Supporting Information

Kawahara et al. 10.1073/pnas.1102116108

SI Text

First-Round Screen with Pooled Compounds. In the first screen, 20 1-d postfertilization (dpf) embryos resulting from a mating of *sapje* heterozygous fish were arrayed, 20 per well, in 48-well plates containing 0.25 mL fish water and eight pooled chemicals (vertical pool of eight chemicals in 96 well-plate) from the Prestwick chemical library. Each pool of chemicals was repeated in a second well of 20 embryos. The chemicals were at a final concentration of 2.4 μg/mL, which was the same concentration that other groups had used in their zebrafish studies (1). As a normal control, 20 embryos were cultured in duplicate without chemicals in every 10 wells. All plates containing embryos were incubated at 28.5 °C for 72 h. At 4 dpf, the birefringence of all fish was tested using a dissecting microscope. Because the muscle phenotype in these mutant fish is transmitted in a recessive manner, approximately 25% of the offspring have the abnormal muscle birefringence phenotype after 4 dpf (Fig. 1*A*). After comparison of the percentage of affected fish at 4 dpf between chemical-treated and nontreated fish, chemical pools with a reduced percentage of affected fish on birefringence assay compared with those in nontreated fish were selected for the secondary screen.

Second-Round Screen Using Individual Compounds. At 1 dpf, 20 embryos per well of each *sapje* and *sapje*-like fish, as described above, were arrayed in duplicate in 48-well plates containing 0.25 mL fish water with individual chemicals from the pooled mixtures that seemed to restore normal muscle structure. The final concentration was 2.4 μg/mL. Embryos were incubated at 28.5 °C for 72 h. At 4 dpf, the birefringence of all fish in the wells was examined using a dissecting microscope. All surviving fish, both normal and those with apparently restored muscle, were killed after anesthesia with 1% tricane. Heads were removed for genotyping, and bodies were used for immunostaining.

1. Zon LI, Peterson RT (2005) In vivo drug discovery in the zebrafish. *Nat Rev Drug Discov* 4:35–44.

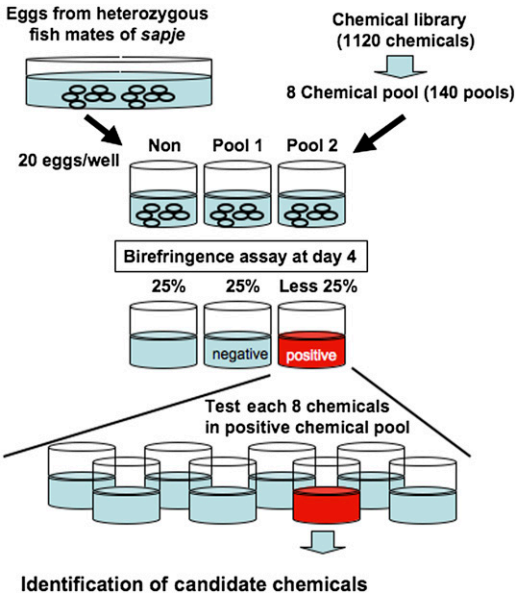


Fig. S1. Schematic of matings and screening.

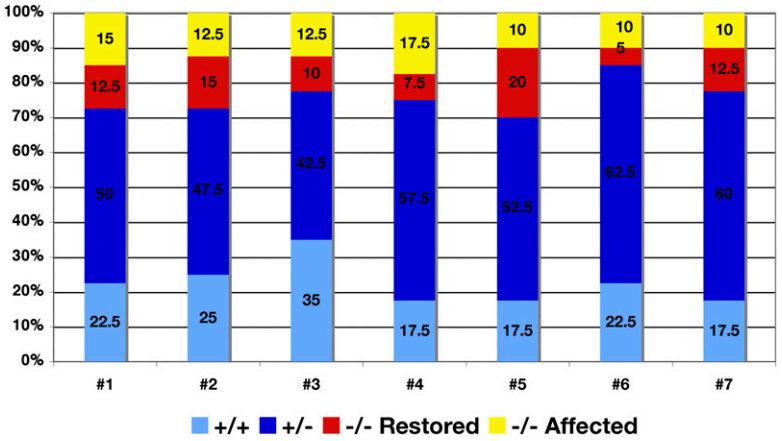
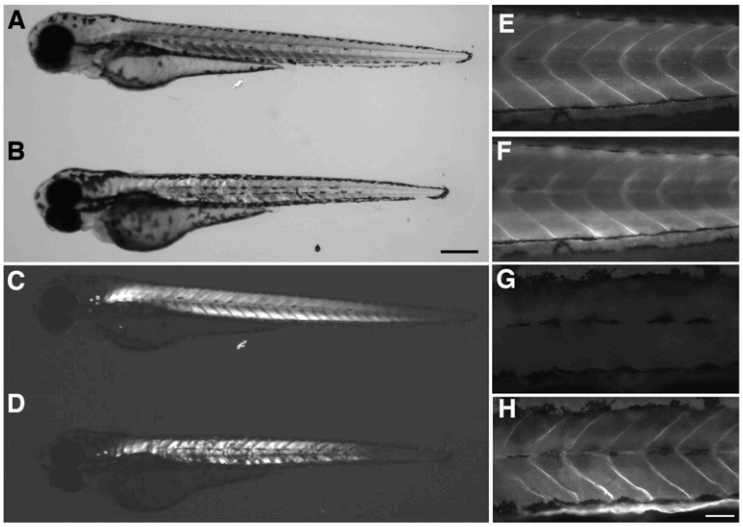
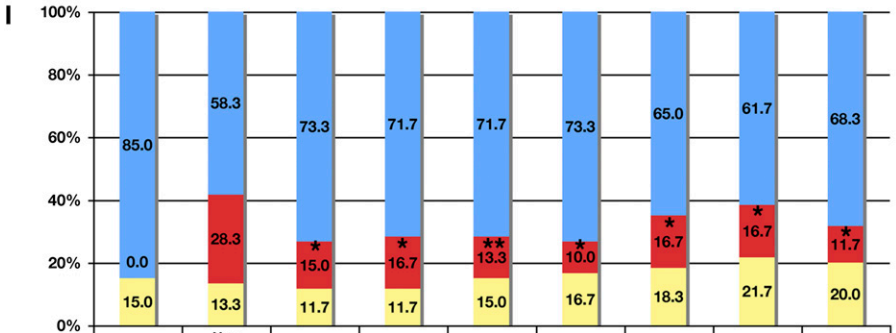


Fig. S2. Genotyping of 40 *sapje*-like fish treated with seven potential candidate chemicals. Yellow, dystrophin-null affected fish (abnormal birefringence). Red, dystrophin-null unaffected fish (normal birefringence). Blue, heterozygous fish (normal birefringence). Light blue, wild fish (40 fish, normal birefringence). Each of the seven chemical-treated fish wells has some fish that show normal birefringence; however, the genotyping results show that these are dystrophin-null fish.





	Wild	Non treatment	#1	#2	#3	#4	#5	#6	#7
Normal	85.0	58.3	73.3	71.7	71.7	73.3	65.0	61.7	68.3
Affected fish	0.0	28.3	15.0	16.7	13.3	10.0	16.7	16.7	11.7
Dead	15.0	13.3	11.7	11.7	15.0	16.7	18.3	21.7	20.0

*p<0.01 **p<0.05 (vs Non treatment)

Fig. S3. Dystrophin morphant by injection of morpholino and the effects of candidate chemicals in recovering the phenotypes of the morphants. The dystrophin morphant has a similar phenotype to the *sapje* fish, which shows reduced birefringence and expression of dystrophin. (*A*, *C*, *E*, and *F*) WT. (*B*, *D*, *G*, and *H*) Dystrophin morphant. (*A* and *B*) Normal light image. (*C* and *D*) Birefringence image. (*E* and *G*) Immunostaining with anti-dystrophin antibody. (*F* and *H*) Immunostaining with anti-laminin antibody. (*I*) Percentage of dead affected and normal fish in morphants with treatment of each of the seven chemicals. In untreated dystrophin morphants, 30% are affected fish. For each chemical treatment, the percentage of affected fish is reduced compared with those of untreated morphants. Blue bar, normal. Red bar, affected fish. Yellow bar, dead fish (*$P < 0.01$, **$P < 0.05$; $n = 3$).

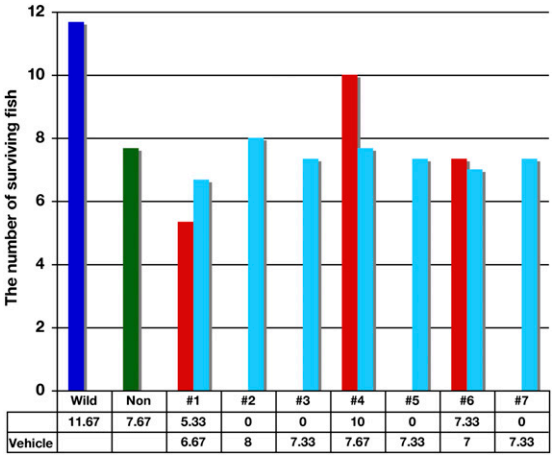


Fig. S4. Twenty embryos containing three strains ($^{+/+}$, $^{+/-}$, and $^{-/-}$ dystrophin) from *sapje* heterozygous fish mates were incubated with each of the seven candidate chemicals from 1 to 30 dpf in triplicate. At 30 dpf, only the fish treated with each chemical 1, 4, and 6 had survived (red bars). Interestingly, more fish treated with chemical 4 were able to survive compared with control fish (light blue bars, vehicle) and untreated fish (green bar). The fish treated with chemicals 1 and 6 were almost the same as control. The remaining four chemicals groups (2, 3, 5, and 7) resulted in death of all three strains ($^{+/+}$, $^{+/-}$, and $^{-/-}$ dystrophin).

Table S1. Tested chemicals and their source

Chemical name	Company	Catalog number
Epirizole	Sigma	M9017
Homochlorcyclizine dihydrochloride	Sigma	H1761
Conessine	Wako	031–16001
Aminophylline	Sigma	A1755
Equilin	Sigma	E8126
Pentetic acid	Sigma	D1133
Proscillaridin A	Sigma	P2428
Enoximone	Sigma	E1279
Milrinone	Sigma	M4659
Ibudilast	Sigma	I0157
Rolipram	Sigma	R6520
Sildenafil citrate salt	Sigma	PZ0003
Dipyridamole	Sigma	D9766