

Tyr
WT CAGCTCGTCTCTCCAGCAGTTCCCCGAGTCTGCA**CCTCCCAGAGTCTCCAGTCC**AAACGCTGCTGTCCAGTCTGG
D_143_13 CAGCTCGTCTCTCCAGCAGTTCCCCGAGTCTGCACCTCC-----AGTCCAAACGCTGCTGTCCAGTCTGG
D_146_1 CAGCTCGTCTCTCCAGCAGTTCCCCGAGTCTGCACCTCC--CAGAAGTCTCCAGTCCAAACGCTGCTGTCCAGTCTGG
D_146_2 CAGCTCGTCTCTCCAGCAGTTCCCCGAGTCTGCACCTCC--AGAAGTCTCCAGTCCAAACGCTGCTGTCCAGTCTGG
D_149_2 CAGCTCGTCTCTCCAGCAGTTCCCCGAGTCTGCACCTCC--GAAGTCTCCAGTCCAAACGCTGCTGTCCAGTCTGG
D_135_18 CAGCTCGTCTCTCCAGCAGTTCCCCGAGTCT-----CTCCAGTCCAAACGCTGCTGTCCAGTCTGG
D_147_9 CAGCTCGTCTCTCCAGCAGTTCCCCGAGTCTGCACCTCC-----CTCCAGTCCAAACGCTGCTGTCCAGTCTGG

InsrA
WT **CCACGCATTTGTTAGACGAGTGG**GGCTCCAAACAGCCGCCAGACACTGGTCGTGGCAGCACTGGCTGTCTTTAGTGCAG**GCGCGGTGCTTGCA****TGAGGATGG**
D_91_80 CCACGCATTTGTTAGAC-----GGATGG
D_88_3 CCACGCATTTGTTAGAG---GTGGGGCTCCAAACAGCCGCCAGACACTGGTCGTGGCAGCACTGGTTGTCTTTAGTGCAGGCGCGGTGCTTGCA**TGAGGATGG**
D_87_5 CCACGCATTTGTTAG-----TGGGGCTCCAAACAGCCGCCAGACACTGGTCGTGGCAGCACTGGTTGTCTTTAGTGCAGGCGCGGTGCTTGCA**TGAGGATGG**
D_169_3 CCACGCATTTGTTAGACGAGTGGGGCTCCAAACAGCCGCCAGACACTGGTCGTGGCAGCACTGGTTGTCTTTAGTGCAGGCGCGGTGCTTGCA**TGAGGATGG**
D_90_76 CCACGCATTTGTTAGAC-----ATGAGGATGG

InsrB
WT CCACCGAATCCA**AGTCTTGGACCAGTCCA** **GAGTGG**ACAAATAGCACAGGTTTGGGTTCTTCTCGATGCGCA**CCGCTCCCCGCGTGATGTTGGTCAA**ACTG
I_111_1 CCACCGAATCCAAGATCTTGGACCAGTCCA**AGTGG**ACAAATAGCACAGGTTTGGGTTCTTCTCGATGCGCACCGCTCCCCGCGTGATGTTGGTCAA**ACTG**
D_112_48 CCACCGAATCCAAGATCTTGGACCAGTCCA-----CCCGCGTGATGTTGGTCAA**ACTG**
D_106_7 CCACCGAATCCAAGATCTTGGACCAGT-----GGACAAATAGCACAGGTTTGGGTTCTTCTCGATGCGCACCGCTCCCCGCGTGATGTTGGTCAA**ACTG**
D_112_1 CCACCGAATCCAAGATCTTGGACCAGTCCA--AGTGGACAAATAGCACAGGTTTGGGTTCTTCTCGATGCGCACCGCTCCCCGCGTGATGTTGGTCAA**ACTG**
D_100_15 CCACCGAATCCAAGATCTTGGAC-----AAATAGCACAGGTTTGGGTTCTTCTCGATGCGCACCGCTCCCCGCGTGATGTTGGTCAA**ACTG**

Figure S1 Alignment of top 5 alleles of the 3 loci in *Tg(actb2:cas9)*; (*U6x:sgRNA(insra/b)*) fish. The mutant alleles are arranged according to their frequency with the top being the most frequent. Protospacers and PAM are highlighted in yellow and cyan in the wild-type sequence, respectively. Micro-homology pairs are labelled by the same types of underline, or in bold in the wild-type sequence. Red letter in the mutant allele indicates insertion. Dashes in the mutant alleles indicate deletion.