Supporting Information

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SI Materials and Methods

Generation of UAS:GAL4 Fish. A sequence encoding for the Gal4activator (KalTA4) was inserted within the 5' UTR of ltk, aug- $\alpha 1$, aug- $\alpha 2$, and aug- β genes. To do so, embryos carrying sox10: mCherry and UAS:eGFP transgenes were injected at the singlecell stage with a mixture containing (i) sgRNAs targeting specific 5' UTR sequences near the endogenous gene's start codon; and (ii) Cas9 mRNA; (iii) a donor plasmid with the equivalent 5' UTR sequence followed by a viral 2A peptide and KalTA4 (32). The donor plasmid, which is based upon Auer et al. (33), was specifically inserted into the 5' UTR of the ltk and augmentor genes using two sgRNAs specific to that region as previously described. This donor plasmid includes a coding sequence of the transcriptional transactivator Gal4 (KalTA4) preceded by an E2A peptide linker for multicistronic expression. Upon injection of the above mixture into a UAS:eGFP transgenic fish, Cas9 cleaves the genomic locus as well as the donor plasmid sequence as specified by the sgRNAs. DNA repair triggers integration of the donor plasmid into the genomic locus through nonspecific ligation of cleaved DNA. Since the indel sequence preceding the Gal4-cDNA cannot be predicted upon integration, the presence of the E2A will allow the in-frame insertions of the Gal4-cDNA (33).

Determination of On-Targets and Off-Targets. Genomic DNA from zebrafish embryos at 24 hpf and from the tails of adult fish was extracted by boiling at 95 °C for 20 min in 50 μ L of 100 mM sodium hydroxide and then neutralized with 10 μ L of 1 M Tris·HCl (pH 7.5). Then 1 uL of genomic DNA was used as template to amplify an ~200-bp sequence surrounding the ontarget and off-target regions identified by CRISPR RGEN Tools (www.rgenome.net) (Fig. S24). Mutations were detected by T7EI assay (31). Mutation frequencies were determined by quantifications of T7EI-undigested and -digested PCR products. For each quantification, the intensity of the PCR bands of the undigested product and of a T7E1-digested fragment of the same size were analyzed by ImageJ (https://imagej.nih.gov/ij/). The

mutation frequencies were calculated by the ratio of T7EIdigested to T7EI-undigested PCR products.

Sox10⁺ Cell Isolation. Sox10⁺ cells were isolated from Sox10: mCherry by using FACS. Sox10:mCherry transgenic zebrafish embryos are embryos expressing mCherry as driven by the sox10 promoter. mCherry-positive and -negative cells were separated as described previously (41). Briefly, 16-hpf and 24-hpf sox10:mCherry embryos and age-matched wild-type embryos were collected and dechorionated, and the yolk was mechanically removed and disassociated with pronase followed by further digestion to single cells with Librase (Roche) at 28 °C for 10 min then trypsin (Gibco) for 5 min. The reaction was terminated by the addition of FBS. Cells were pelleted, washed with suspension buffer containing Leibovitz medium, 0.8 mM calcium chloride, penicillin streptomycin, and 0.5% FBS (all from Gibco), and filtered with a 35-µm cell strainer into 5-mL FACS tubes. FACS was performed by a five-laser BD FACSAria II cell sorter equipped with BD FACSDiva software v8.0.1 (BD Biosciences). DAPI was added to the sample before FACS to collect only viable cells. Viable mCherry-positive and -negative cells were sorted using a 70-µm nozzle at 60 psi. Sorted cells were collected, and total RNA was extracted using TRIzol reagent (Life Technologies).

Whole-Mount in Situ Hybridization. Zebrafish embryos at different stages were fixed, and whole-mount in situ hybridization was performed as previously described (42). To make riboprobes, fragments for riboprobe synthesis were amplified from wild-type full-length zebrafish cDNA and cloned into pBluescript II KS (-) (Agilent Technologies). To generate digoxingenin (DIG)-labeled antisense riboprobes, linearized constructs were reverse transcribed by T3 RNA polymerase with a DIG Labeling Kit (Roche). Meanwhile, sense-sequence riboprobes were generated using T7 RNA polymerase with the DIG Labeling Kit. The DIG signal was revealed by the NBT/BCIP system (Roche), and images were acquired with Leica Application Suite (Leica) using the Leica MacroFluo system and Leica LED5000RL light (Leica).

Α

Full-Length Augmentors

		angan aginomore	an Eongan
11 59 11	MCFALSLSIYKHTGTPPNKCRLNSALKRNRATVRLDIGQRGSLCPPFSMSAVRPPVF-IG MRAEKRWHILLS * * : : :	sh_Aug_al 1 sh_Aug_a2 1 sh_Aug_b 1	ebrafish_Aug_a ebrafish_Aug_a ebrafish_Aug_k
63 11 7	LVLLICTAAQSDASANKVEKTLRRIMEIMROVENSADDESAQKTESAPEPKDT LLLIILTTGYCKPRDRDETSLLELLMDRVROTQEHHSEGNTQHPPQIIEHSLETKDV MILLIITSSQCMDSKEVKESERKTLLNLILÖVIGEKPASRRVTSGLYSVSQDAKFSSREN ::**: *:	.sh_Aug_al 13 .sh_Aug_a2 60 .sh_Aug_b 13	ebrafish_Aug_a ebrafish_Aug_a ebrafish_Aug_k
123 175 132	-HHLKTASGETILEIFPRDLSRKEKFITIL-TGPLYFGPKCKKDVYRLYHNTRDCTIPAH NKVTKSYQHERILEVFPRDLRQXDKFLKHL-TGPLYFSPKCSKLFYKLYNNTRDCTIPAY TAHLPKPDNSRPIEVPRDTSMKDKFIEHFTAGPVKFPSECRTHFHRIMENTRDCSRPTY :*:.*** *::::::::::::::::::::::::::::::	sh_Aug_al 66 sh_Aug_a2 117 sh_Aug_b 73	ebrafish_Aug_a ebrafish_Aug_a ebrafish_Aug_k
143 193 152	YKRCARLLTRLAGTRKCQEG YKRCARLLTRLAGSQRCTEG YKRCARLLTRLAMSPLCTOS	.sh_Aug_a1 124 .sh_Aug_a2 176 .sh_Aug_b 133	ebrafish_Aug_a ebrafish_Aug_a ebrafish_Aug_t

Full-Length Aug-α

ze

man_AUG_a brafish_AUG_a1 brafish_AUG_a2	1 1 1	MRCPGHPLI-LG MRAPULJ-LG MCFALSLSIYKHTGTPPNKCRLNSALKRNRATVRLDIGQRGSLCPPFSMSAVRPVF-IG * * *: : : *	11 12 59	
man_AUG_a brafish_AUG_a1 brafish_AUG_a2	12 13 60	LLLVIGAACRGRGGAEPREPADGQAL RLVVELVQELRKHHSAEHKGLQLLGRDCALG LVLLICTAAQSDASANKVEKTIRIMEIMRQVENSADDESAQKTESAPEPK LLLLILTGYCKPRDRDETSLELLMDRVRQTQEHHSEGNTQHPPQIIENSLETK *:*::::::::::::::::::::::::::::::::::	69 63 114	Percent Identity Natrix - created by Clustal2.1
man_AUG_a brafish_AUG_a1 brafish_AUG_a2	70 64 115	RAEAAGLGPSPEQRVEIVPRDLRMKDKFLKHLTGPLYESPKCSKHFHRLYHNTRDCTIPA DT-HHLKTASGETILEIPPRDLSKKEKFITILTGPLYESPKCKDVYRLYHNTRDCTIPA DVNKVTKSYQHERILEVPPRDLRQKDKFLKHLTGPLYESPKCSKLFYKLYNNTRDCTIPA	129 122 174	1: human_AUG_a 100.00 44.29 48.97 2: zebrafish_Aug_a1 44.29 100.00 50.70 3: zebrafish_Aug_a2 48.97 50.70 100.00
man_AUG_a brafish_AUG_a1 brafish_AUG_a2	130 123 175	YYKRCARLLTRLAVSPVCMEDKQ HYKRCARLLTRLAGTRKCQEC YYKRCARLLTRLAGSQRCTEC :************ : * *.	152 143 195	

Aug-α Aug Domain

hAUG_a_AugDomain zAug_a1_AugDomain zAug_a2_AugDomain	1 1 1	EQRVEIVPRDLRMKDKFLKHLTGPLYFSPKCSKHFHRLYHNTRDCTIPAYYKRCARLLTR ETILEIFPRDESKEKFITILTGPLYFSPKCKDVYKLYHNTRDCTIPAHYKRCARLLTR ERILEVFPRDLRQKDKFLKHLTGPLYFSPKCSKLFYKLYNNTRDCTIPAYYKRCARLLTR	60 60 60	Percent Identity Matri	x - crea	ted by C	lustal2.1
hAUG_a_AugDomain zAug_a1_AugDomain zAug_a2_AugDomain	61 61 61	LAVSPVCMEDKQ LAGTRKCOEG LAGSQRCTEG ** : * *.	72 70 70	1: zAug_a1_AugDomain 2: hAUG_a_AugDomain 3: zAug_a2_AugDomain	100.00 68.57 72.86	68.57 100.00 78.57	72.86 78.57 100.00

Full-Length Aug-β

human_Aug_b zebrafish_Aug_b	1 1	MRPLKPGAPIPALFILAIALSPHGAHGRPRGRRGA MRAEKRWHILLSMILLLITSSQCMDSKEVKESERKTLINLILCVIGEKPASRRVTSGL ** * * :: * ** *	35 58	
human_Aug_b	36	RVTDKEPKPLLFLPAAGAGRTPSGSRSAEIFPRDSNLKDKFIKHFT-GPVTFSPECSKHF	94	percent identical: 41.29%
zebrafish_Aug_b	59	YSVSQD-AKFSSRENTAHLPKPDNSRPIEIVPRDTSMKDKFIEHFTAGPVKFPSECRTHF	117	
human_Aug_b	95	HRLYYNTRECSTPAYYKRCARLLTRLAVSPLCSQT	129	
zebrafish_Aug_b	118	HRIYENTROCSRPTYYKRCARLLTRLANSPLCTQS	152	

Aug-β Aug Domain

hAUG_b_AugDomain	1	SG SRSABIFPRD SNLKDKFIKHFI-GPVTFSPECSKHFHRLYNTRECSTPAYYKRCARL	59	percent identical: 69 44%
zAug_b_AugDomain	1	DNSRPIBIVFRDTSMKDKFIEHFIRGEVKFPSECRTHFFRIMHNTRDCSRPTYKRCARL	60	
hAUG_b_AugDomain	60	LTRLAVSPLCSQT	72	
zAug_b_AugDomain	61	LTRLAMSPLCTQS	73	

В

Zebrafish Ltk Primary Sequence:	Ltk
MDYITRQTFVKLALFIFTVVRSSCA <mark>LLEKAAESPVHPNPLQSSPAEDSDV<mark>SFCDFESPCS</mark></mark>	
WTLSSHSTGGEWFITSAQQHRSNRRDTQPIRDYSTGKSEGHFLLLKPSSSHLSAARCSFH	MAM #1
MTSPVVLSSGPFCHLQLARFQPEPHAGNISAFVKHTDSIDIKPIDLTIKEQESDSSQWEV	
<mark>LEAVIGQLNEPFQVTVQYSACSSHEVGFLAFDSLELKNCVM</mark> GDDYVDLGS <mark>DCEKYSSLQC</mark>	×
HSGGCIEKQRVCDFHTDCPEGEDEGLICSTLPLG <mark>SYCSFELGSCGWSAADTQSSWRLVSG</mark>	LDLa
QQLIEDTHLLGTTLKNTQGHFLFLKVRGHGDEREALVQSPALPSTISNQDCQLQFSLYRY	
GDFNGTVLLSVVESGASAPALIWERSGHWKDAWQEITLPITEILNGFHLKVQAFWTSGSK	
ADIALDDISLSAACFDTELNELLHEGLPHDLDFSPLPEPSASEASPITWWFTSCGASGPF	MAM #2
GPTQAQCDSAYRNTNVSVVVGKEGPLRGVQMWKVPATNTYKISAYGAAGGKGAKNHNKRS	
HGVFISATFPLEKGDILYILIGHQGEDACPGRNPQTHKICLGESSVIEDGFDSDGSALKW	
AGGGGGGGGGATYIYRMENGQPLPLLIAAGGGGKAYLEDPESSQDQSFREQYENDTTVSGV	
SGRSGAAGGGGGWSDVSSLSWAGKSLVEGGQGGSSCPEALSVLGWATFGGFGGGGGGACSA	
<mark>GGGGGGYRGG</mark> DAPLLDDISADGQDGLSFVHPMGKIFLQSLAAMESHG <mark>EAEIVVYLNCSHC</mark>	GlvR
KTQSCKRDEDTKLILCLCDSDEVLAPDNVTCAGTKHSLCQFINKHLQHNSSPLVCPPLVV	
PMGSLADGPPSLVFIMAVIVSTVVTGVVLTCASLTLIYYRKKNHLHAVRIRLQSPEYKLS	
KIRSSTIMTDYNPNYGYFGKAASLSELKEVPRKN <mark>ITLLRALGHGAFGEVYEGQVLGMNGE</mark>	
${\tt NTAMQVAIKTLPEICSEQDEMDFLMEALIMSKFSHQNIVRCIGVSLQILPRFILLELMTG}$	
GDMKSFLRLNRPRTNHSSSLSMLELLHMARDIALGCRYLEENHFIHRDIAARNCLLTCPG	EGF-like
PDRVAKIGDFGMARDIYRASYYRKGGRAMLPVKWMPPEAFLEGIFTCKTDTWSFGVLLWE	Plasma Membrane
IFSLGYMPYPCKTNQEVLEFVTGGGRMDPPKSCPGPVYRIMTQCWQHCPEHRPNFTTILE	
RINYCTQDPDVINTPLPVECGPPVEEEGGTVIRPDGSGSMTPLLVARSLSQDASPRASIT	
SVTPQALKPRLQLQRPVHLTQEVGTYRETLEPCWAEPVPASGVCPGPWLQVPEHRPCSRS	KD
SSSSGSQKLKNKTKNLWNPTYGSWVLESFGRGKSALCHTQSMPLSCNPTSVSAPSSTSEH	
TDPVVEVNANVSASPPPSAAPSQTTLTPTAAPSRKSPTGAAGVSLATVMDLAKLQSFPCG	
NVNYAYDEQSYETESLPVVVSKSLEPSTSSAATSSLVALSQASSFTHKPLVKRHASYGHE	
DVRRYTQPEKPTRDRDSGFSLSEDLSVTPV	V

#2

Signal Peptide MAM domain		LDLa Repeat	Glycine Rich
EGF-like motif	Transmembrane	Protein Kinase	

Sequence Alignment

-DLa MAM #1	hALK_MAM_1 zLtk_MAM_1 zLtk_MAM_1 zLtk_MAM_1 zLtk_MAM_1 zLtk_MAM_1 hALK_LDLa zLtk_LDLa	1 1 61 56 111 115	LECSFDFPCELEYSPPLHDLRNQSWSWRRIPSEEASQMDLLDGPGAERSKEMPRGSFLLI SFCDFSPCSWTLSSHSTGGEWFITSAQOHRSNRRDTOPIRDYSTGKSECHFLLI ***********************************	60 55 110 114 164 171 37 39	Percent Identical: 21.5% Percent Identical: 37.5%
MAM #2	hALK_MAM_2 zLtk_MAM_2 hALK_MAM_2 zLtk_MAM_2 hALK_MAM_2 zLtk_MAM_2	1 52 58 112 115	FYCNFEDGFCGWTQGTLSPHTPOWOVRTLKDARFODHODHALLSTTDVPA SVGSFELGSCGWGAADTQSSWRLVSGQQLIEDTHLGTTLKNTQGHFLFKVRCHGD ************************************	51 57 111 114 159 162	Percent Identical: 28.0%
GIJR	human ALK GlyR human LTK GlyR zebrafish_Ltk_G human ALK GlyR human_LTK_GlyR zebrafish_Ltk_G human ALK GlyR human_LTK_GlyR zebrafish_Ltk_G	1 1 57 61 61 61 117 121 121	GGGGGGGGATYVFKMKDGVPVPLIIAAGGGGRAYGAKTDTFHPERLENNSSVLGLN GGGGGGGGATYVFRVRAGELEPLLVAAGGGGRAYLRPRDRGRTQASPEKLENRSEAPGSG GGGGGGGATYVFRVRAGELEPLLVAAGGGGRAYLRPDSSQDQSFREQYENDTTVSGVS **********************************	56 60 60 116 120 120 125 129 129	Percent Identity Matrix - created by Clustal2.1 1: human_LTK_GlyR 100.00 60.00 59.69 2: Human_ALK_GlyR 60.00 100.00 64.00 3: zebrafish_Ltk_GlyR 59.69 64.00 100.00

Fig. S1. (Continued)

С

Alk

Sequence ID: Query_44940 Length: 426 Number of Matches: 2

Score		Expe	ct	Identities	Gaps	Strand	
130 bi	ts(70)	2e-3	4	70/70(100%)	0/70(0%)	Plus/Plus	
Query	1	GTGTTTGCAGC	TGTCAR	GTGGATATTTCACACC	TGTGGAGCGACAGGAC	CAAGATGGCCCA	60
Sbjct	99	GTGTTTGCAGC	TGTCAA	AGTGGATATTTCACACC	TGTGGAGCGACAGGAC	CAAGATGGCCCA	158
Query	61	ACACCAACCC	70				
Sbjct	159	ACACCAACCC	168				

Range	2: 173	to 217 Graphics		Vext Match	Previous Match	First Match
Score		Expect	Identities	Gaps	Strand	
84.2 b	its(45)	1e-20	45/45(100%)	0/45(0%)	Plus/Plus	
Query	136	GGGATCCAAATGTGGG	AGGTTCCAGAGACAAGGA	AATACAGGTAC 18	30	
Sbjct	173	GGGATCCAAATGTGGC	AGGTTCCAGAGACAAGGA	AATACAGGTAC 21	17	

Amino Acid Alignment

- WT: 370 WIFHTCGATGQDGPTPTQCSNSYRNTNVNVTVGTKGPFKGIQMWQVPETRKYRITAYGA
- ALK KO: 370 WIFHTCGATGQDGPTPTRLGSKCGRFQRQGNTGSRHMEQLVDAVFWRFTSHMGCT



Ltk

aug-a1

Sequence ID: Query_68687 Length: 356 Number of Matches: 1

Range 1	1: 64 to	230 Graphics					🔻 Next Match 👔	Previous Match
Score		Exp	pect	Identitie	s	Gaps	Strand	
252 bit	ts(136)) 4e-	-71	166/178	(93%)	11/178(6%) Plus/Pl	us
Query	46	GCAGATGTAG	TTTCCA	CATGACCA	GTCCTGTAG	TCTCAGCAGTGGG	CCATTTTGTCATC	105
Sbjct	64	GCAGATGTAG	TTTCCA	CATGACCA	GTCCTGTAG	TCTCAGCAGTGGG	CCATTTTGTCATC	123
Query	106	TCCAACTCGC	TCGCTT	FCAACCAG	AGCCACACGO	TGGAAACATATCA	GCCTTCGTGAAGC	165
Sbjct	124	TCCAACTCGC	TCGCTT	FCAACCAG	AGCCACACGO	TGGAAACATATC-	AGC	172
Query	166	ACACCGACTO	TACCGA	CATCAAAC	CCATTGACC	тасаатсааадаа	CAAGAAAGCGA	223
Sbjct	173	ACACCGACTO	TATCGA	CATCAAAC	CCATTGACC	TACAATCAAAGAA	CAAGAAAGCGA	230

Amino Acid Alignment

- WT: 115 RCSFHMTSPVVLSSGPFCHLQLARFQPEPHAGNISAFVKHTDSTDIKPIDLTIKEQES
- LTK KO: 115 RCSFHMTSPVVLSSGPFCHLQLARFQPEPHAGNISAHRLYRHQTH-



Sequence ID: Query_7615 Length: 318 Number of Matches: 1

F	Range 1	: 98 to	233 Graphics			🔻 Next Match 🔺	Previous Match
	Score	re Expect Identities Gaps		Strand			
2) ,,,	150 bit	s(81)	8e-41	131/152(86%)	16/152(10%)	Plus/Minu	S
ç	Query	2	CACTCTGGTTTCGG	GAGAGATGCGCGCGCTGCG	AGCCCCGGTGCTGG	TAATGGGGGCTCGT	61
5	Sbjct	233	CACTCTGGTTTCGG	GAGAGATGCGCGCGCGCTGCG	AGCCCCGG	GGCTCGT	186
ç	Query	62	ATTGTTAATCTGCA		CAGCGCGAACAAAG	IGGAGAAAACGCT	121
5	Sbjct	185	ATTGTTAATCTGCA	AT-C-GTT-AAT-CGATGC	CAGCGCGAACAAAG	TAGAGAAAACGCT	130
ç	Query	122	CAGACGGATCATGG	AAATCATGAGACAGGTGG	153		
5	Sbjct	129	CAGACGGATCATGG	AAATCATGAGACAGGTGG	98		
Amino Ac	id A	lign	ment				

- WT: 1 MRALRAPVLVMGLVLLICTAAQSDASANKVEKTLRRIMEIMRQVENSADDESAQKTESAPEPKDT
- AugKO: 1 MRALRAPGLVLLICNR-

stop

Sequence ID: Query_210167 Length: 411 Number of Matches: 1 aug-a2

Score		Expect	Identities	Gaps	Strand	
335 bits(181)) 1e-95	213/227(94%)	8/227(3%)	Plus/Minus	5
uery 1	34	CACCTTTCAGCATGAG	IGCAGTGCGCCCACCTGT	C-TTC-ATAGGGCT	CCTACTGCTGAT	191
Sbjct 3	18	CACCTTTCAGCATGAG	IGCAGTGCGCCCACCTGI		CCTACTGCTGAT	259
uery 1	92	CCTCACCACCGGCTAC	IGCAAACCGAGAGACAGA	GACGAAACCAGTCI	GCTGGAGCTCTT	251
bjct 2	58	CCTCACCACCGGC		GACGAAACGAGTCI	GCTGGAGCTCTT	205
uery 2	52	AATGGACAGAGTGAGA	CAGACACAGGAGCATCAC	AGTGAGGGAAACAC	ACAGCATCCTCC	311
Sbjct 2	04	AATGGAGAGAGTGAGA	CAGACACAGGAGCATCAC	AGTGAGGGAAACAC	ACAGCATCCTCC	145
Query 3	12	TCAGATCATCGAGCAT	ГСӨСТӨӨАААСААААӨАС	GTTAATAAAGTCA	358	
bjct 1	44	TCAGGTCATCGAGCAT	I I I I I I I I I I I I I I I I I I I	GTTAATAAAGTCA	98	

- WT: 1 MCFALSLSIYKHTGTPPNKCRLNSALKRNRATVRLDIGQRGSLCPPFSMSAVRPPVFIGLLLLIL
- AugKO: 1 MCFALSLSIYKHTGTPPNKCRLNSALKRNRATVRLDIGQRGSLCPPFSMSAVRPPVLL-



aub-b Sequence ID: Query_197261 Length: 418 Number of Matches: 2

Score 183 hits	(99)	Expect	Identities 112/117(96%)	Gaps 5/117(4%)	Strand Plus/Plus
105 010	5(55)	50 50	112/11/(5070)	5/11/(470)	1103/1103
Query	106	ACTCTGCTTAATCTA	ATCCTGCAAGTGATCGGG	GAAAAGCCCGCGTCCAG	ACGCGTCACC 165
Sbjct	164	ACTCTGCTTAATCTA		 GAAAAGCCCGCGTCCA-·	GTCACC 218
Query	166	AGTGGCCTCTACTCT	GTATCTCAAGATGCTAAG	TTTTCCTCGCGTGAAAA	CACTGCA 222
Shict	219				
00,000	217		0111010101110011100	1111001000010101010	
Range 2: 95 to		155 Graphics		🔻 Next Match 🔺	Previous Match 🔺 First Ma
Score		Expect	Identities	Gaps	Strand
108 bits	s(58)	2e-27	60/61(98%)	0/61(0%)	Plus/Plus
Query	1	А ТGÇĞĞĞÇŢĞAĞAAĞ	А ĢАŢĢĢĊĄĊ АŢĊŢŢ ĢŢŢĢ	А ĢŢ ĂŢĢĂŢŢĊŢŢŢŢ ĞĊŢ(САТСАССТСС 60
Sbict	95	ATTCGGGCTGAGAAG			CATCACCTCC 154
0.000	61	A 61			
Query	01				
Sbjct	155	À 155			
Naid N	lia	nmont			
	TTTA				
Z RAF	TKKM	HILLSMILLL	SSUCHDSKEVKESI	RKITPINFIPÖATG	EKPASKKVTSGLIS
Z RAE	SKRW	HILLSMILLLIT	STSI	TLLNLILQVIG	EKPASSHOWPLLCI

WT: QDAKFSS

AugKO

Amino

stop

Fig. S1. (A) Comparison of the full-length and augmentor domain primary sequence of zebrafish and human augmentors. (B) Ltk primary sequence with domains highlighted. Sequence comparison of Ltk is more closely related to human ALK than to LTK. (C) Sequencing of knockout Alk, Ltk, and augmentor fish resulting in frame-shift mutations (red text denotes the mutant amino acid sequences) and subsequent successful introduction of a premature stop codon (red arrowheads).

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Fig. 52. (*A*) Off-target sites were identified as previously described (43). Each sgRNA off-target sequence contains two or fewer nucleotide mismatches as indicated by lowercase letters in red. (*B*) On-target mutations were amplified by PCR and heteroduplex reaction, followed by the T7EI assay. Products of T7EI digestion are labeled as "+." and products without T7EI are labeled as "-." Gel images from two off-target mutations of each sgRNA are presented. (*C*) Mutation frequencies were quantified for each on-target site and all its correspondent off-target sites. Quantifications are plotted in the chart.



Fig. S3. (*A*) The mRNA expression of *Ltk*, *Aug-\alpha1*, *Aug-\alpha2*, and *Aug-\beta* in embryos at 7, 24, 48, and 72 hpf. (*B*) The location of *Ltk* mRNA in the eye region was determined by in situ hybridization in 24-hpf embryos. Black arrows indicate *Ltk*⁺ cells. (*C*) The mRNA expression level of *Ltk*, *Aug-\alpha1*, *Aug-\alpha2*, and *Aug-\beta* in Sox10⁺ cells isolated from 16- and 24-hpf embryos.

Gene	sgRNA
sgRNA seque	nces to introduce a premature stop codon within each gene
Aug-α1	taatacgactcactataGGGCCCCGGTGCTGGTAATGgttttagagctagaa
	taatacgactcactataGGCGCTGGCATCGCTTTGTGgttttagagctagaa
	taatacgactcactataGGGAGCCCCGGTGCTGGTAAgttttagagctagaa
Aug-α2	taatacgactcactataGGCTCTCGGTTTGCAGTAGCgttttagagctagaa
	taatacgactcactataGGGCGCCCACCTGTCTTCATgttttagagctagaa
	taatacgactcactataGGATGCTCGATGATCTGAGGgttttagagctagaa
Aug-β	taatacgactcactataGGGGCCACTGGTGACGCGTCgttttagagctagaa
	taatacgactcactataGGGTCCAGACGCGTCACCAGgttttagagctagaa
	taatacgactcactataGGTGTCCATGCACTGGCTGGgttttagagctagaa
ALK	taatacgactcactataGGGGTAGGAGTTACTGCACTgttttagagctagaa
	taatacgactcactataGGTAACATTGGTGTTGCGGTgttttagagctagaa
	taatacgactcactataGGCACTAAGGGGCCATTCAAgttttagagctagaa
LTK	taatacgactcactataGGGAGTTGGAGATGACAAAAgttttagagctagaa
	taatacgactcactataGGAGTCGGTGTGCTTCACGAgttttagagctagaa
	taatacgactcactataGGGTTTGATGTCGGTAGAGTgttttagagctagaa
sgRNA design	ed to integrate Gal4 into the 5' UTR
Aug-α1	ttaatacgactcactataGGGTTTTGGAAACACCAGAAgttttagagctagaa
Aug-α2	ttaatacgactcactataGGGGTCCAATTTGGCACGAGgttttagagctagaa
Aug-β	ttaatacgactcactataGGCTGACAAACGAACAAACgttttagagctagaa
Aug-β	ttaatacgactcactataGGCTGACAAACGAACAAAACgttttagagctagaa
ALK	ttaatacgactcactataGGGAAATGAAGACTGGCCAGgttttagagctagaa
LTK	ttaatacgactcactataGGCAGTCTCCGACGCACCATgttttagagctagaa

 Table S1.
 sgRNA sequences used to generate loss-of-function mutants

The first set of lowercase letters (taatacgactcactata) indicates the T7 promoter sequence, uppercase letters indicate the CRISPR binding site that is specific to each gene target, and the second set of lowercase letters (gttttagagctagaa) indicates the annealing sequence to the 5' universal primer as previously described (29, 30).

Table S2. Primers used for sequencing, cloning, and qPCR analysis

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Gene	Forward (5' \rightarrow 3')	Reverse (5' \rightarrow 3')		
Primers used f	for sequencing for subsequent digestion by T7 assay			
Aug-α1	GCACTCTGGTTTCGGG	CCACCTGTCTCATGATTTCC		
Aug-α2	GCTCTCTTTGCCCACC	GACTTTATTAACGTCTTTTGTTTCC		
Aug-β	CGGGCTGAGAAGAGATGG	GCAGTGTTTTCACGCGAG		
ALK	GGTACGTTTGGTGTATCTGG	CACACATAACAAGATGTGTGTG		
LTK	GCAGATGTAGTTTCCACATGAC	CGCTTTCTTGTTCTTTGATTG		
Primers used f	for 6-FAM PCR analysis [X = fluorescein (6-FAM)]			
Aug-α1	XGCACTCTGGTTTCGGG	CCACCTGTCTCATGATTTCC		
Aug-α2	XCACCTTTCAGCATGAGTGC	GACTTTATTAACGTCTTTTGTTTCC		
Aug-β	XCGGGCTGAGAAGAGATGG	GCAGTGTTTTCACGCGAG		
ALK	XGGTACGTTTGGTGTATCTGG	CACACATAACAAGATGTGTGTG		
LTK	XGCAGATGTAGTTTCCACATGAC	CGCTTTCTTGTTCTTTGATTG		
Primers used t	to clone the 5' UTR of each gene into eGFP Bait-E2A-KalTA4-	pA donor vector digested with BamHI and EcoRV		
Aug-α1	actgactgGGATCCCCACTTCCAGGCATCG	actgactgGATATCTCATGGTGGCGGCCCACAGCAGCCTGAGG		
Aug-α2	actgactgGGATCCCACGGCGGAATAGTGC	actgactgGATATCTCATGGTGGCGGCAAATAGACTCCGTTGGCG		
Aug-β	actgactgGGATCCGCAGTCACAAGACCCG	actgactgCATATGTCATGGTGGCGGCGATTATATATTGGTAGGTGCTTCTG		
ALK	actgactgGGATCCCCCTCAAGTGAATACAAGTGC	actgactgGATATCTCATGGTGGCGGCTCTCAGATACCCACGCTG		
LTK	actgactgGGATCCCCACTTACATATAAATAGAACGCCAG	actgactgGATATCTCATGGTGGCGGCGCGGGAAAAAAAGATTTATATTAGC		
Primers used f	for qPCR analysis			
Actin	GATCTGGCATCACACCTTCTAC	TCTTCTCTGTTGGCTTTGG		
Aug-α1	GGTGCTGGTAATGGGG	GTTTCTCCACTTGCCG		
Aug-α2	CACCACCGGCTACTGC	CGAAGATCTCTCGGAAAGAC		
Aug-β	CAGCAAGGAGGTAAAGGAGT	GTCCTGCATTCAGATGGA		
ALK	CCGTGGACAACTTCACTCTG	ATTGGTGTTGCGGTAGGAGT		
LTK	TGGTTTATCACTTCAGCA	GTGTGCTTCACGAAGG		

Uppercase letters represent gene-specific sequences while lowercase letters indicate either BamHI or EcoRV restriction site sequences.