Table S1. Endpoint behaviors tested in this study.

Endpoint (units)	Definition	Interpretation
Total distance traveled (cm)	Total distance the zebrafish traveled within the novel tank $$\Sigma\sqrt{(X_2\text{-}X_1)^2+(Y_2\text{-}Y_1)^2+(Z_2\text{-}Z_1)^2}$$	Reflects general motor/ neurological phenotypes.
Average swimming speed (cm s^{-1})	$$\left(\frac{\text{Total distance (cm)}}{\text{Total time (s)}}\right)$$	Reflects motor aspects of zebrafish swimming, may be increased or decreased depending on the nature of behavioral test.
Maximum speed (cm s^{-1})	Maximum speed fish capable to reach	
Minimum speed (cm s^{-1})	Minimum speed fish capable to reach	
Freezing time (s)	Total time when speed less than 1 cm s^{-1}	Indicates increased anxiety and freezing time is generally higher in stressed zebrafish.
Swimming time (s)	Total time when speed 1-10 cm s^{-1}	
Rapid movement time (s)	Total time when speed more than 10 cm s^{-1}	
Turning angle (°)	Total turning angle in 1 frame $$\left(\frac{1}{\left(\tan\left(\frac{\Delta Y}{\Delta X}\right)\times180\right)\times\pi^{-1}}\right)$$	Reflects motor aspects of zebrafish swimming, may be increased or decreased depending on the nature of behavioral test.
Angular velocity (° ms^{-1})	Magnitude and direction of zebrafish angular speed $$\frac{\text{(Turning angle} \times \text{frame rate)}}{1000}$$	
Absolute turn angle	Total turning angles without considering the sign of angular direction	
Fast type latent time (s)	Total time when Angular velocity above 0.5° ms^{-1}	
Slow type latent time (s)	Total time when Angular velocity below 0.5° ms^{-1}	
Meandering (° m^{-1})	Movement without a fixed direction or path (zig-zag movement) $$\left(\frac{\text{Absolute turn angle (°)}}{\text{Total distance (cm)}} \times 100\right)$$	
Time spent in the top (%)	Total time spent in the top portion of the novel tank	More top entries indicates lower anxiety level.
Time spent in the bottom (%)	Total time spent in the bottom portion of the novel tank	More bottom entries indicates higher anxiety level.

Table S2. Statistical analysis of locomotion in 2% ethanol-treated adult zebrafish based on 3D swimming assay.

		Distance and Speed		Absolute Turn Angle (°)	Average Angular Velocity (° s⁻¹)	Meandering (° m⁻¹)	Movement Time Ratios			Time in Top Ratio (%)	Time in Bottom Ratio (%)
		Total distance (cm)	Average speed (cm s⁻¹)				Freezing Time Ratio (%)	Swimming Time Ratio (%)	Rapid movement time ratio (%)		
Wild Type (Control)	Fish 1	1556.4	5.2	1288.4	4.3	82.78	1%	95%	4%	91%	9%
	Fish 2	1483.8	4.9	1536.3	5.1	103.54	1%	95%	4%	74%	26%
	Fish 3	1668.7	5.6	1371.0	4.6	82.16	1%	93%	6%	70%	30%
	Fish 4	1770.2	5.9	1486.3	5.0	83.96	1%	90%	9%	72%	28%
	Fish 5	1373.7	4.6	1829.1	6.1	133.15	2%	94%	4%	52%	48%
	Fish 6	2045.4	6.8	1658.2	5.5	81.07	1%	85%	14%	76%	24%
Wild Type + EtOH 2%	Fish 1	2280.6	7.6	1684.8	5.6	73.87	1%	82%	17%	3%	97%
	Fish 2	2597.7	8.7	1369.4	4.6	52.72	1%	79%	21%	3%	97%
	Fish 3	2802.9	9.3	2076.7	6.9	74.09	1%	77%	22%	4%	96%
	Fish 4	2620.4	8.7	4608.6	5.7	65.45	0%	79%	20%	5%	95%
	Fish 5	2690.3	9.0	2342.0	7.8	87.06	0%	79%	21%	1%	99%
	Fish 6	2402.1	8.0	1493.4	5.0	62.18	1%	81%	18%	2%	98%

Table S3. Comparison of different methods to measure zebrafish behavior

	Require more than 1 camera?	Major software platform to run movement tracking	Require special script to run software?	Is able to track multiple objects ?
This study	No	idTracker	No	Yes
Zhu and Weng (2007)	No	C#	Yes (Custom Made Tracking System)	Yes
Cachat and colleagues (2011)	Yes	EthoVision XT7	No	Not reported in original article
Ladu and colleagues (2015)	Yes	MATLAB	Yes (Custom Made Tracking System)	Not reported in original article
Li and colleagues (2016)	Yes	MATLAB	Yes (Custom Made Tracking System)	Not reported in original article
Macri and colleagues (2017)	Yes	MATLAB	Yes (Custom Made Tracking System)	Not reported in original article
Mwaffo and colleagues (2017)	Yes	MATLAB	Yes (Custom Made Tracking System)	Not reported in original article