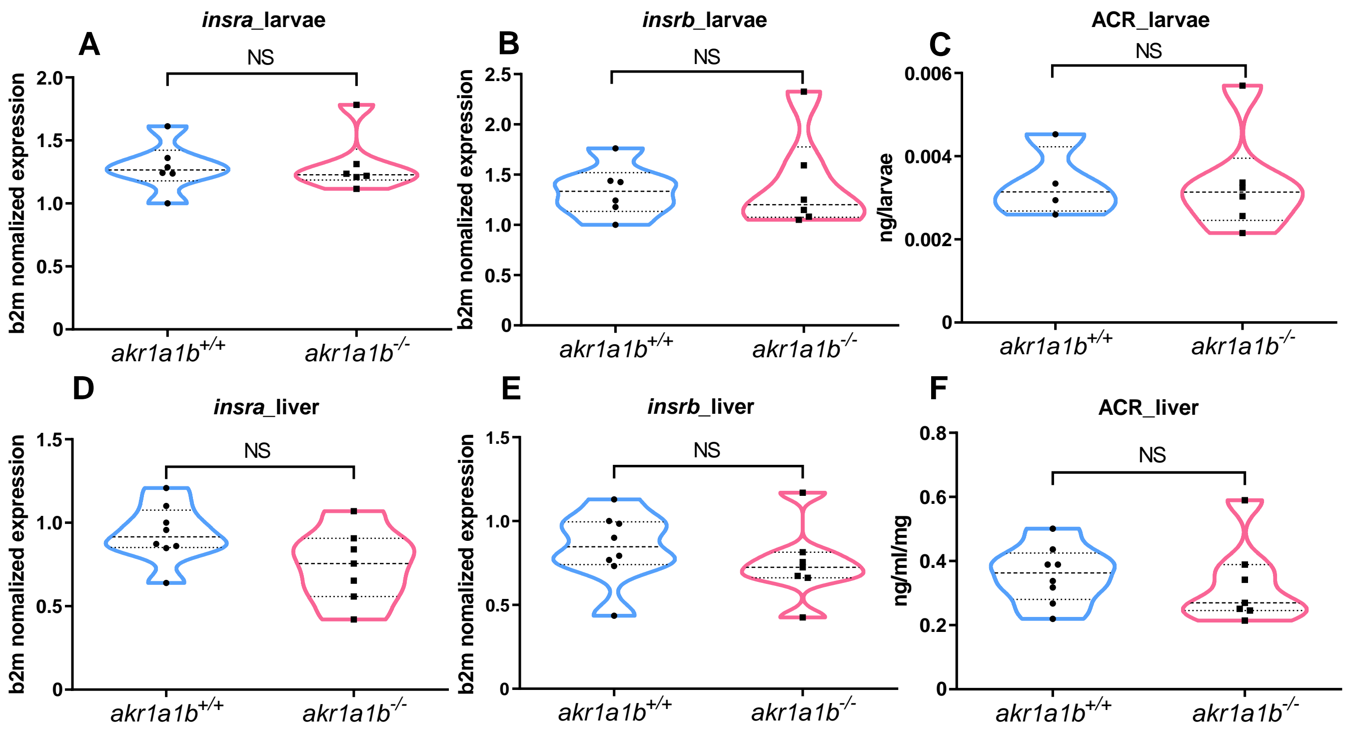


Figure.S7 ***insra/insrb*** **expression and ACR concentrations were unaltered in** ***akr1a1b*** **mutants.**
(A,B). *insra* and *insrb* mRNA expression were unaltered in *akr1a1b*$^{-/-}$ larvae at 5dpf. n = 6 clutches with 30 larvae. (C). ACR was unchanged in *akr1a1b*$^{-/-}$ larvae. n = 4-6 clutches with 50 larvae. (D,E). *insra* and *insrb* mRNA expression were unaltered in *akr1a1b*$^{-/-}$ liver. n = 7-8. (F). ACR was unchanged in *akr1a1b*$^{-/-}$ liver. n = 7-8. For statistical analysis Student's t-test was applied. NS, not significant.

Figure.S8 Hyaloid vasculature is unaltered in *akr1a1b*$^{-/-}$ larvae at 5dpf.
(A). Representative confocal images of hyaloid vasculature. White scale bar: 20 µm. (B,C). Quantification of hyaloid vasculature showed unalterted branches and sprouts in *akr1a1b*$^{-/-}$ larvae at 5dpf. n = 9-10. For statistical analysis Student's t-test was applied. NS, not significant.

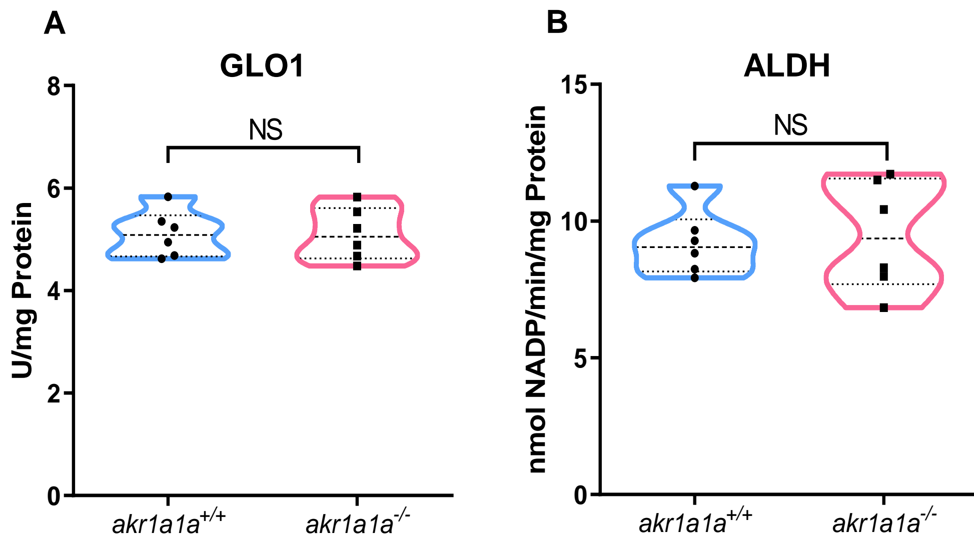
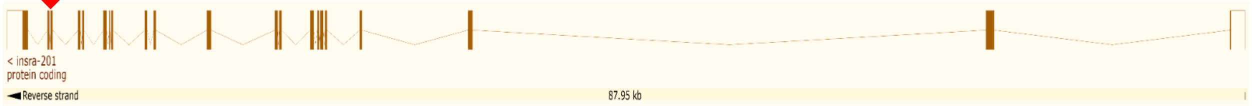


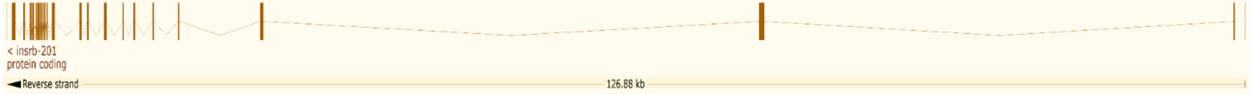
Figure.S9 Glo1 and ALDH enzyme activity were unaltered in *akr1a1a*$^{-/-}$ larvae.
(A,B). Glo1 and ALDH enzyme activities were unaltered in *akr1a1a*$^{-/-}$ larvae. n = 6 clutches with 50 larvae. n = 6 clutches with 50 larvae. For statistical analysis Student's t-test was applied. NS, not significant.

A

SB-*insra*-MO(exon3-intron3):CACACAAGCAGCAGGGTACTTACGT

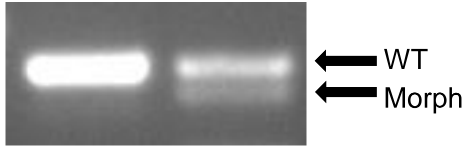


SB-*insrb*-MO(exon7-intron7):ACTGAAAGGACCACACTCACGCTTC



B

Control-MO SB-*insra*-MO



Control-MO SB-*insrb*-MO

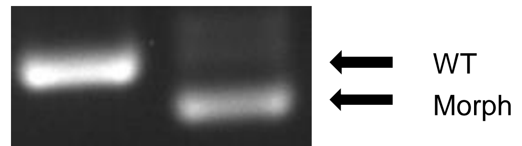


Figure.S10 *Insra/insrb* morpholino design and validation.
(A). SB-*insra*-MO and SB-*insrb*-MO targeting exon3-intron3 and exon7-intron7 junctions of *insra/insrb,* respectively. (B). Validation of splice-blocking morpholinos: SB-*insra*-MO and SB-*insrb*-MO. RT-PCR of Control-MO, SB-*insra*-MO and SB-*insrb*-MO injected larvae showed wild type and generation of morphant *insra/insrb* signals at 24 hpf. 2 ng of morpholinos: Control-MO, SB-*insra*-MO and SB-*insrb*-MO were injected into the one-cell stage of zebrafish embryos, respectively. WT, wild type; MO, morpholino; Morph: morphant.

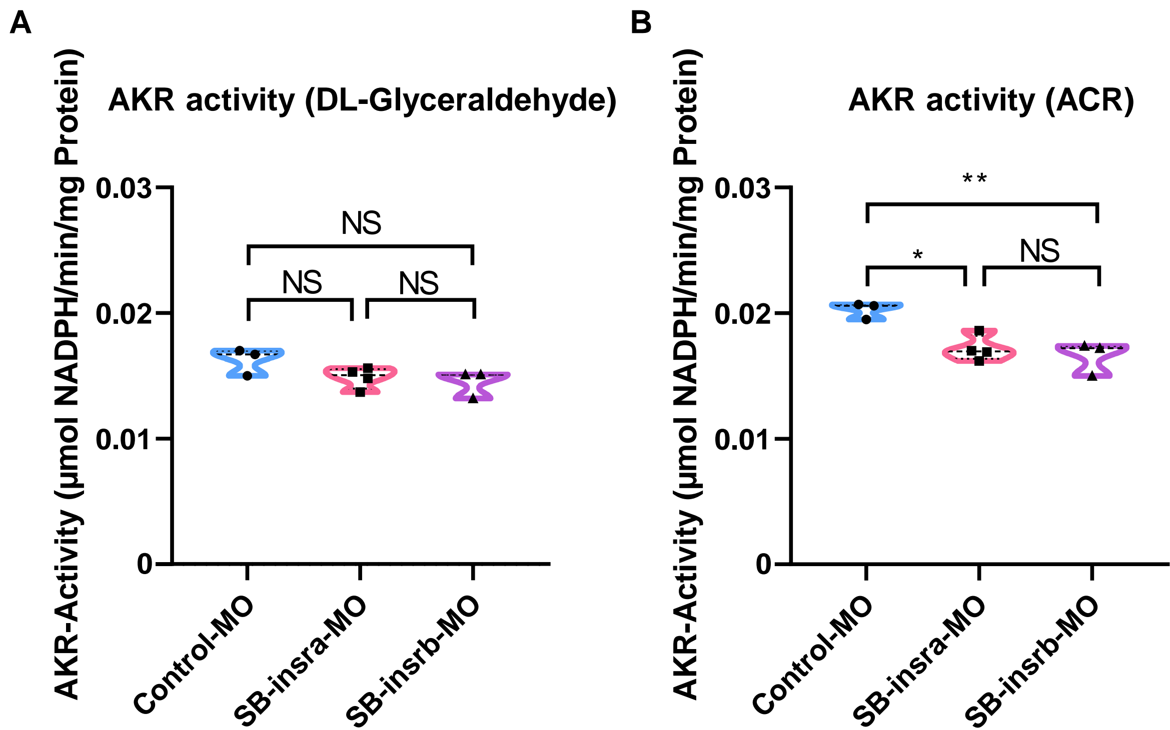
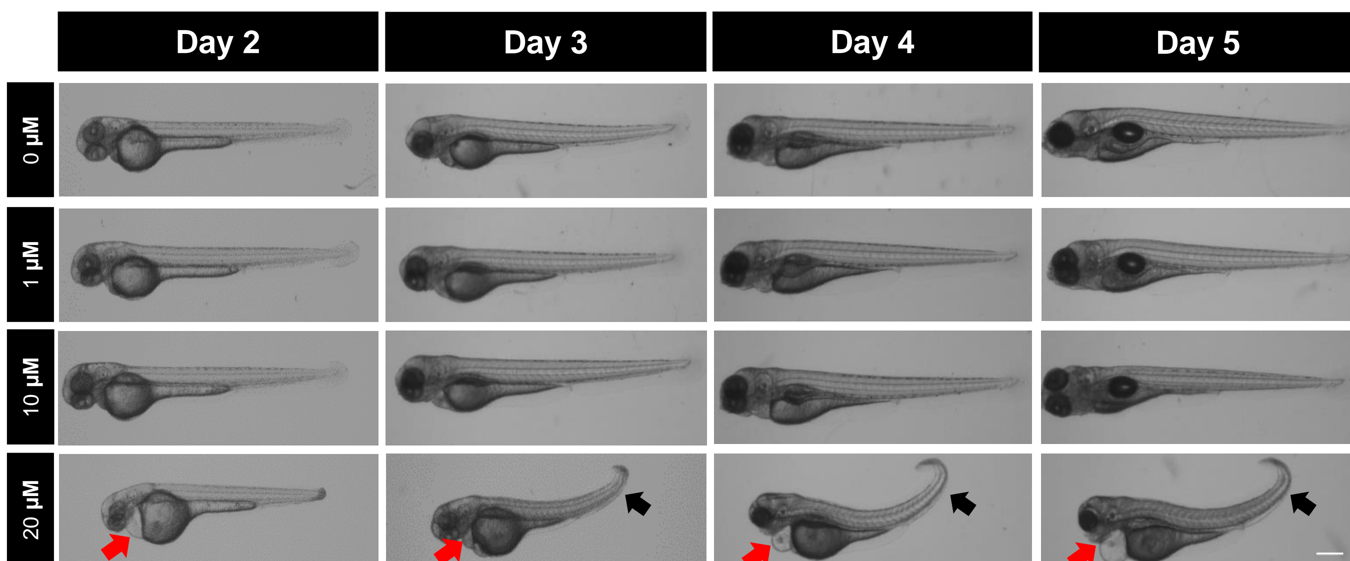
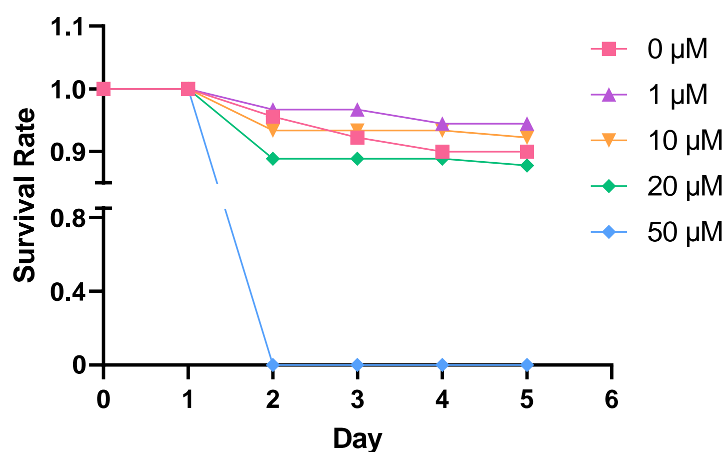


Figure.S11 Reduced AKR activity using ACR as substrate in *insra/insrb* morphants.
(A). *insra/insrb* morphants showed unaltered AKR enzyme activity (DL-Glyceraldehyde served as substrate) measured by spectrophotometric analysis in zebrafish lysates at 96 hpf; n = 3-4 clutches with 50 larvae. (B). *insra/insrb* morphants showed decreased AKR enzyme activity (ACR served as substrate) measured by spectrophotometric analysis in zebrafish lysates at 96 hpf; n = 3-4 clutches with 50 larvae. For statistical analysis one-way ANOVA followed by Tukey's multiple comparison test was applied, *$p<0.05$. **$p<0.01$. NS, not significant.





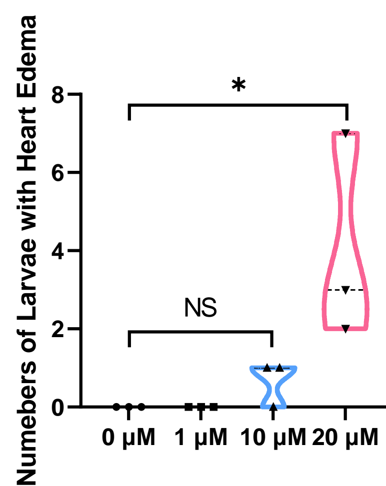
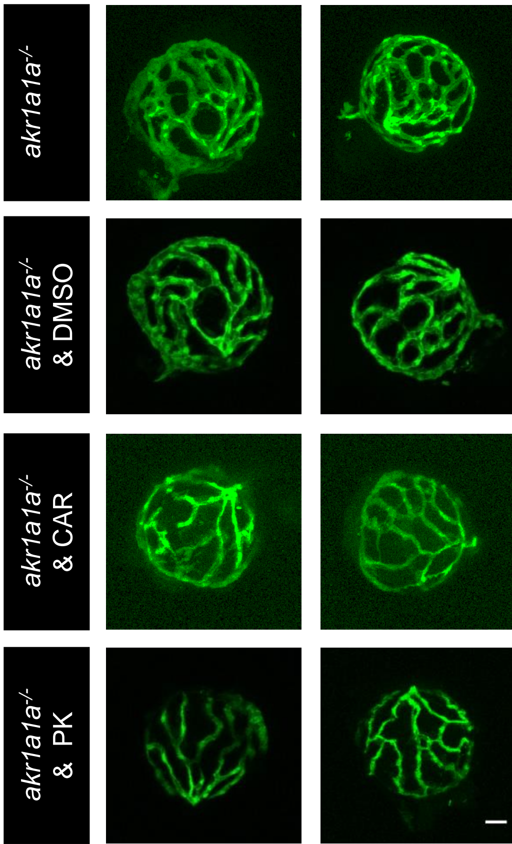
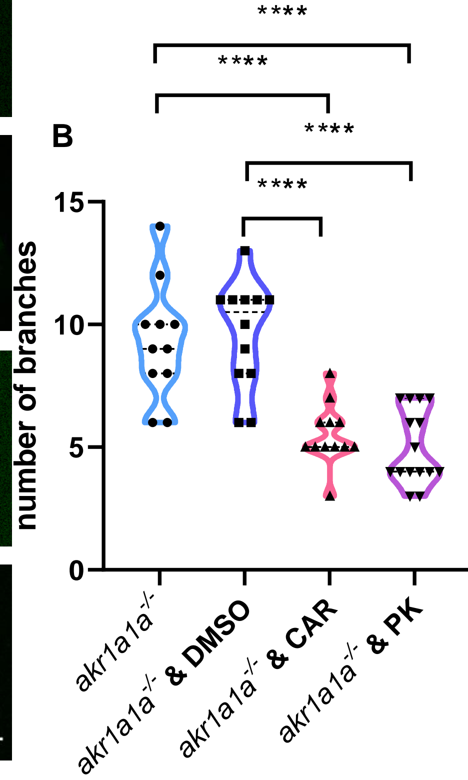


Figure.S12 High concentrations of ACR harm development of zebrafish larvae.
(A). Representative microscopic images showed destructive effects of 20μM on wildtype zebrafish larvae. Red arrow: heart edema. Black arrow: curly tail. Scale bar: 200 μm. (B). Survival rate showed strong lethality of 50μM ACR. (C). The number of larvae with heart edema increased significantly with 20μM ACR treatment. For statistical analysis one-way ANOVA followed by Tukey's multiple comparisons test was applied. *$p < 0.05$. NS, not significant.





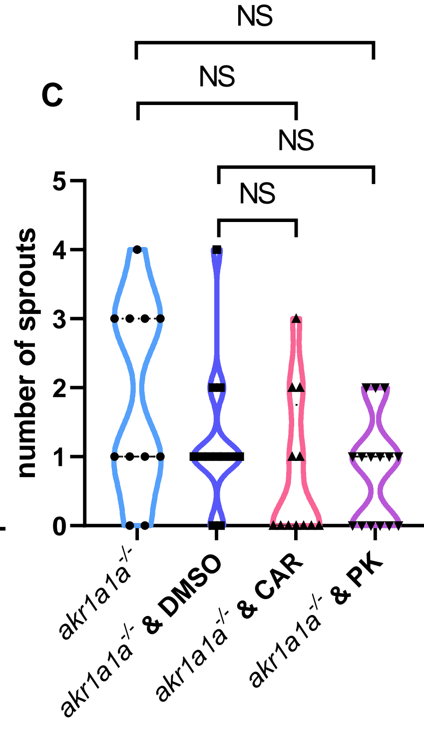


Figure.S13 Carnosine and PK11195 can alleviate the angiogenic retinal hyaloid vasculature in *akr1a1a$^{-/-}$* larvae at 5dpf.
(A). Representative confocal images of hyaloid vasculature. White scale bar: 20 μm. (B,C). Quantification of hyaloid vasculature showed Carnosine (dissolved in egg water) and PK11195 (dissolved in DMSO) can reverse the increasing numbers of branches in *akr1a1b$^{-/-}$* larvae at 5dpf. n = 11-15. For statistical analysis one-way ANOVA followed by Tukey's multiple comparisons test was applied. ****p<0.0001. NS, not significant. DMSO, dimethylsulfoxid. CAR, carnosine. PK, PK11195.

CRISPR-construct	Oligonucleotide sequence(5‘ to 3‘)
akr1a1a-CRISPR-for	TAGGTCAGAGGATGCCAACGGT
akr1a1a-CRISPR-rev	AAACACCGTTGGCATCCTCTGA
Genotyping primer	**Primer sequence (5‘ to 3‘)**
akr1a1a-Crisp-Genotype-for	TCATTTGGGCAGGAAAACGT
akr1a1a-Crisp-Genotype-rev	GTAGCCACAGTCTAAAGCTGC

Table S1. CRISPR construct and genotyping primers for zebrafish *akr1a1a*.

qPCR primer name	Primer sequence
b2m-qPCR-for	ACTGCTGAAGAACGGACAGG
b2m-qPCR-rev	GCAACGCTCTTTGTGAGGTG
insa-qPCR-for	GGTCGTGTCCAGTGTAAGCA
insa-qPCR-rev	GGAAGGAAACCCAGAAGGGG
Insra-qPCR-for	AGAGGCCAGCGAGCTCTAC
Insra-qPCR-rev	CACTTGTGTGGGGGCTCT
Insrb-qPCR-for	GCCTCTGCGGATCACTACAT
Insrb-qPCR-rev	CTCCTGCGTGGTCTTGAAC
pfkla-qPCR-for	ACTGCCACTCCAGCGTTAAA
pfkla-qPCR-rev	CAGAGCTGGAGTTCACCCTC
pfklb-qPCR-for	GCCGTTCAACATTCACGACC
pfklb-qPCR-rev	TGCAGTCGAACACTCCTTGG
pkl-qPCR-for	TCCTGGAGCATCTGTGTCTG
pkl -qPCR-rev	GTCTGGCGATGTTCATTCCT
hK1-qPCR-for	ATGATAGCGGCACAGCTTCT
hk1-qPCR-rev	GTTGGTGTCTCGTGCCAATC

Table S2. qPCR primers.

Morpholinos	sequence
SB-*insra*-MO	CACACAAGCAGCAGGGTACTTACGT
(exon3-intron3 junction)	
SB-*insrb*-MO	ACTGAAAGGACCACACTCACGCTTC
(exon7-intron7 junction)	
Control-MO	CCTCTTACCTCAGTTACAATTTATA
Genotyping primer	**Primer sequence**
SB-*insra*-MO	GAGCTCCACAACAAGTGCAA
	CTCCAGCTGTCCCAGGTTAG
SB-*insrb*-MO	ACACAAATCCGCACCATGAG
	CAATGGCCCAGCTGTTAGAG

Table S3. Morpholinos and the genotyping primers for zebrafish *insra/insrb* morpholinos.