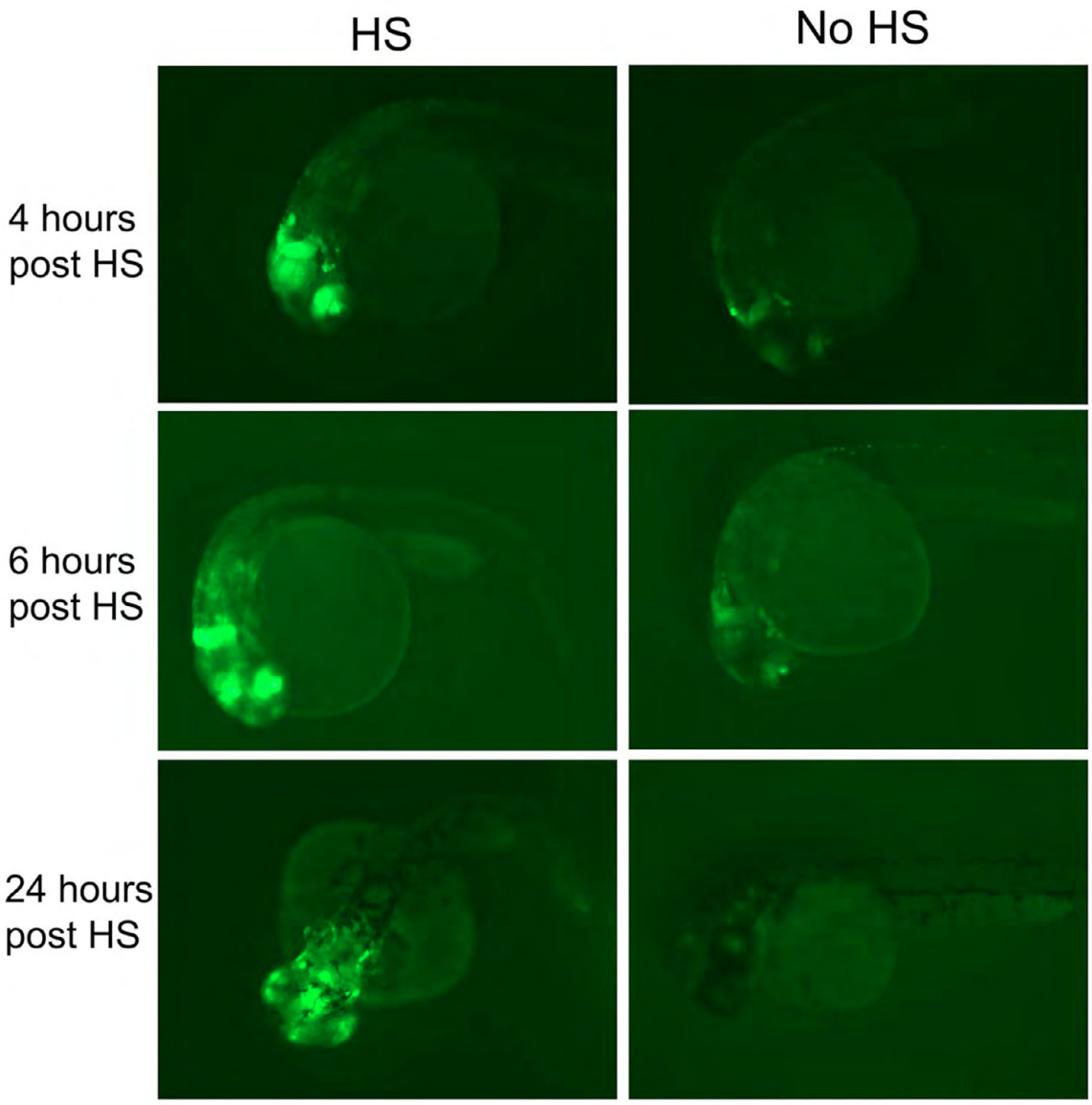

Fig. S1. *dusp6* activation in *Tg(hsp70-HRAS^{G12v}; dusp6-d2EGFP)* embryos with and without heat shock at different time points. Transgenic embryos were kept at 28.5°C until 22 hpf. Some received 37°C heat shock for 1 hour; some were kept as controls. At 4, 6 and 24 hours post-heat shock, EGFP expression was observed under a fluorescence microscope. Representative images for each time point for heat shocked and control are shown.

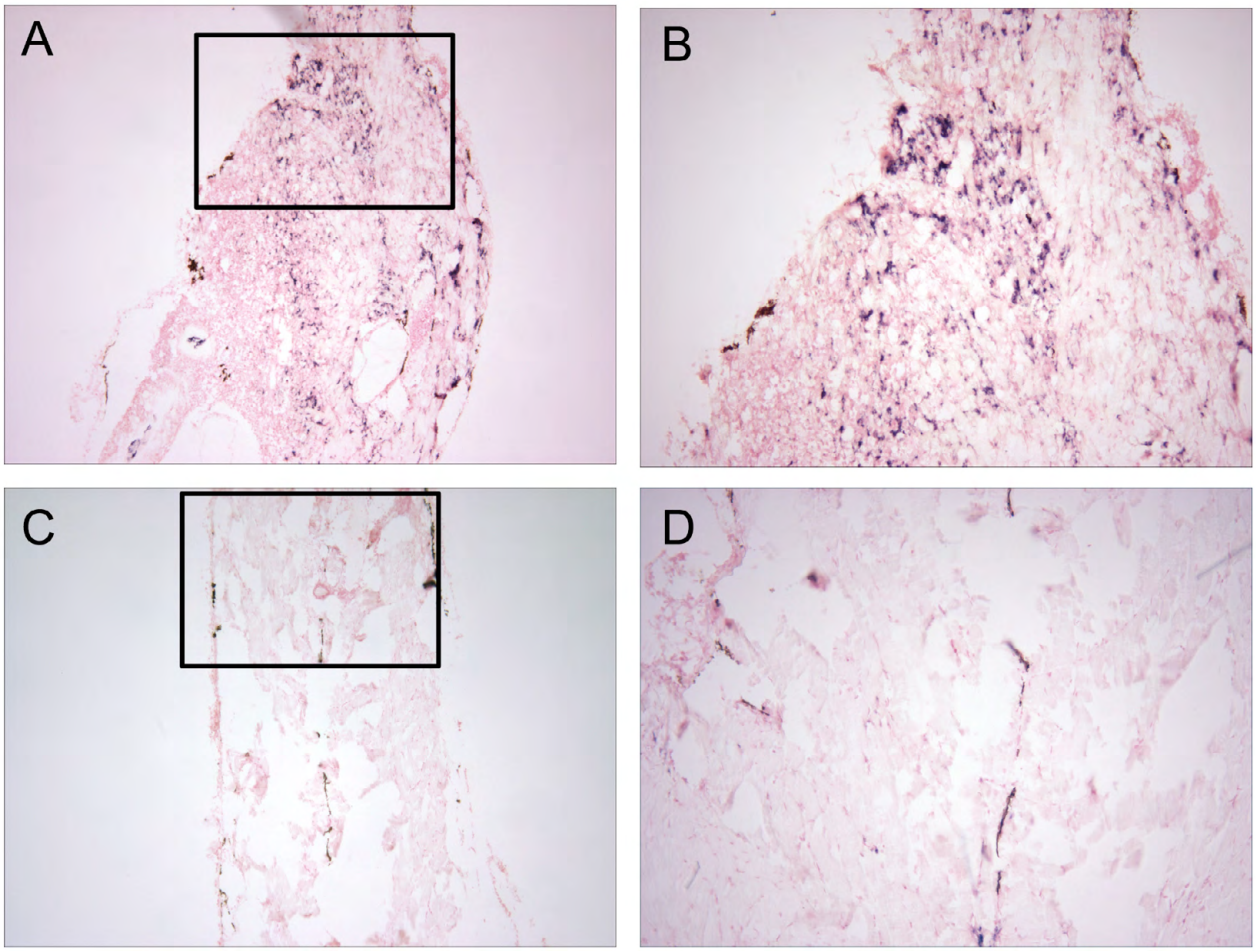

Fig. S2. *dusp6* expression is upregulated in zebrafish rhabdomyosarcoma. *dusp6* expression was assessed by ISH. (**A**,**B**) Sections of zebrafish ERMS. Wild-type zebrafish embryos were injected with *rag2-KRAS*^{G12D} at the one-cell stage to induce rhabdomyosarcoma. (**C**,**D**) Sections of normal musculature. Wild-type siblings without injection as controls. (A,C) 10×, (B,D) 40×.

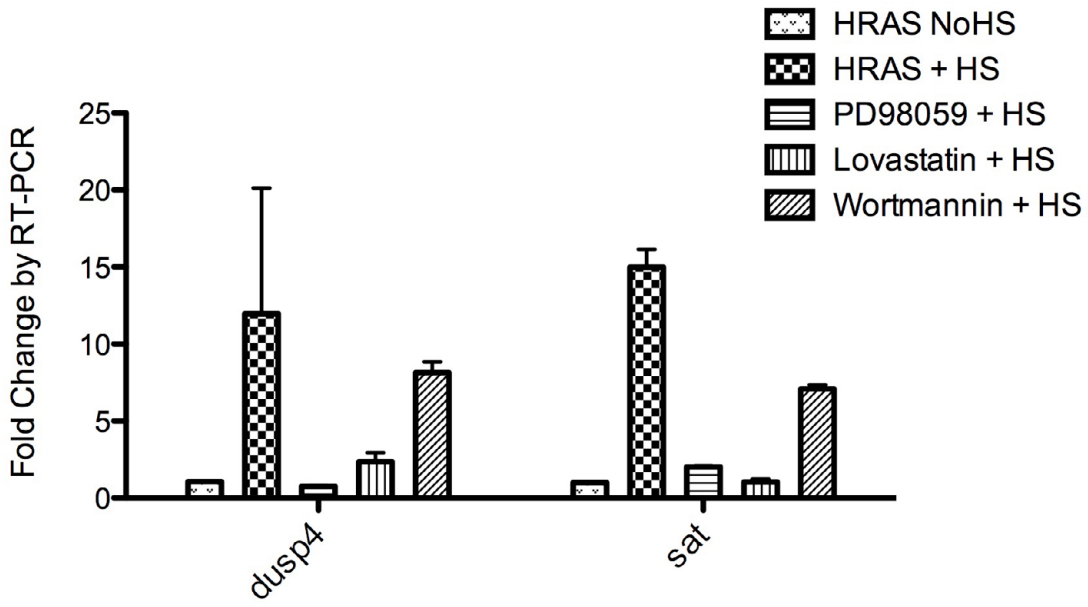

Fig. S3. Quantitative RT-PCR analysis of *dusp4* and *sat* expression levels in response to pathway-specific chemical inhibitors. *Tg(hsp70-HRAS*^{G12V}*)* embryos were incubated with chemicals from 22-24 hpf, heat shocked for 1 hour, and analyzed at 30 hpf for expression.

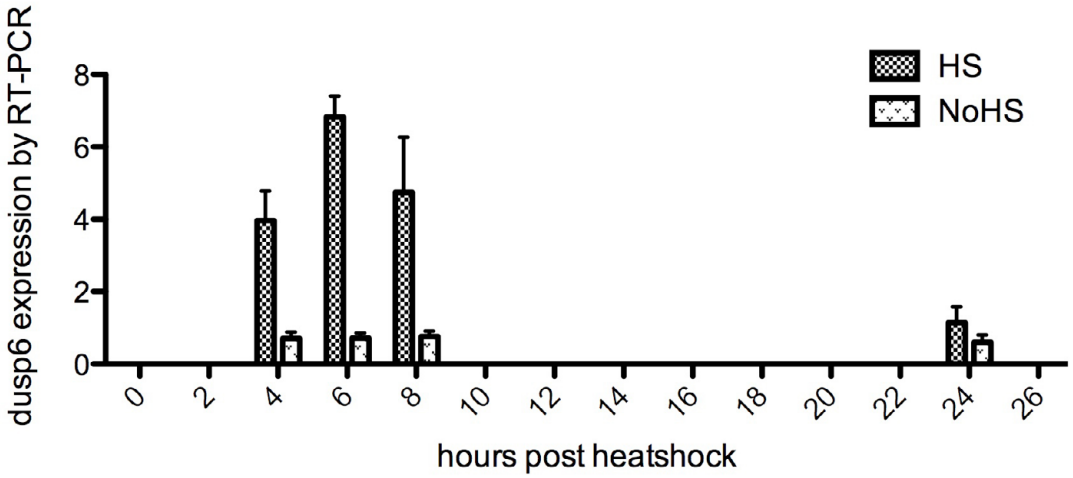

Fig. S4. Quantitative RT-PCR analysis of *dusp6* activation levels in *Tg(hsp70-HRAS^{G12V})* at various time points after 1 hour of heat shock. Transgenic embryos were kept at 28.5°C until 22 hpf. Some received 37°C heat shock for 1 hour; some were kept as controls. At 4, 6, 8 and 24 hours post-heat shock, the embryos were harvested for RNA extraction and RT-PCR analysis. Transgenic embryos kept at 28.5°C without heat shock were used as controls.

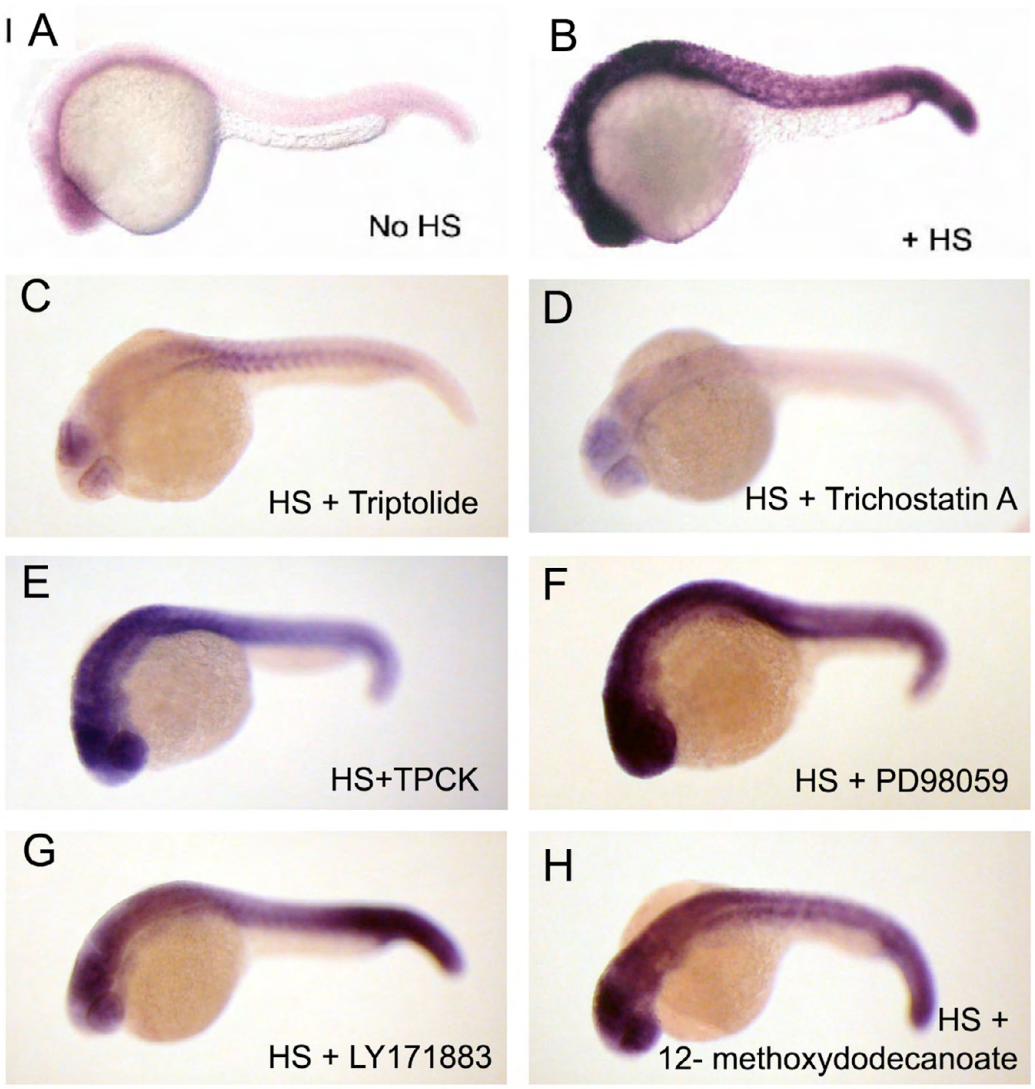

Fig. S5. *In situ* hybridization of *Cre* in transgenic *Tg(hsp70-Cre)* embryos with chemical treatment. (**A**,**B**) *Tg(hsp70-Cre)* embryos with or without heat shock were fixed and subjected to ISH for *Cre* as controls. (**C**,**D**) *Tg(hsp70-Cre)* embryos were incubated in chemicals (Triptolide and TSA) from 22 hpf, heat shocked from 24-25 hpf, fixed at 30 hpf, and analyzed by ISH for *Cre*. The *Cre* expression levels were suppressed in comparison to B, indicating that these chemicals interfere with heat shock promoter activation or transcription. (**E-H**) *Tg(hsp70-Cre)* embryos were incubated in chemicals (TPCK, PD98059, LY171883 and 12-methoxydodecanoate) from 22 hpf, heat shocked from 24-25 hpf, fixed at 30 hpf, and analyzed by ISH for *Cre*. The *Cre* expression levels were not suppressed in comparison to B, indicating that these chemicals do not interfere with heat shock promoter activation or transcription; therefore, these chemicals were confirmed as suppressors of the RAS pathway.

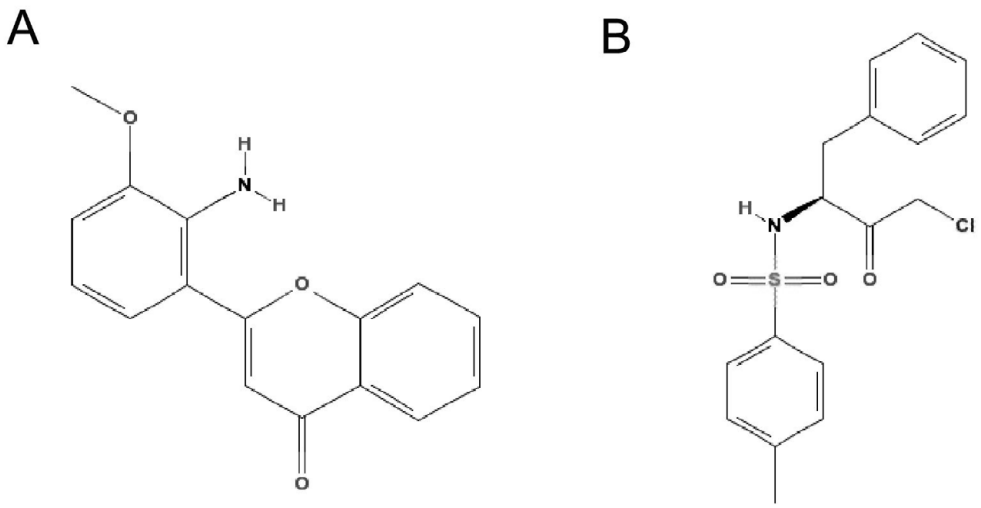

Fig. S6. Chemical structures of PD98059 and TPCK. (**A**) PD98059 and (**B**) TPCK. Modified from PubChem (http://pubchem.ncbi.nlm.nih.gov/).

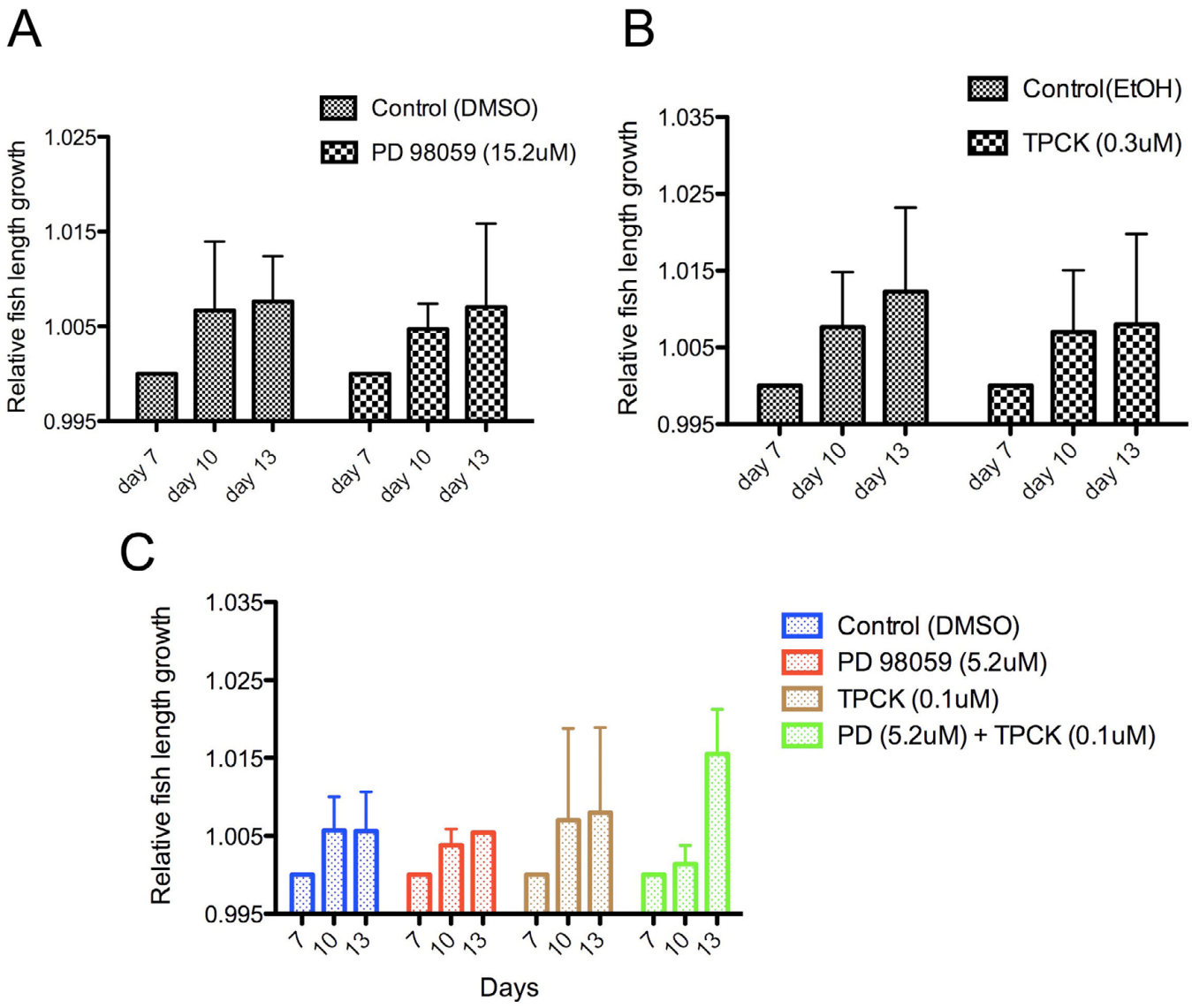

Fig. S7. Overall fish growth was not altered by chemical treatment in tumor-bearing fish. (**A**) Relative growth of fish (total length) was compared between the PD98059-treated group (1.005±0.0097, *n*=13 at day 10; 1.007±0.025, *n*=8 at day 13) and vehicle (DMSO)-treated group (1.0067±0.023, *n*=10 at day 10; 1.0077±0.015, *n*=10 at day 13). *P*>0.90, ANOVA. (**B**) Relative increase in total fish length was compared between the TPCK-treated group (1.007±0.031, *n*=15 at day 10; 1.008±0.029, *n*=6 at day 13) and vehicle (EtOH)-treated group (1.008±0.020, *n*=8 at day 10; 1.012±0.015, *n*=2 at day 13). *P*>0.90, ANOVA. (**C**) Relative increase in total fish length was compared in four groups : PD98059-treated group (1.004±0.0075, *n*=13 at day 10; 1.0054, *n*=1 at day 13), TPCK-treated group (1.007±0.031, *n*=7 at day 10; 1.008±0.029, *n*=7 at day 13), combination-treated group (1.0014±0.012, *n*=27 at day 10; 1.015±0.017, *n*=9 at day 13) and vehicle (DMSO)-treated group (1.006±0.015, *n*=12 at day 10; 1.006±0.015, *n*=9 at day 13). *P*>0.85, ANOVA.

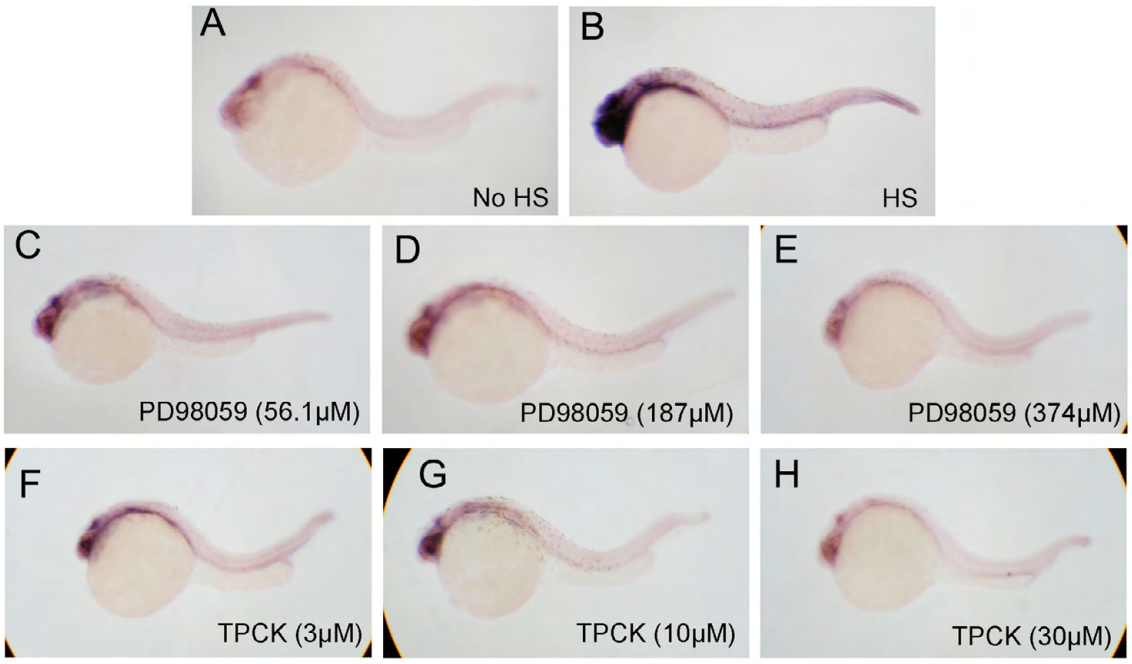

Fig. S8. PD98059 and TPCK suppress *dusp6* upregulation in *Tg(hsp70-HRAS^{G12V})* embryos in a dose-dependent manner. *dusp6* expression was assessed by ISH. (**A**,**B**) *Tg(hsp70-HRAS^{G12V})* receiving heat shock or no heat shock treatment provided positive and negative controls. (**C-E**) *Tg(hsp70-HRAS^{G12V})* receiving PD98059 treatment in an increasing dose demonstrated a decreasing expression level of *dusp6*. (**F-H**) *Tg(hsp70-HRAS^{G12V})* receiving TPCK treatment in an increasing dose demonstrated a decreasing expression level of *dusp6*.

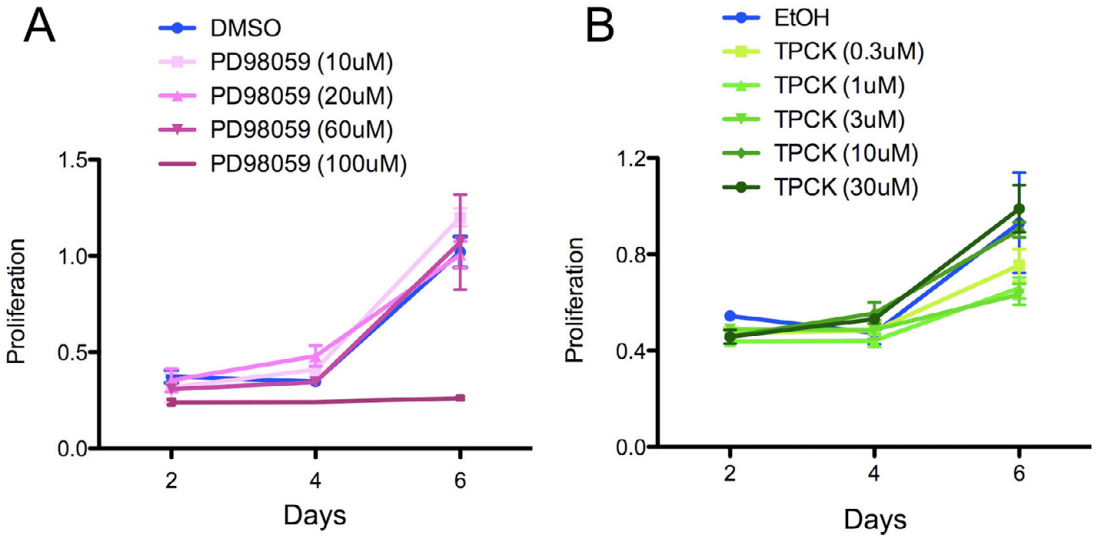

Fig. S9. PD98059 and TPCK did not suppress cell proliferation in mouse embryonic fibroblasts. Cells were plated in 96-well tissue culture plates at day –1. Cells were treated with a range of concentrations of (**A**) PD98059 (10-100 μM) or (**B**) TPCK (0.3-30 μM) starting at day 0 and continuing throughout the 6-day treatment. Medium/chemicals were changed on days 0, 2 and 4 to ensure continued chemical activity and adequate nutrients for cell growth. Cell proliferation was measured by MTT assay at days 2, 4 and 6. *y*-axis represents absolute OD from the MTT assay.

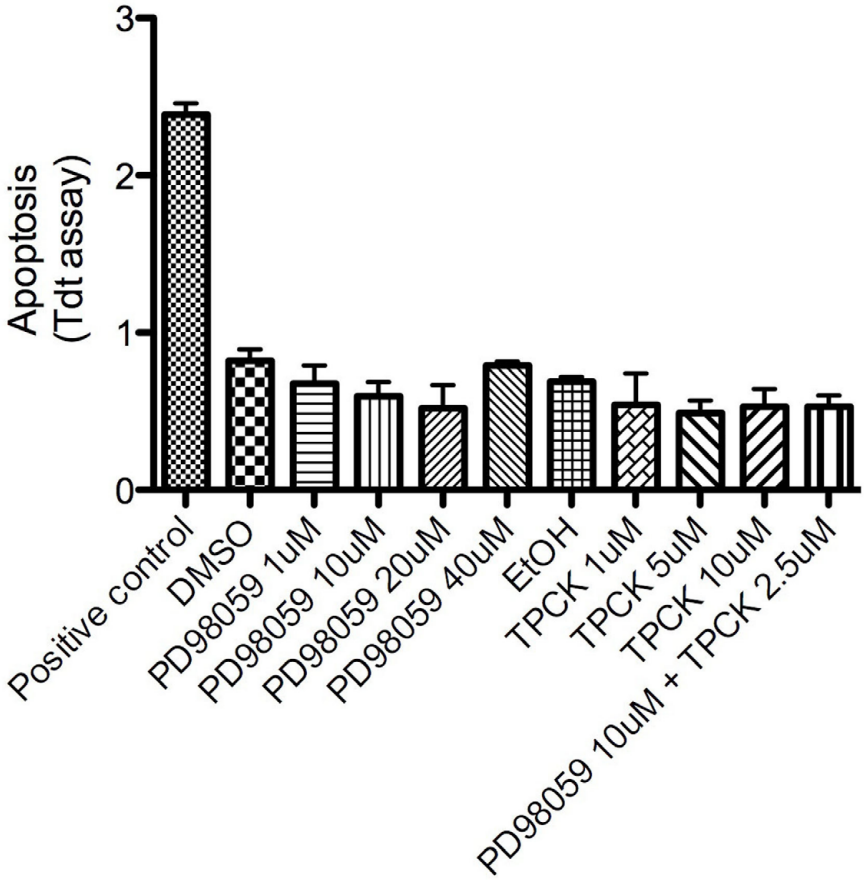

Fig. S10. Apoptosis levels, measured by TdT assay, were not altered by chemical treatments in RD cells. In comparison to vehicle controls (DMSO or EtOH), PD98059 (10-40 μM) treatment or TPCK (1-10 μM) or a combination (10 μM PD98059 + 2.5 μM TPCK) demonstrated similar apoptosis levels after 4 days of continued chemical exposure.

Table S1. Primers for RT-PCR of 17 genes tested in *Tg(hsp70-HRAS^{G12V})* and wild-type zebrafish embryos

Gene	Forward primer (5'-3')	Reverse primer (5'-3')
ctsl	GATGCAGGACATGAATCCTT	TAGCCATGTAAATGTAGCCT
ddx18	CAGCCATGGCGGACATGCAGA	CCGGTCCTCAAACGCACCTGT
mcl1	AATGTGTCCGGTAGACCGTA	TACATCTGATGAAGTCCAGA
csflr	ATGGATATCCAGCTCCCAGCA	TCTGGATCGGCTGCTGCAGATCT
dusp4	AGCTCCGATACAAACACCAT	TACTGGACTGAGAACTGGACA
dusp6	TACTGCTGAGGAGAATGAA	ACTGCTGCTTGACGAACCGT
gbp1	GAGTGCACTCACCTGAGGT	GCACCTCAGGCTCTACACAT
arpc1	ACACTGGTCATTCTCAGGATCA	TAAAGTCGCACGATCCAGCT
calr	TGGCACTGGCAGCCAATGCA	TAACCGTAAACTGGATCACCA
fgfr3	TGGCATCAAGCTACGACATCA	AGGCTGAGCATCACTGTACA
msn	ACCATCGAGGTCCAGCAGAT	TCCTGAGCACGTTTACGCT
pdia3	TCGCACCCTGGTGTGGTCACT	CGGCACTTCCTCCGTCAGCG
psmb2	ATCCAGATGAAGCACGATTA	ACATGATATGGAGTCCGACT
sat	ATCAGTCCATCGAAGGTTATA	TGAAGTGCATACCGCTGCAT
snrpd3	AGTAAGATCAGGTTCCTCATC	TGGTCCACCACGACCACGT
ssb	AGCTGCAGTTAGACGACGGA	ATTCCTCGTTGTTCTCAGGA
lrrfip1	TGGATGCAACTGGTGCTTAC	GTCCAGCTGTGCATTGGACA

Table S2. Upregulated gene list

Download Table S2

Table S3. Downregulated gene list

Download Table S3

Table S4. Eighteen compounds confirmed as RAS signaling pathway inhibitors

PD98059
TPCK
XK469
Lovastatin
Dipyrone
Tyrphostin AG 126
Lisofylline
CinnGEL
SB 431542
Clofibrate
LY 171883
Bithionol
Benzyl isothiocyanate (BITC)
Catechin
Epigallocatechin
Hesperetin
Mycophenolic acid
12-methoxydodecanoate