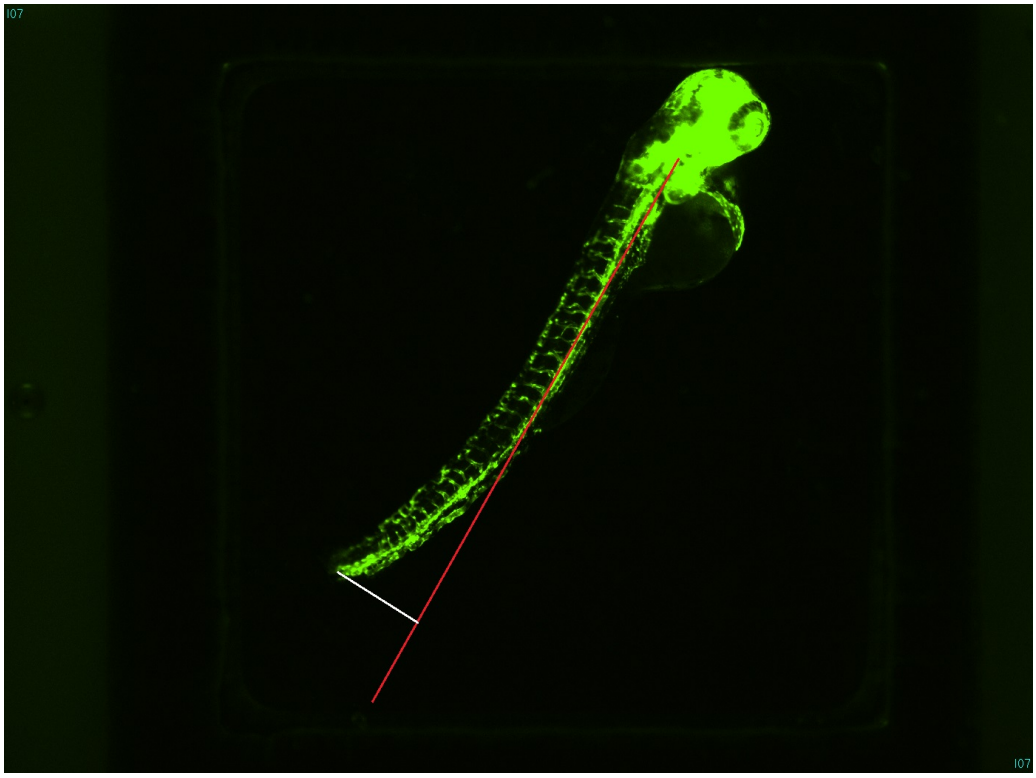

Figure S1. Representative image of a 72-hpf embryo treated with 100 nM RA. RA disrupted normal trunk and caudal fin development in the absence of detectable effects on cardiac development and function. Therefore, the distance from the caudal fin to the anteroposterior axis was manually quantified for RA-exposed embryos within MetaXpress. Red line denotes the anteroposterior axis, and white line denotes the distance from the caudal fin to anteroposterior axis. Images were acquired using a 2X objective and FITC filter cube within our ImageXpress Micro Widefield High-Content Screening System.

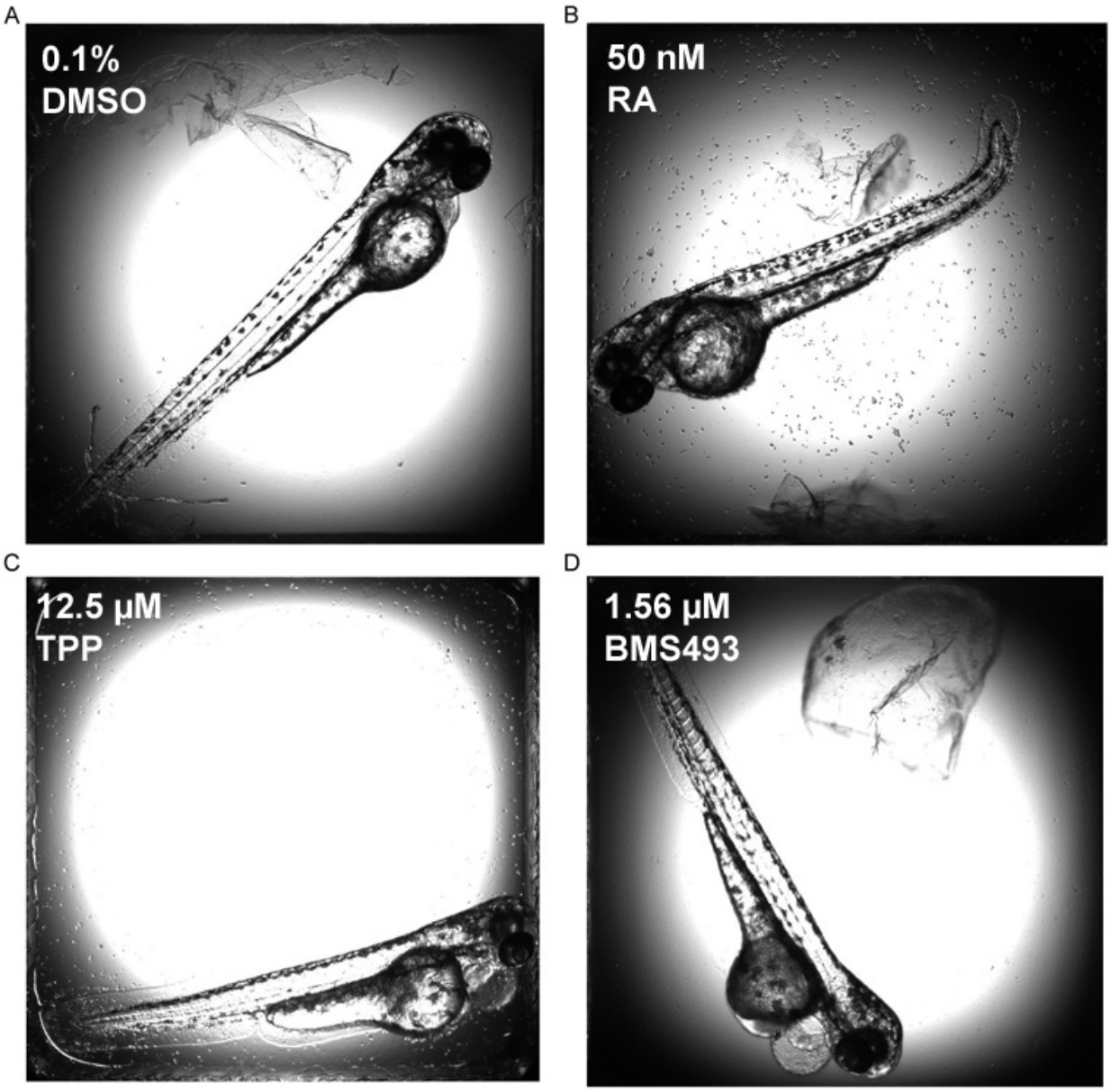

Figure S2. Representative images of 72-hpf embryos treated with 0.1% DMSO, 50 nM RA, 12.5 µM TPP, or 1.56 µM BMS493 from 5-72 hpf. RA, TPP, and BMS493 treatments represent the highest concentration that did not significantly impact body length based on Figures 1-3. Images were acquired using a 2X objective and transmitted light within our ImageXpress Micro Widefield High-Content Screening System.

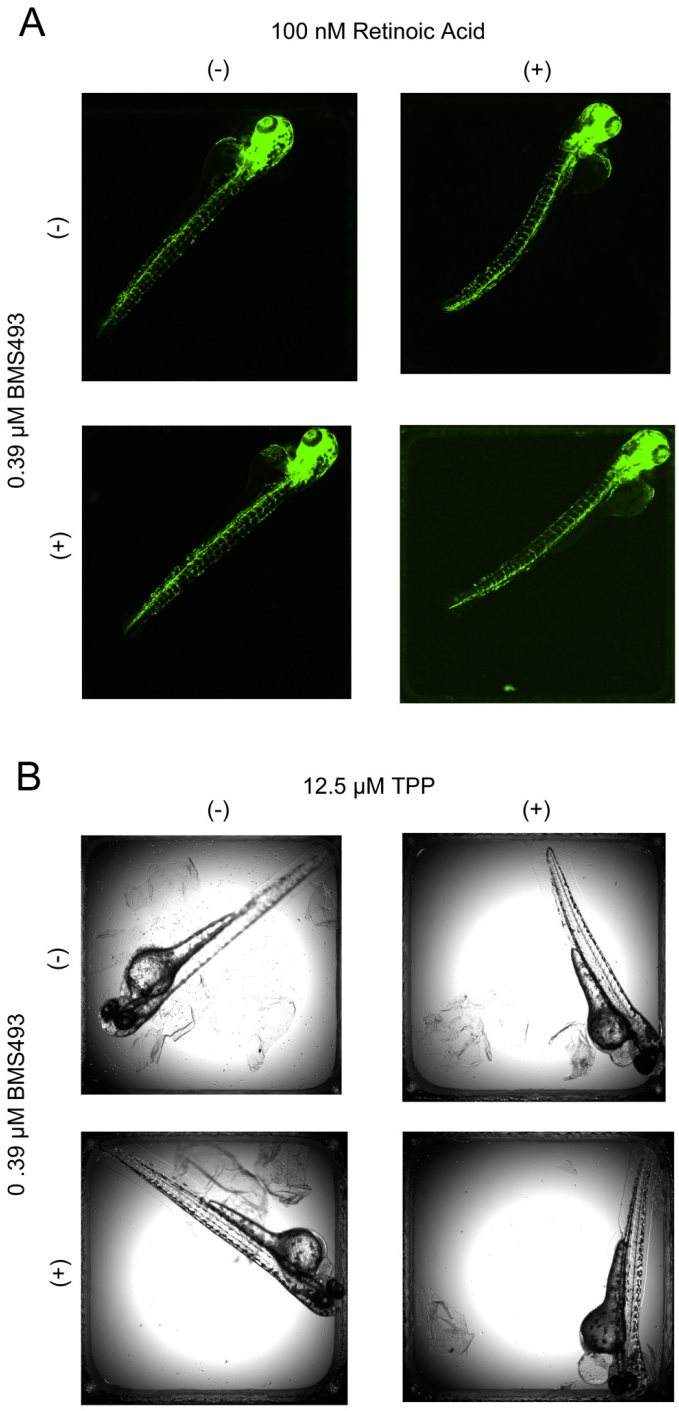

Figure S3. (A) Representative images of 72-hpf embryos treated with 100 nM RA in the presence or absence of 0.39 µM BMS493. Images were acquired using a 2X objective and FITC filter cube within our ImageXpress Micro Widefield High-Content Screening System. (B) Representative images of 72-hpf embryos treated with 12.5 µM TPP in the presence or absence of 0.39 µM BMS493. Images were acquired using a 2X objective and transmitted light within our ImageXpress Micro Widefield High-Content Screening System.

Table S1. Cell viability (percent live cells) for reference antagonist and TPP exposures within three different human RAR reporter assays. BMS195614 was used as a reference antagonist for RARα reporter assays, whereas CD2665 was used as a reference antagonist for RARβ and RARγ reporter assays.

		RARα		RARβ		RARγ	
Compound	Concentration	% Live Cells	% CV	% Live Cells	% CV	% Live Cells	% CV
DMSO	0.10%	100	1.7	100	5.3	100	0.4
TPP	3.13 µM	92	4.9	97	1.3	100	2.0
	6.25 µM	90	1.9	96	1.3	101	4.7
	12.5 µM	96	3.9	98	0.8	105	5.6
	25 µM	98	2.0	102	0.4	106	6.4
	50 µM	94	4.3	98	2.8	103	4.0
	100 µM	75	1.2	74	3.5	78	4.0
BMS195614	0.0006 µM	86	1.2	n/a	n/a	n/a	n/a
	0.002 µM	98	0.5	n/a	n/a	n/a	n/a
	0.01 µM	93	0.8	n/a	n/a	n/a	n/a
	0.04 µM	94	2.6	n/a	n/a	n/a	n/a
	0.16 µM	96	2.6	n/a	n/a	n/a	n/a
	0.63 µM	102	2.0	n/a	n/a	n/a	n/a
	2.5 µM	99	2.1	n/a	n/a	n/a	n/a
	10 µM	107	4.7	n/a	n/a	n/a	n/a
CD2665	0.0006 µM	n/a	n/a	89	0.7	89	4.8
	0.002 µM	n/a	n/a	95	0.7	95	0.8
	0.01 µM	n/a	n/a	96	0.9	97	1.1
	0.04 µM	n/a	n/a	99	1.0	100	2.5
	0.16 µM	n/a	n/a	100	1.3	102	0.5
	0.63 µM	n/a	n/a	101	1.1	100	1.2
	2.5 µM	n/a	n/a	106	0.9	107	1.0
	10 µM	n/a	n/a	114	4.8	113	2.4

% CV = percent coefficient of variation based on triplicate assay wells
n/a = not applicable