**Supplementary Figure 1.** **CRISPR-Cas 9 targeting of *tyr* confirms high-efficiency gene targeting.** (A) Wild-type uninjected (*left*) and fish in which tyr gRNA and Cas9 encoding RNA were injected (*right*) at 48 (*top*) and 96 hpf (*bottom*). The fish in which the *tyr* gene was targeted show reductions in melanophores. (B) Bar graph showing a pixel density analysis of uninjected (n=21), *tyr* CRISPR*-*injected (n=36), or PTU-treated larvae (n=20). *tyr* CRISPR-injected larvae show reduced pixel density at both developmental ages, indicating effective gene knock-down. Each group measured against pixels detected above an arbitrary threshold determined *a priori*. Ordinary One-Way ANOVA was used \*\*\*\*=p<0.0001. (C) Fragment Analysis of WT (*top*) or *tyr* injected embryos (*bottom*). While PCR analysis of wild-type larvae reveals a single peak of ~278 base pairs, PCR analysis across this same region of tyr-CRISPR-injected larvae shows several peaks, indicating the presence of indels in the target region.

**Supplementary Table 1. Targeting and Genotyping primer information for the 8 GABAA receptor α subunits.** The primer information and exon location for each gene is shown. “Target” columns refer to the primer information that is used to generate sgRNAs. “Flanking” columns refer to the primer information used to genotype embryonic samples by fluorescent PCR after behavioral analysis was performed.

**Supplementary Figure 2. Protein sequences confirm premature stop codons in all generated F2 mutants.** The translated protein sequence of the 3 alleles used for behavioral analysis and/or electrophysiology is shown. (A) The first allele of the transheterozygous α3 mutant is depicted. A 7 base pair deletion leads to a frameshift and 9 different amino acids compared to wild-type before the first stop codon. Total protein size = 147 amino acids. (B) The second allele of the transheterozygous α3 mutant is shown. An 18 base pair insertion creates 6 different amino acids, with the 5th amino acid being a stop codon. Total protein size = 137 amino acids. (C) The α4 mutant allele. An 11 base pair deletion leads to a frameshift, creating 70 different amino acids before a stop codon. Total protein size = 194 aa. A *purple M* denotes the start methionine. *Red text* shows the sequence alignmed to the wild-type protein. *Green text* illustrates those amino acids that are different from wild-type. *Yellow highlights* indicate the transition to mutant-specific amino acid sequences. *Black text* past the stop codon shows size differences compared to wild-type proteins.

**Supplementary Table 2. Examples of mutational efficiency in targeting the 8 GABAA receptor α subunits.** CRISPR-STAT analysis was used to assess mutational efficiency for each round of injections, however only representative examples are shown. Peak information shows Relative Fluorescent Units (RFUs). *Red, bold text* indicates the expected peak size. The table only shows the first 10 peaks over 1,000 RFU. Peaks labeled with an asterisk represent instances when samples exhibited more than 10 additional peaks. In the case of a3 and a6a the expected peak fell outside of 10 initial peaks. some cases the expected peak size.

**Supplementary Figure 3. Comparisons among the 36 F0 somatic mutants subunit combinations.** Boxplots of F0 somatic mutants. Analysis is shown for (A) 48 hour duration, (B) 48 hour C-bend, (C) 96 hour duration, and (D) 96 hour C-Bend. Alternating box coloring used to optimize visualization. Ordinary one-way ANOVA and Dunnett’s multiple comparisons reveal restricted significant differences compared to Wild-type. \*\*\*\* = p<0.0001. These data are also depicted in the heat matrices shown in Figures 2, 3 and 4.

**Supplementary Movie 1.** **Representative movie of a wild-type larva touch response at 48 hpf.** The video was recorded at 250 frames/second (1.18 seconds in length) and was used to generate the trajectory trace shown in Figure 2D. Wild-type larvae respond to touch by performing a C-bend followed by rhythmic, smaller amplitude body bends to propel the animal away from the touch stimulus.

**Supplementary Movie 2. Representative movie of an α3/α5 F0 somatic mutant at 48 hpf.** The video was recorded at 250 frames/second (5.48 seconds in length) and was used to generate the trajectory trace shown in Figure 2D. α3/α5 mutants exhibit both increased swimming durations and C-bends per response.

**Supplementary Movie 3. Representative movie of an α4/α5 F0 somatic mutant at 48 hpf.** The video was recorded at 250 frames/second (3.93 seconds in length) and was used to generate the trajectory trace shown in Figure 2D. α4/α5 mutants exhibit increased swimming durations without a statistically significant increase in the number of C-bends.

**Supplementary Movie 4. Representative movie of an α3/α4 F0 somatic mutant at 48 hpf.** The video was recorded at 250 frames/second (0.99 seconds in length) and was used to generate the trajectory trace shown in Figure 3C. α3/α4 mutants exhibit increased C-bends without a statistically significant increase in length of response durations.