

**Table S1. Quantitative results in figures.**

	Group	Age (hpf)	Marker	Mean $\pm$ SEM	Unit	n	P value
Figure 1	Control	26	HCS-1	4.5 $\pm$ 0.2	#HCS-1 <sup>+</sup>	10	N.A.
	miR-182	26	HCS-1	7.7 $\pm$ 0.7	#HCS-1 <sup>+</sup>	13	
	miR-182 (dup-ear)	26	HCS-1	9.3 $\pm$ 0.5	#HCS-1 <sup>+</sup>	15	
	miR-96	26	HCS-1	11.2 $\pm$ 0.5	#HCS-1 <sup>+</sup>	12	
Figure 2	Control	48	HCS-1	39.9 $\pm$ 0.9	#HCS-1 <sup>+</sup>	30	
	miR-182	48	HCS-1	45.4 $\pm$ 1.0	#HCS-1 <sup>+</sup>	29	<0.05
	miR-96	48	HCS-1	30.1 $\pm$ 2.2	#HCS-1 <sup>+</sup>	26	<0.05
	Control	48	Sox2	61.6 $\pm$ 1.4	Length ( $\mu$ m)	15	
	miR-182	48	Sox2	75.3 $\pm$ 1.7	Length ( $\mu$ m)	15	<0.05
	miR-96	48	Sox2	69.5 $\pm$ 4.3	Length ( $\mu$ m)	5 *	<0.05
	Control	48	HuC	3585.9 $\pm$ 102.2	Area ( $\mu$ m <sup>2</sup> )	15	
	miR-182	48	HuC	2623.9 $\pm$ 217.6	Area ( $\mu$ m <sup>2</sup> )	15	<0.05
	miR-96	48	HuC	839.1 $\pm$ 109.3	Area ( $\mu$ m <sup>2</sup> )	7 *	<0.05
Figure 4	Control	48	HCS-1	19.3 $\pm$ 0.4	#HCS-1 <sup>+</sup>	43	
	96 MO	48	HCS-1	11.9 $\pm$ 0.7	#HCS-1 <sup>+</sup>	16	<0.05
	182/183 MO	48	HCS-1	11.2 $\pm$ 0.5	#HCS-1 <sup>+</sup>	58	<0.05
	all3 MO	48	HCS-1	9.1 $\pm$ 0.9	#HCS-1 <sup>+</sup>	14	<0.05
	Control	48	Sox2	2561.8 $\pm$ 41.0	Area ( $\mu$ m <sup>2</sup> )	12	N.S.
	96 MO	48	Sox2	2491.0 $\pm$ 55.4	Area ( $\mu$ m <sup>2</sup> )	16	
	all3 MO	48	Sox2	2429.4 $\pm$ 58.7	Area ( $\mu$ m <sup>2</sup> )	14	
	Control	48	Sox2	2278.9 $\pm$ 54.9	Area ( $\mu$ m <sup>2</sup> )	16	
	182/183 MO	48	Sox2	2158.5 $\pm$ 58.1	Area ( $\mu$ m <sup>2</sup> )	21	N.S.
	Control (LC)	48	Prox1	35.8 $\pm$ 1.4	#Prox1 <sup>+</sup>	24	
	96 MO(LC)	48	Prox1	28.1 $\pm$ 1.6	#Prox1 <sup>+</sup>	16	
	182/183 MO (LC)	48	Prox1	22.4 $\pm$ 2.0	#Prox1 <sup>+</sup>	22	
	all3 MO(LC)	48	Prox1	13.5 $\pm$ 3.0	#Prox1 <sup>+</sup>	14	
	Control	48	HuC	4001.1 $\pm$ 76.8	Area ( $\mu$ m <sup>2</sup> )	27	
	96 MO	48	HuC	3360.2 $\pm$ 85.0	Area ( $\mu$ m <sup>2</sup> )	16	<0.05
	182/183 MO	48	HuC	3525.2 $\pm$ 89.0	Area ( $\mu$ m <sup>2</sup> )	26	<0.05
	all3 MO	48	HuC	3446.5 $\pm$ 112.7	Area ( $\mu$ m <sup>2</sup> )	14	<0.05
Figure 5	Control	48	HCS-1	19.4 $\pm$ 0.4	#HCS-1 <sup>+</sup>	21	
	96 MO	48	HCS-1	14.1 $\pm$ 0.6	#HCS-1 <sup>+</sup>	30	<0.05
	96 MO/miR-182	48	HCS-1	16.4 $\pm$ 0.5	#HCS-1 <sup>+</sup>	42	<0.05

N.A. = not available; N.S. = not significant.

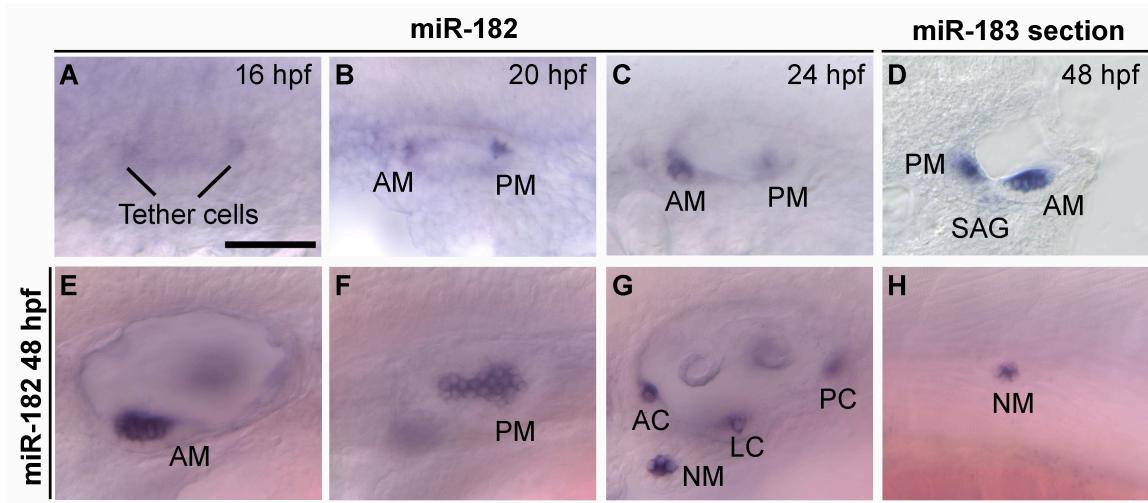
\*: a subset of data were used to show exceptional examples.

**Table S2. Effects of various combinations of MOs against miR-183, miR-96 and miR-182 on the number of hair cells in the PM.** Embryos are between 48 to 50 hpf. Control (CO) embryos were uninjected or injected with standard MOs. The ratio of the number of HCS-1<sup>+</sup> or ET4<sup>+</sup> cells in the PM between morphants and CO was calculated for each manipulation.

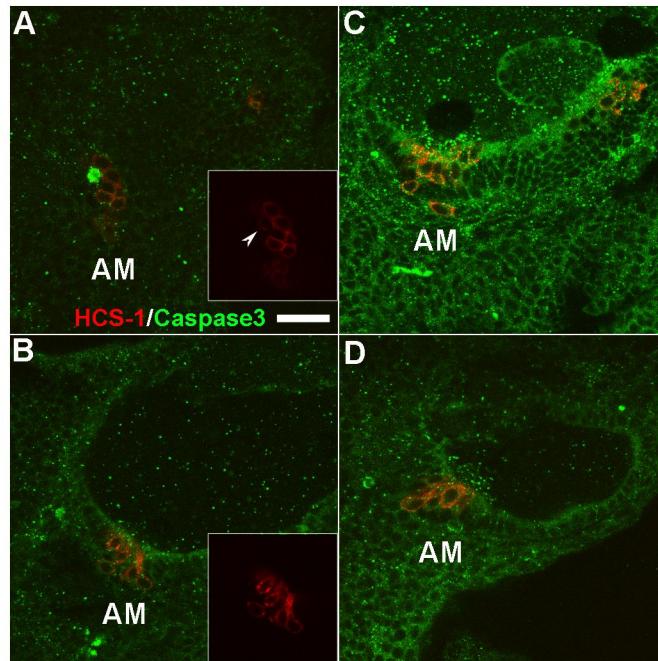
Experiment (MO against)	Total Conc. (mM)	Age (hpf)	Marker	Control (CO)		Morphant (MO)		MO/CO (# HCS- 1 <sup>+</sup> )
				Mean $\pm$ SEM	n	Mean $\pm$ SEM	n	
pre-miR-182	1	48	HCS-1	17.0 $\pm$ 0.5 *	8	11.8 $\pm$ 0.9	9	0.69
miR-182	1	50	HCS-1	21.2 $\pm$ 0.7 **	16	12.0 $\pm$ 1.4	7	0.56
miR-182	0.67	49	HCS-1	22.1 $\pm$ 1.6	7	15.2 $\pm$ 0.9	13	0.68
miR-182	0.33	50	HCS-1	17.6 $\pm$ 1.4	10	13.2 $\pm$ 1.3	10	0.75
pre-miR-183	1	48	HCS-1	17.0 $\pm$ 0.5 *	8	9.6 $\pm$ 0.7	9	0.56
miR-183	1	50	HCS-1	21.2 $\pm$ 0.7 **	16	13.5 $\pm$ 1.6	8	0.64
pre-miR- 182+pre-miR- 183	1	48	HCS-1	15.1 $\pm$ 0.5	37	11.1 $\pm$ 0.5	32	0.73
miR-182+miR- 183	2	48	HCS-1	19.4 $\pm$ 0.5	31	11.2 $\pm$ 0.5	58	0.58
miR-182+miR- 183	2	48	ET4	27.6 $\pm$ 0.9	17	15.3 $\pm$ 1.0	15	0.55
miR-182+miR- 183+miR-96	1.5	48	HCS-1	19.2 $\pm$ 0.4	12	9.1 $\pm$ 0.9	14	0.47

\* The same group of control embryos

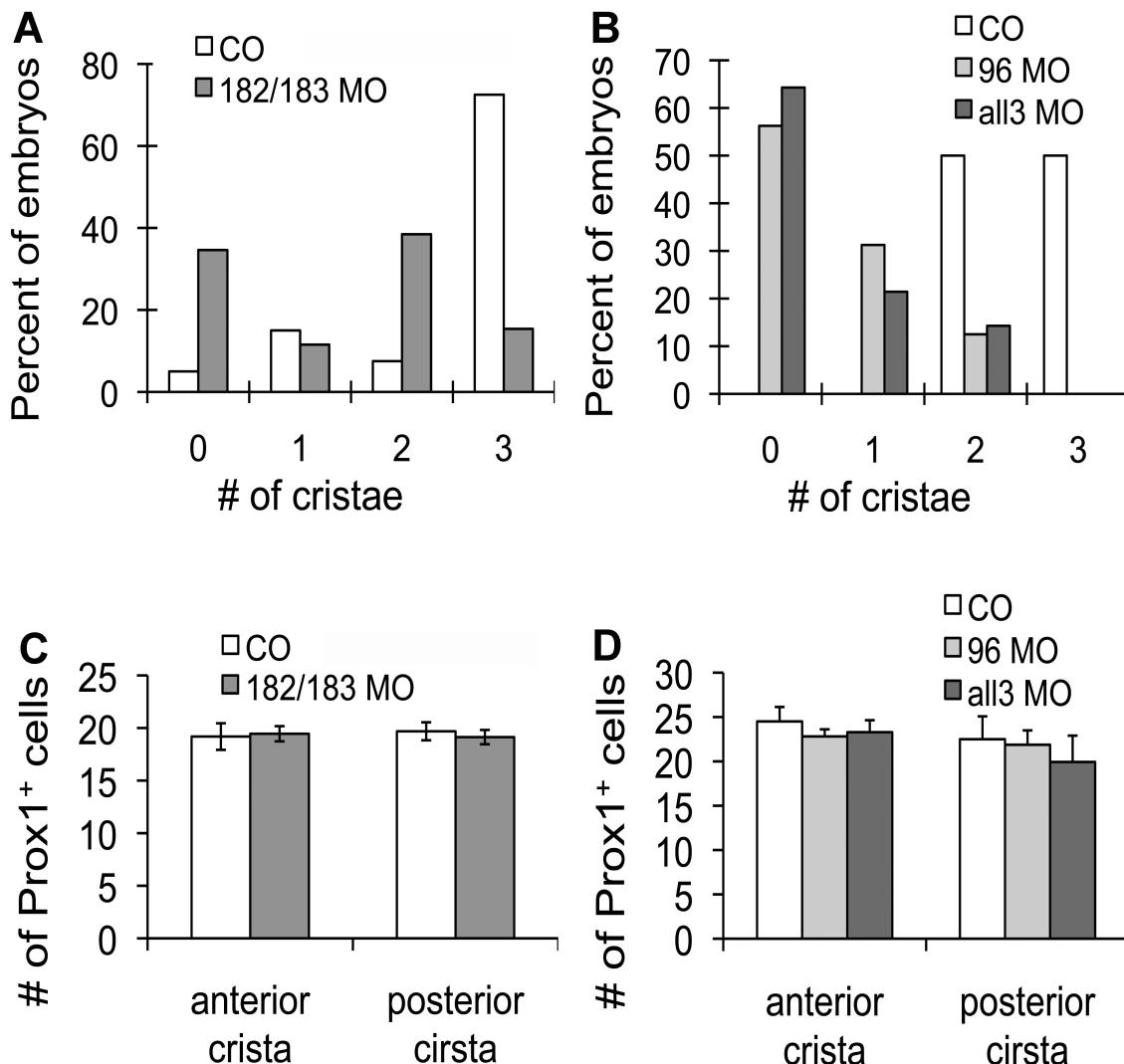
\*\* The same group of control embryos



**Figure S1. Expression of miRNAs in the developing zebrafish inner ear and lateral line by *in situ* hybridization.** Expression of miR-182 in the tether cells at (A) 16 hpf, (B) 20 hpf, (C) 24 hpf. (E-H) Expression of miR-182 in hair cells of the inner ear at 48 hpf, showing (E) the anterior macula (AM), (F) the posterior macula (PM), (G) the anterior crista (AC), lateral crista (LC), posterior crista (PC), anterior lateral line neuromast (NM), and (H) the posterior lateral line NM. (D) Transverse section of a 48 hpf inner ear, showing expression of miR-183 in the hair cell layer of the AM and PM, and in the SAG. All images, except (D), are lateral views. Anterior is left, dorsal is up. (D) Medial is left, dorsal is up. Scale bar: 50  $\mu$ m.



**Figure S2. Ectopic hair cells in the supporting cell layer of miR-96-injected embryos are not undergoing apoptosis.** (A-D) Immunostaining of miR-96-injected embryos with HCS-1 (red) and activated Caspase3 (green) at 48 hpf. White arrow in (A) shows a Caspase3<sup>+</sup> hair cell in the normal anterior macula (AM). Background green fluorescence was deliberately raised to visualize the epithelial boundaries. Lateral views. Anterior is left, dorsal is up. Scale bar: 25  $\mu$ m.



**Figure S3. Appearance of hair cells in the cristae is disrupted in the miR-183 family morphants.** Embryos are at 48 hpf. Control (CO): uninjected or injected with standard

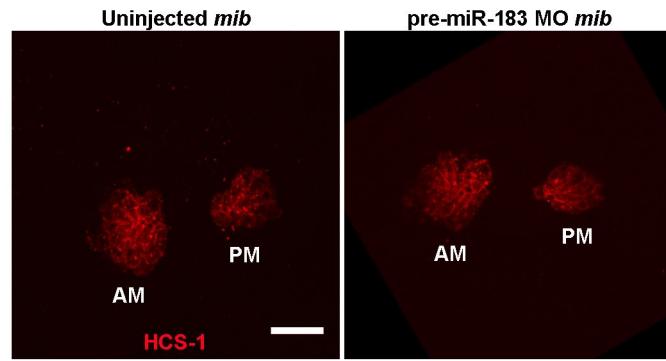
MOs (1 mM). (A) Histogram of the number of cristae counted by viewing HCS-1<sup>+</sup> or ET4<sup>+</sup>

foci (Parinov et al., 2004) (quantification: mean  $\pm$  SEM, CO  $2.5 \pm 0.1$ , n = 40; 182/183

MO  $1.3 \pm 0.2$ , n = 52). (B) Histogram of the number of cristae counted by viewing HCS-

1<sup>+</sup> foci (quantification: mean  $\pm$  SEM, CO  $2.5 \pm 0.2$ , n = 12; 96 MO  $0.6 \pm 0.2$ , n = 16; all3

MO  $0.5 \pm 0.2$ , n = 14). (C) Quantification of the number of Prox1<sup>+</sup> cells of the AC (mean  $\pm$  SEM, CO  $19.2 \pm 1.3$ , n = 16; 182/183 MO  $19.4 \pm 0.7$ , n = 22) and PC (mean  $\pm$  SEM, CO  $19.7 \pm 0.8$ , n = 16; 182/183 MO  $19.1 \pm 0.7$ , n = 22), and (D) Quantification of the number of Prox1<sup>+</sup> cells of the AC (mean  $\pm$  SEM, CO  $24.5 \pm 1.6$ , n = 8; 96 MO  $22.8 \pm 0.8$ , n = 16; all3 MO  $23.3 \pm 1.4$ , n = 14) and PC (mean  $\pm$  SEM, CO  $22.5 \pm 2.5$ , n = 8; 96 MO  $21.9 \pm 0.9$ , n = 16; all3 MO  $20.0 \pm 1.9$ , n = 14) indicate normal prosensory regions of the AC and PC in morphants. Abbreviations: AC, anterior crista; LC, lateral crista; PC, posterior crista.



**Figure S4. Knockdown of miR-183 in *mindbomb* (*mib*) embryos affects formation of hair cells.** Embryos are at 30 hpf and stained with HCS-1 for hair cells. Lateral views. Anterior is left, dorsal is up. (*left*) uninjected *mib* embryo; (*right*) miR-183 *mib* morphant. Abbreviations: AM, anterior macula; PM, posterior macula. Scale bar: 25  $\mu$ m.

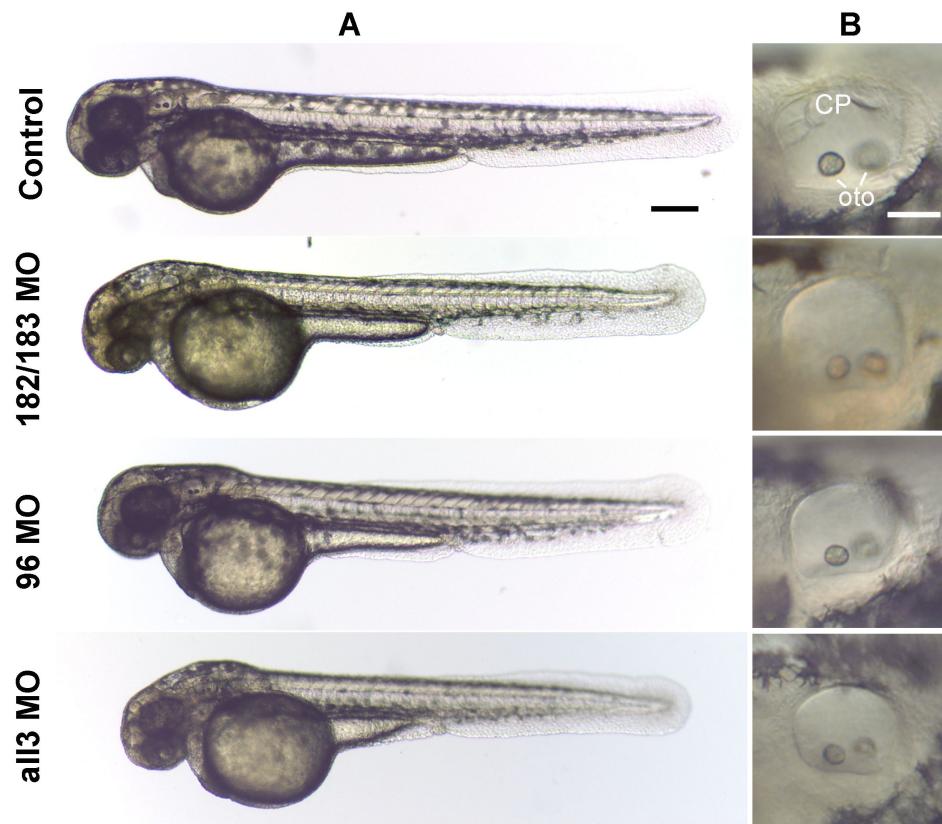
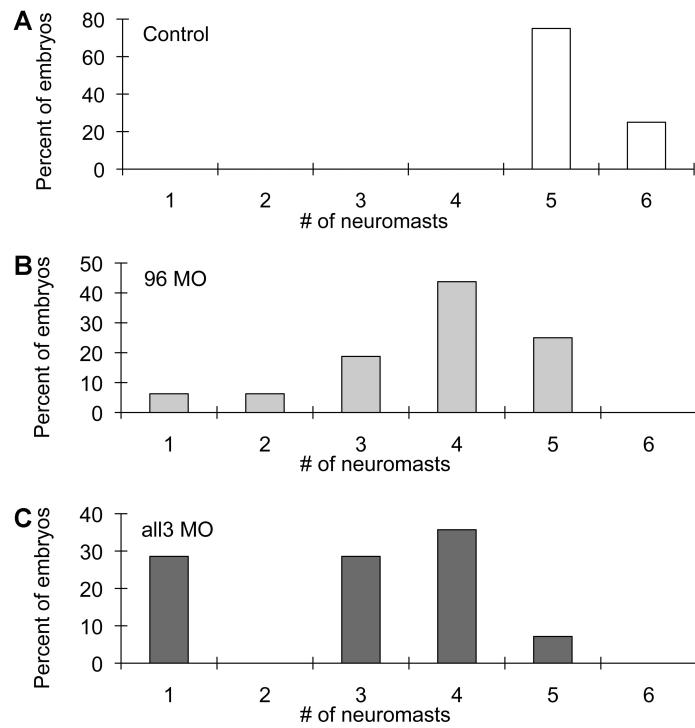
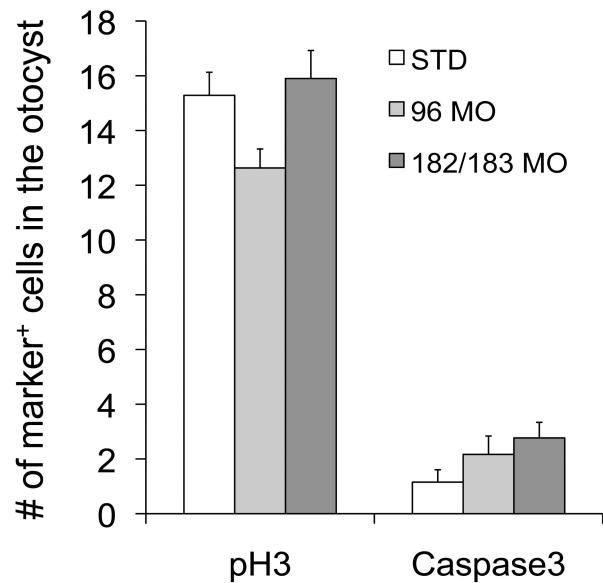


Figure S5. **Overall development of the miR-183 family morphants is normal.**

Embryos are at 48 hpf. Control: uninjected. Column (A) shows bright field images of live embryos; column (B) shows bright field images of the otocyst. Slight differences in pigmentation and length of yolk tube between controls and morphants were observed. Anterior is left, dorsal is up. Abbreviations: CP, canal pillars; oto, otoliths. Scale bar: (A) 200 µm, (B) 50 µm.



**Figure S6. Knockdown of miR-96 or the three miR-183 family members adversely affects formation of neuromasts on the lateral line.** Embryos are at 48 hpf. Control: uninjected. Histogram of the number of neuromasts on the posterior lateral line in (A) controls, (B) 96 MO and (C) all3 MO embryos. (quantification: mean  $\pm$  SEM, CO 5.2  $\pm$  0.1, n = 12; 96 MO 3.8  $\pm$  0.3, n = 16; all3 MO 2.9  $\pm$  0.4, n = 14).



**Figure S7. Cell proliferation and cell death in the inner ear of the miR-183 family morphants are not significantly changed.** Embryos are at 32 hpf. STD: injected with standard MOs (1 mM). Quantification of the number of pH3<sup>+</sup> cells and activated Caspase3<sup>+</sup> cells in the otocyst (mean  $\pm$  SEM, pH3: STD  $15.3 \pm 0.8$ , n = 21; 182/183 MO  $15.9 \pm 1.0$ , n = 20; 96 MO  $12.6 \pm 0.7$ , n = 19; Caspase3: STD  $1.2 \pm 0.4$ , n = 13; 182/183 MO  $2.8 \pm 0.6$ , n = 13; 96 MO  $2.2 \pm 0.7$ , n = 12).

**Reference:**

Parinov S, Kondrichin I, Korzh V, Emelyanov A (2004) Tol2 transposon-mediated enhancer trap to identify developmentally regulated zebrafish genes *in vivo*. Dev Dyn 231:449-459.