

Supplementary Material

A Novel AURKA Mutant-Induced Early-Onset Severe Hepatocarcinogenesis Greater than Wild-Type via Activating Different Pathways in Zebrafish

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A

AURKA(WT)

ATG GACCGATCTAAAGAAAACCTGCATTTCCAGGACCTGTTAAGGCTACAGCTCCAGTTGGAGGTCCAAAACGTGTTCTCGTGACTCAG
CAATTTCTTGTGTCAGAATCCATTACCTGTAATAAGTGGCCAGGCTCAGCGGGTCTTGTGTCCTTCAAATTTCTCCAGCGCATTCCCT
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ACCAGAGTCTACCTAATTTCTGGAATATGCACCACTTGGAAACAGTTTATAGAGAACTTCAGAAAACCTTCAAAGTTTGTAGGAGAGAGA
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TATGAATTTTGTAGTTGGGAAGCCTCCTTTTGGAGCAACACATACCAAGAGACCTTACAAAAGAATATCACGGGTTGAATTCACATTC
CCTGACTTTTACAGAGGGAGCCAGGGACCTCATTTCAGACTGTTGAAGCATATCCAGCCAGAGGCCAATGCTCAGAGAAGTA
CTTGAACACCCTGGATCACAGCAAATTCATCAAACCATCAAATTCGCAAAAACAAGAATCAGCTAGCAACAGTCTTAG

AURKA(V352I)

ATG GACCGATCTAAAGAAAACCTGCATTTCCAGGACCTGTTAAGGCTACAGCTCCAGTTGGAGGTCCAAAACGTGTTCTCGTGACTCAG
CAATTTCTTGTGTCAGAATCCATTACCTGTAATAAGTGGCCAGGCTCAGCGGGTCTTGTGTCCTTCAAATTTCTCCAGCGCATTCCCT
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CTTGAACACCCTGGATCACAGCAAATTCATCAAACCATCAAATTCGCAAAAACAAGAATCAGCTAGCAACAGTCTTAG

B

AURKA(WT)

1 MDRSKENCIS GPKVATAPVG GPKRVLVTQQ FPCQNPLPVN SGQAQRLVCP SNSSQRIPLQ
61 AQLVSSHKP VQNQKQKQLQ ATSVPHVSR PLNNTQKSKQ PLPSAPENNP EEELASKQKN
121 EESKRRQWAL EDFEIGRPLG KGKFGNVYLA REKQSKFILA LKVLFAQLE KAGVEHQLR
181 EVEIQSHLRH PNILRLYGYF HDATRVYLIL EYAPLGTVYR ELQKLSKFDE QRTATYITEL
241 ANALSYCHSK RVIHRDIKPE NLLLSAGEL KIADFGWSVH APSSRRTTLC GTLDYLPPEM
301 IEGRMHDEKV DLWSLGVLCY EFLVGKPPFE ANTYQETYKR ISRVEFTFPD FVTEGARDLI
361 SRLLKHNPSQ RPLREVLEH PWITANSSKP SNCQNKESAS KQS*

AURKA(V352I)

1 MDRSKENCIS GPKVATAPVG GPKRVLVTQQ FPCQNPLPVN SGQAQRLVCP SNSSQRIPLQ
61 AQLVSSHKP VQNQKQKQLQ ATSVPHVSR PLNNTQKSKQ PLPSAPENNP EEELASKQKN
121 EESKRRQWAL EDFEIGRPLG KGKFGNVYLA REKQSKFILA LKVLFAQLE KAGVEHQLR
181 EVEIQSHLRH PNILRLYGYF HDATRVYLIL EYAPLGTVYR ELQKLSKFDE QRTATYITEL
241 ANALSYCHSK RVIHRDIKPE NLLLSAGEL KIADFGWSVH APSSRRTTLC GLDYLPPEM
301 IEGRMHDEKV DLWSLGVLCY EFLVGKPPFE ANTYQETYKR ISRVEFTFPD FTEGARDLI
361 SRLLKHNPSQ RPLREVLEH PWITANSSKP SNCQNKESAS KQS*

Figure S1. Sequence of AURKA (WT) and AURKA (V352I). (A) mRNA sequence of AURKA (WT) and AURKA (V352I), the red indicates the mutated nucleotide sequence. (B) Protein sequence of AURKA (WT) and AURKA (V352I), the red box indicates the mutated amino acid sequence.

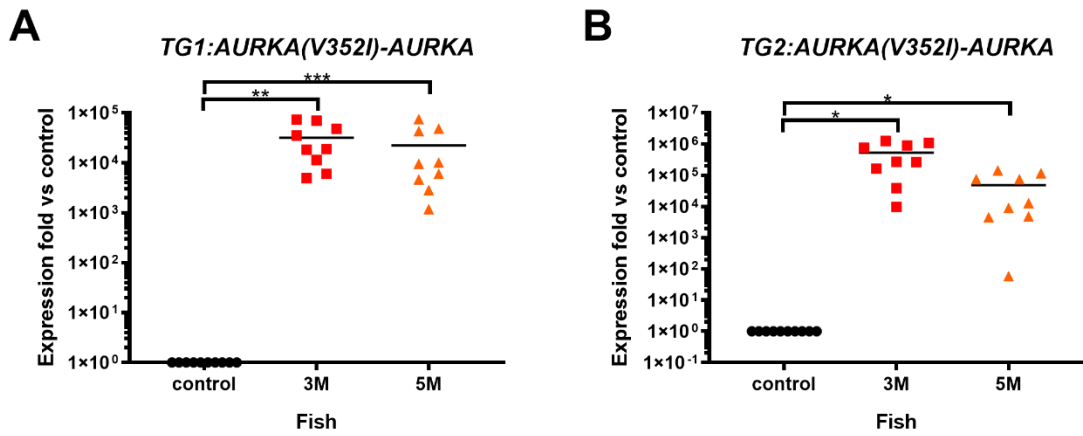


Figure S2. Expression of AURKA in TG1-AURKA(V352I) and TG2-AURKA(V352I) transgenic zebrafish compared with control fish. qPCR analysis of AURKA in TG1-AURKA(V352I) and TG2-AURKA(V352I) transgenic zebrafish compared to control fish at 3 M and 5 M. **(A)** TG1: Tg(fabp10a:AURKA(V352I)-EGFP-mCherry, myl7:EGFP). **(B)** TG2: Tg(fabp10a:AURKA(V352I)-EGFP-mCherry, myl7:EGFP). Expression fold compared to control fish (Tg(fabp10a:EGFP-mCherry)) was shown in red (3M) and orange (5M). Statistical analysis of results was performed using a two-tailed Student's *t*-test. Asterisks (*) represent the level of significance. *: *p*-value ≤ 0.05 ; **: *p*-value ≤ 0.01 ; ***: *p*-value ≤ 0.001 .

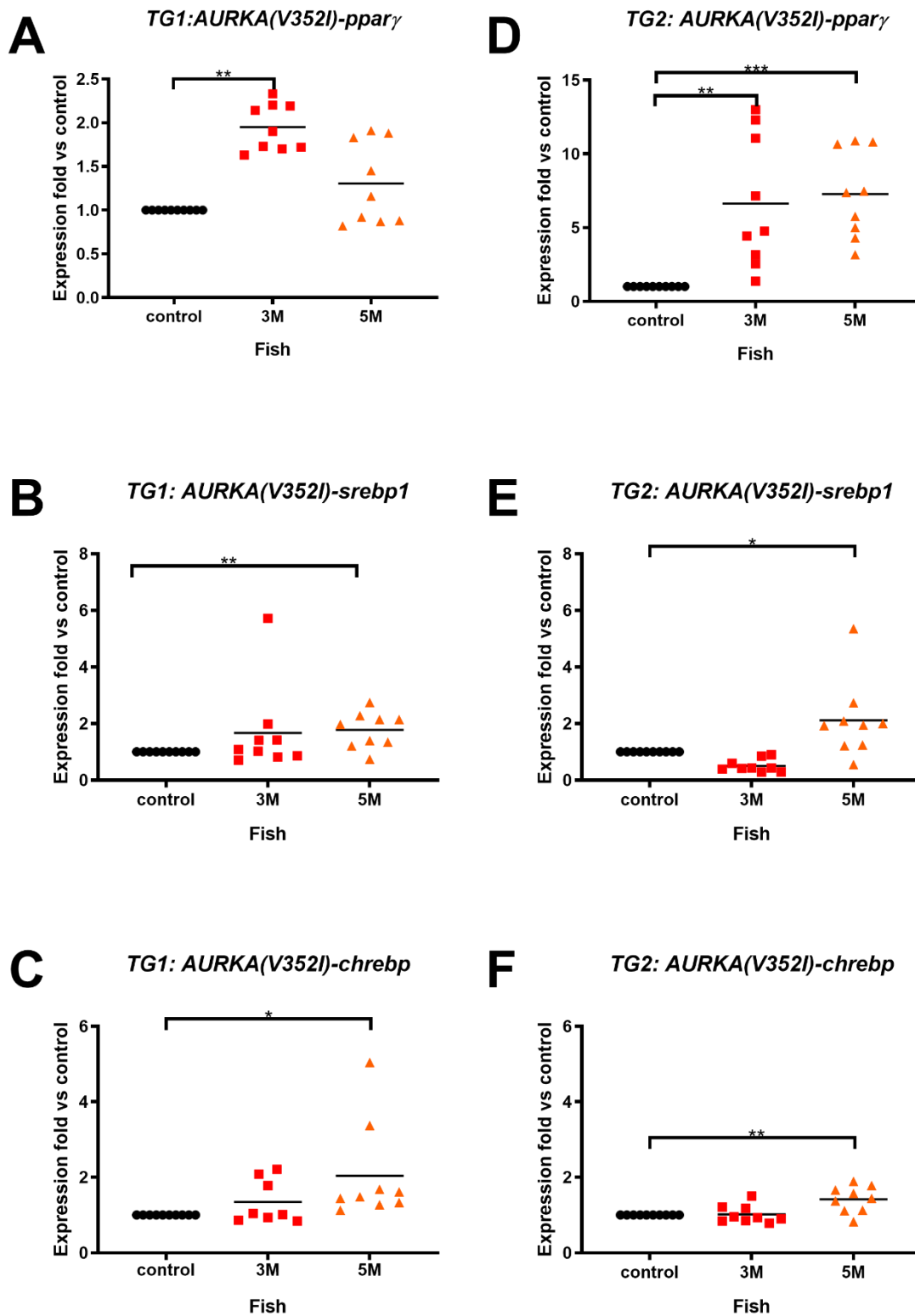


Figure S3. Expression of lipogenic factors (*ppar γ* , *srebp1*, *chrebp*) in TG1-AURKA(V352I) and TG2-AURKA(V352I) transgenic fish were higher than control. qPCR analysis of lipogenic factors (**A**, **D**) *ppar γ* ; (**B**, **E**) *srebp1*; (**C**, **F**) *chrebp* in TG1-AURKA(V352I) and TG2-AURKA(V352I) transgenic zebrafish compared to control fish at different time points. Statistical analysis of results was performed using a two-tailed Student's *t*-test. Asterisks (*) represent the level of significance. *: p -value ≤ 0.05 ; **: p -value ≤ 0.01 ; ***: p -value ≤ 0.001 .

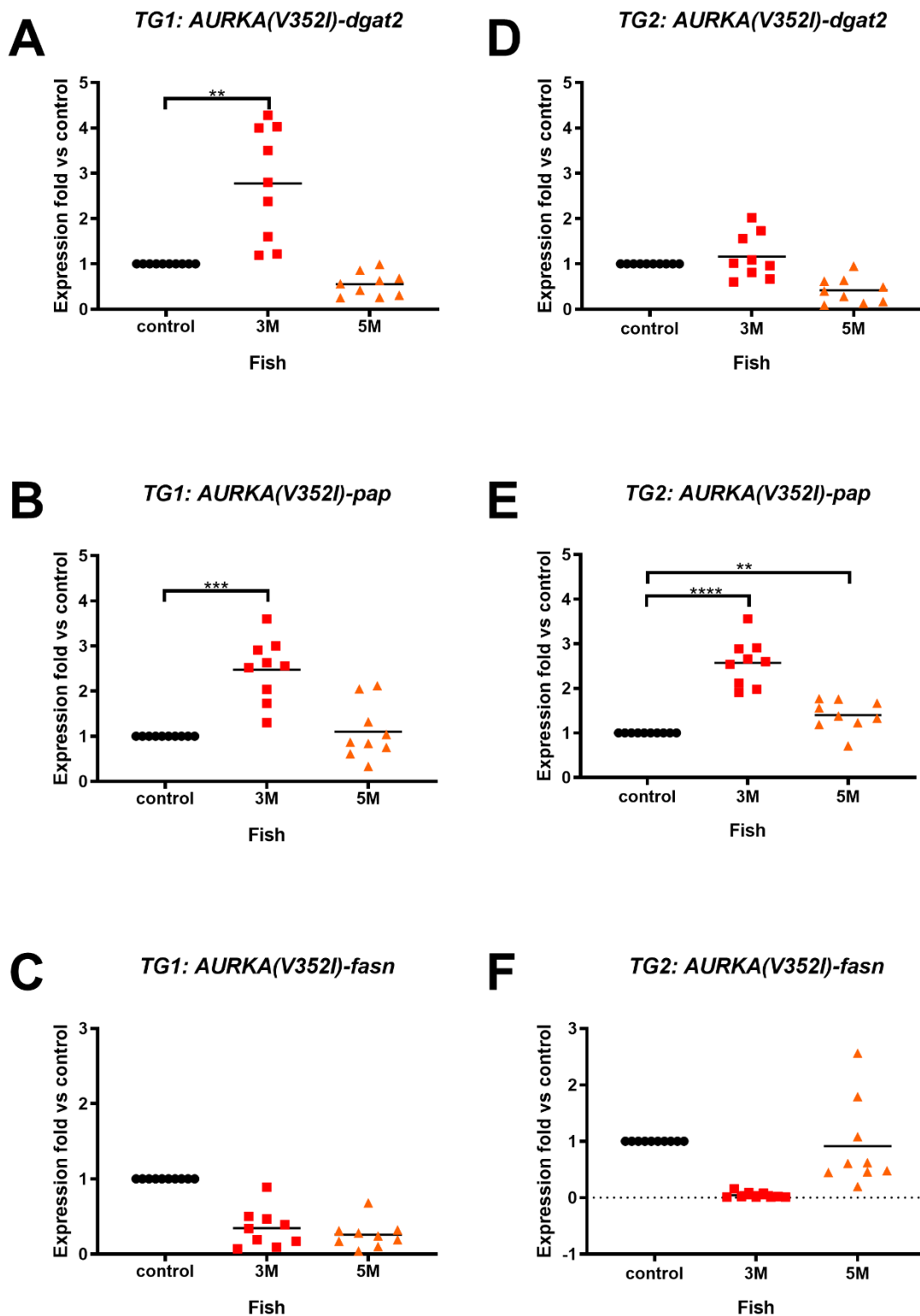


Figure S4. Expression of lipogenic enzymes (*dgat2*, *pap*, *fasn*) in TG1-AURKA (V352I) and TG2-AURKA (V352I) transgenic fish were higher than control. qPCR analysis of lipogenic enzyme (A, D) *dgat2*; (B, E) *pap*; (C, F) *fasn* in TG1-AURKA(V352I) and TG2-AURKA(V352I) transgenic zebrafish compared to control fish at different time points. Statistical analysis of results was performed using a two-tailed Student's *t*-test. Asterisks (*) represent the level of significance. **: *p*-value ≤ 0.01 ; ***: *p*-value ≤ 0.001 .

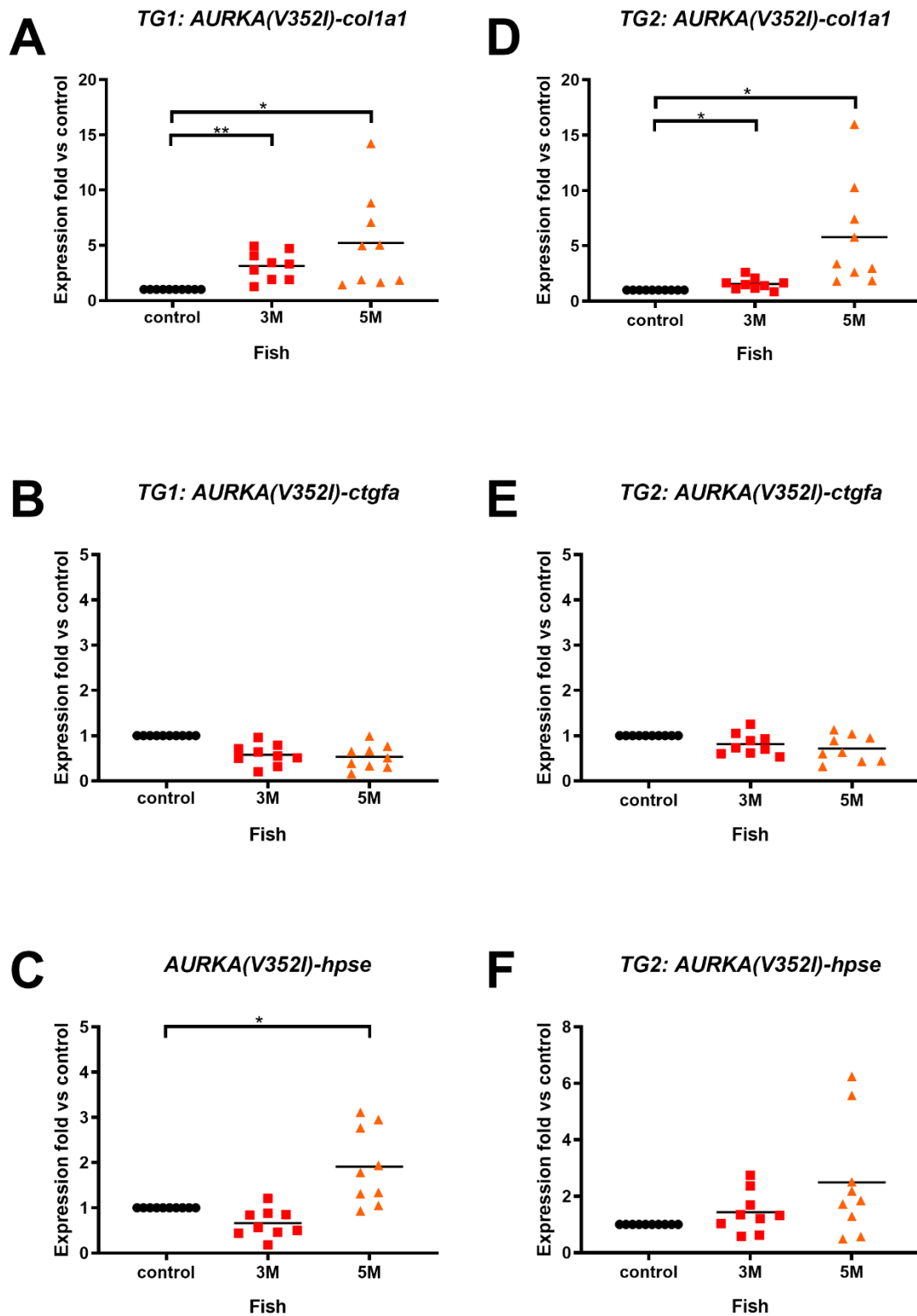


Figure S5. Expression of fibrosis markers (*col1a1*, *ctgfa*, *hpse*) in TG1-AURKA (V352I) and TG2-AURKA (V352I) transgenic fish compared to control. qPCR analysis of fibrosis markers (A, D) *col1a1*; (B, E) *ctgfa*; (C, F) *hpse* in TG1-AURKA(V352I) and TG2-AURKA(V352I) transgenic zebrafish compared to control fish at different time points. Statistical analysis of results was performed using a two-tailed Student's *t*-test. Asterisks (*) represent the level of significance. *: *p*-value ≤ 0.05; **: *p*-value ≤ 0.01.

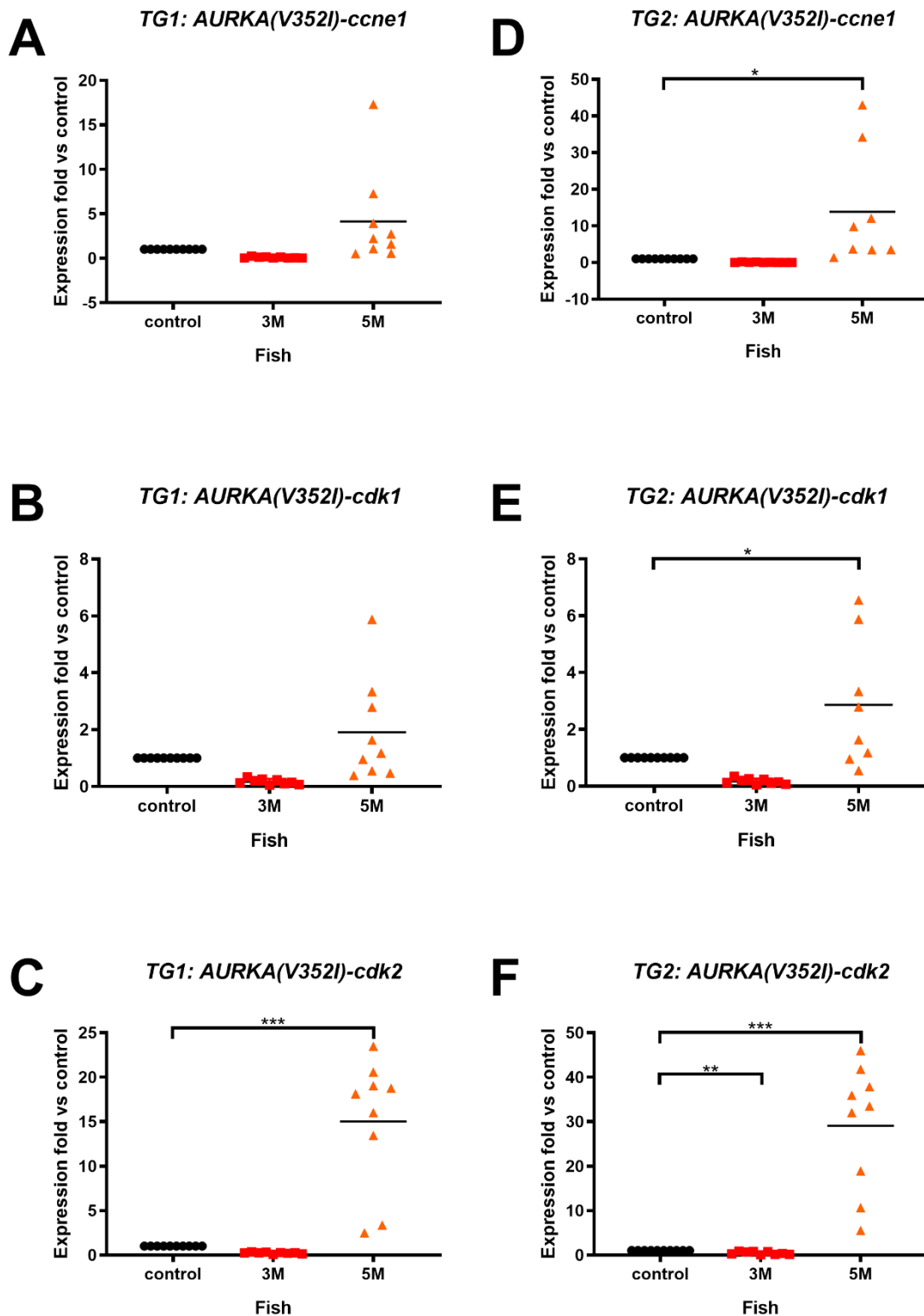


Figure S6. Expression of cell cycle related genes (*ccne1*, *cdk1*, *cdk2*) was higher in TG1-AURKA (V352I) and TG2-AURKA (V352I) transgenic fish. qPCR analysis of cell cycle/proliferation markers (A, D) *ccne1*; (B, E) *cdk1*; (C, F) *cdk2* in TG1-AURKA(V352I) and TG2-AURKA(V352I) transgenic zebrafish compared to control fish at different time points. Statistical analysis of results was performed using a two-tailed Student's *t*-test. Asterisks (*) represent the level of significance. *: p -value ≤ 0.05 ; **: p -value ≤ 0.01 ; ***: p -value ≤ 0.001 .

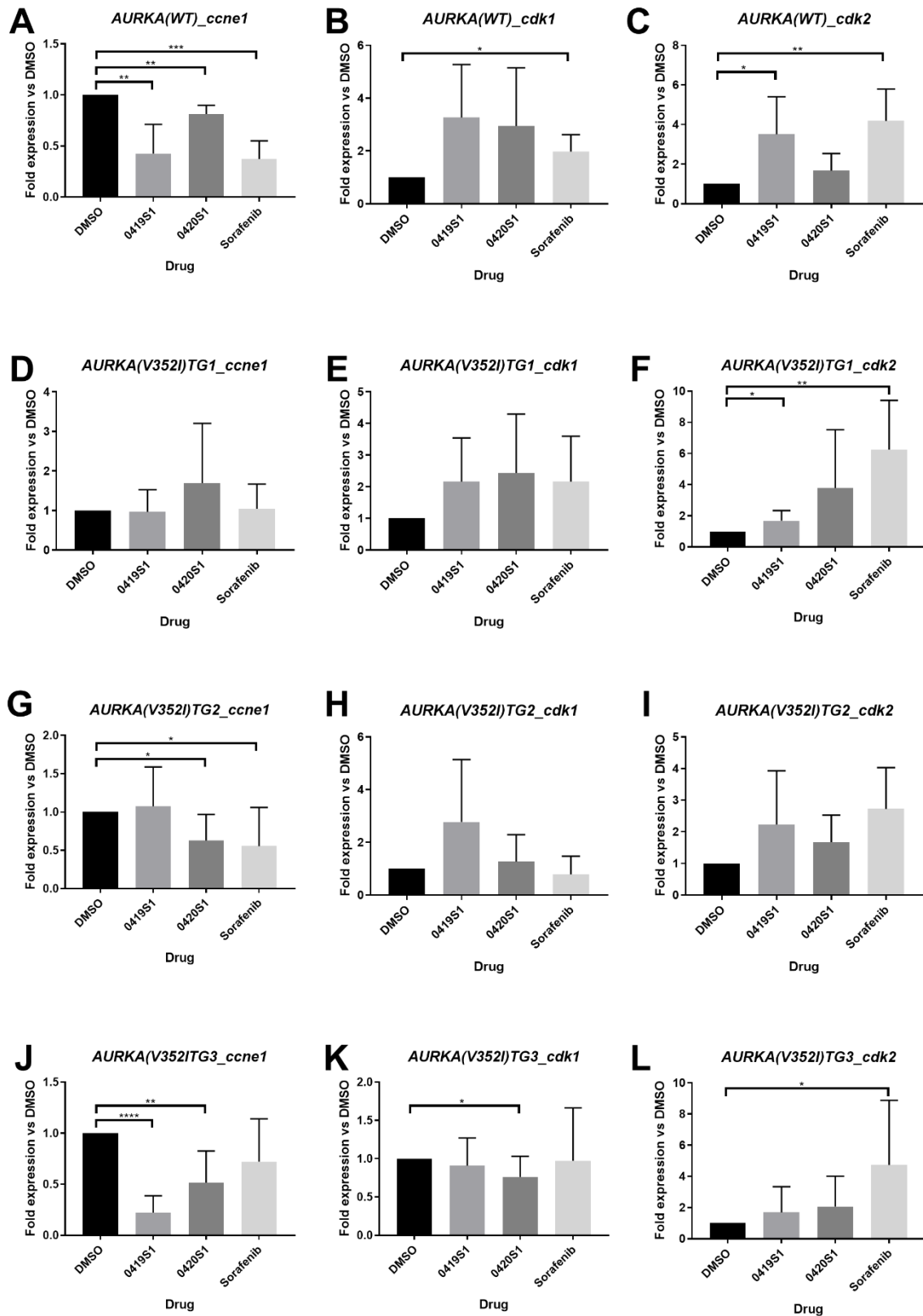


Figure S7. Expression of cell cycle related genes (*ccne1*, *cdk1*, *cdk2*) in AURKA(WT), TG1-AURKA(V352I), TG2-AURKA(V352I), and TG3-AURKA(V352I) transgenic fish after oral feeding of 0419S1, 0420S1 and Sorafenib for one month. qPCR analysis of cell cycle/proliferation markers (**A**, **D**, **G**, **J**) *ccne1*; (**B**, **E**, **H**, **K**) *cdk1*; (**C**, **F**, **I**, **L**) *cdk2* in AURKA (WT), TG1-AURKA(V352I), TG2-AURKA(V352I), and TG3-AURKA (V352I) transgenic zebrafish compared to control fish at different time points. Statistical analysis of results was performed using a two-tailed Student's *t*-test. Asterisks

(*) represent the level of significance. *: p -value ≤ 0.05 ; **: p -value ≤ 0.01 ; ***: p -value ≤ 0.001 ; ****: p -value ≤ 0.0001 .