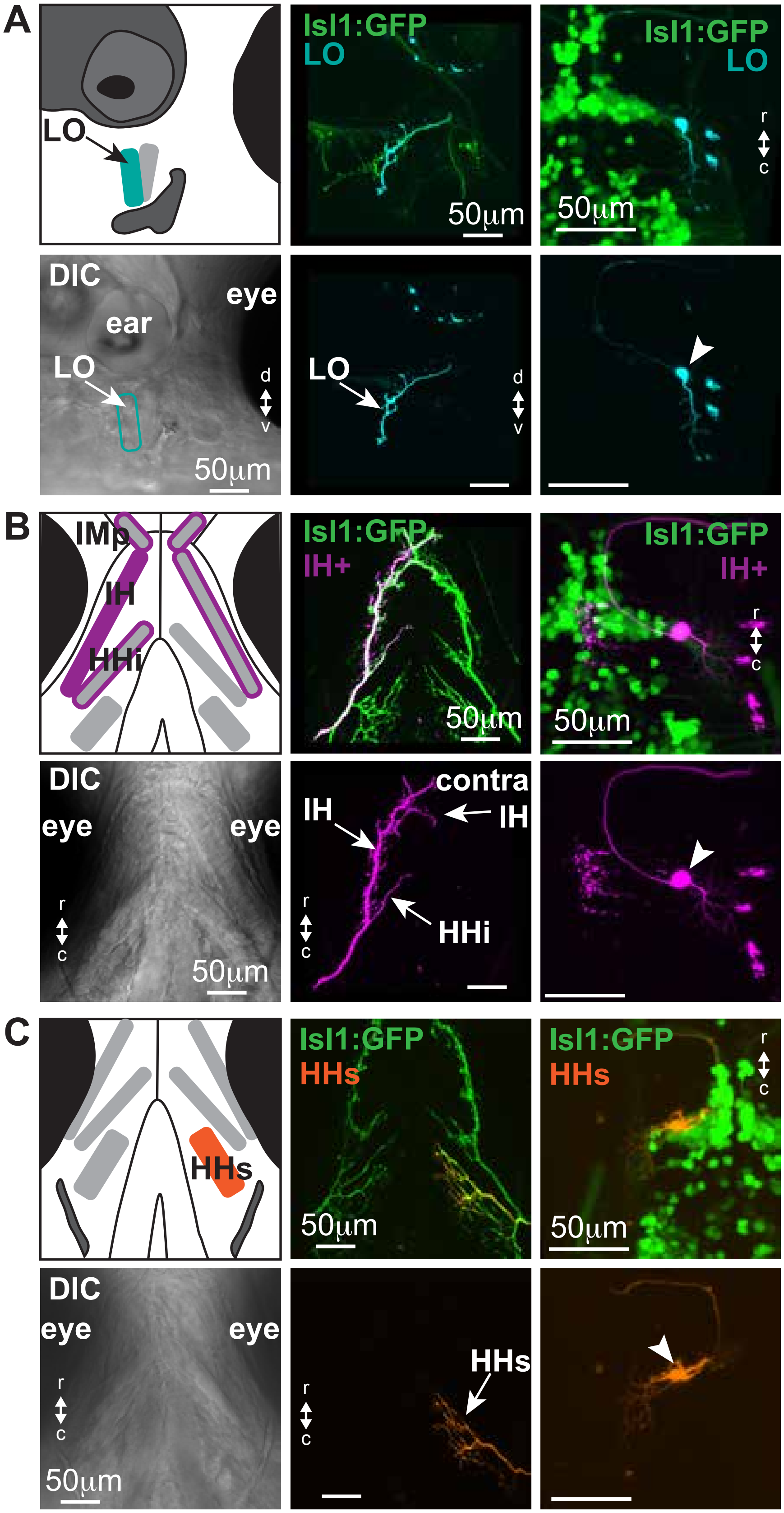

Figure S1. In zebrafish larvae, some facial motor neurons innervate a single muscle, while others innervate multiple muscles; see also Figures 1 and 2. In each section (A-C), a schematic of the cranial musculature is shown in the upper left, with a corresponding DIC view in the lower left. The axon terminal from a single-cell is shown in the middle (upper and lower) panels, with the corresponding filled cell body shown in the right (upper and lower) panels. (A) Both backfills and single-cell fills are consistent with the existence of an LO-only facial motor pool. Electroporation targeting the LO facial nerve branch did not fill other nerve branches, and the axons of filled neurons projecting to the LO do not also terminate on other muscles. (B) Both backfills and single-cell fills are consistent with the existence of a multi-muscle motor pool innervating both ipsilateral and contralateral IH plus the ipsilateral HHi and the bilateral IMp (a muscle typically associated with trigeminal innervation). As shown here, a single facial motor neuron axon can terminate on all of these muscles. Some of these muscles will fuse later in development [S1]. (C) Both backfills and single-cell fills are consistent with the existence of an HHs-only facial motor pool, though this muscle will separate into two separate muscles later in development [S1]. Electroporation targeting the HHs facial nerve did not fill other nerve branches, and the axons of filled neurons projecting to the HHs do not typically terminate on other muscles. Rarely, a small branch might be seen to extend onto the IH, but these small branches were not elaborated and might not be functional. LO = levator operculi, IH = interhyoideus, HHi = hyohyoideus inferior, HHs = hyohyoideus superior, IMp = intermandibularis posterior.

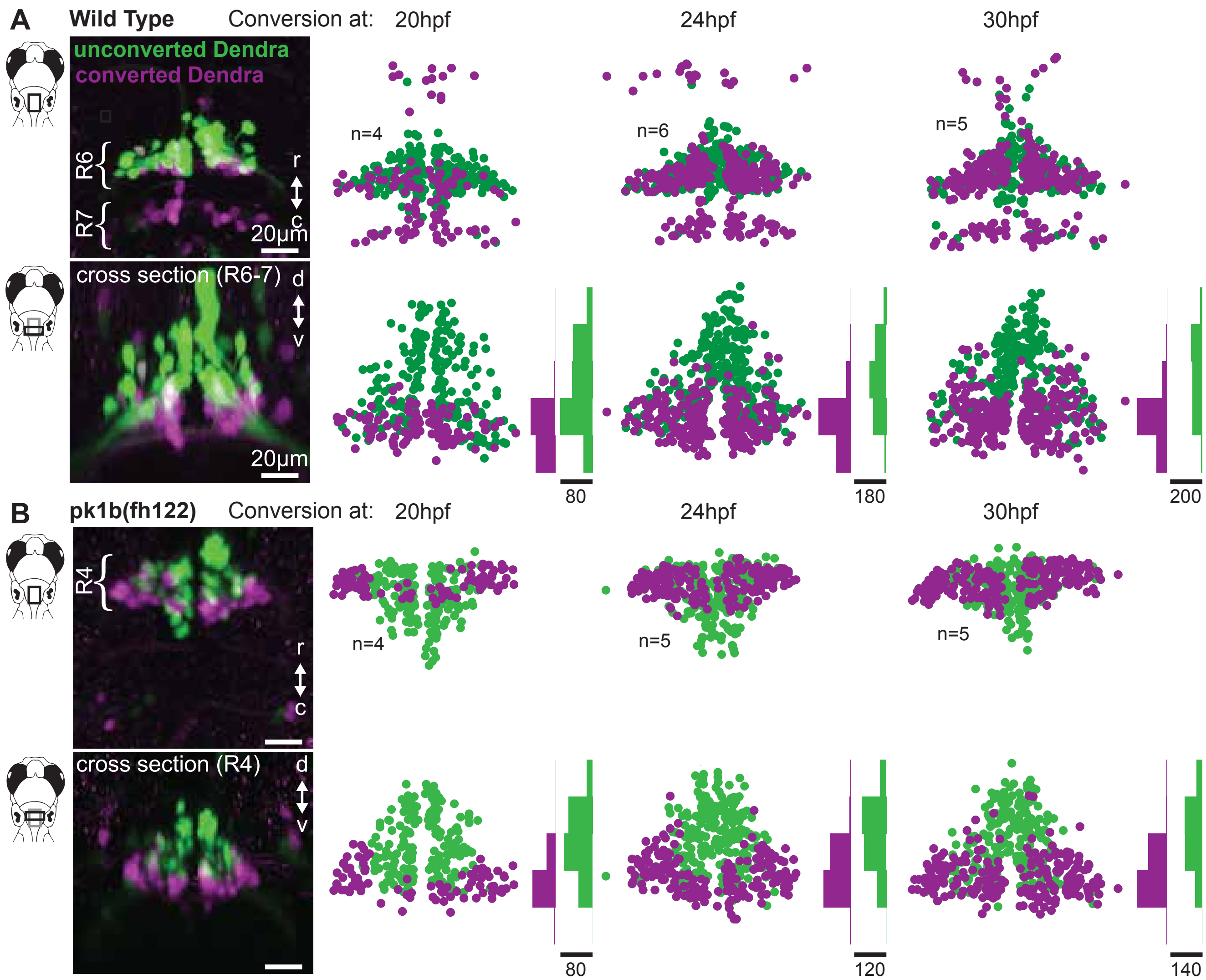

Figure S2. Facial motor neuron age topography in wild type and pk1b(fh122) mutants. Related to Figure 3. Zebrafish embryos expressed the photo convertible protein Dendra in cranial motor neurons and were exposed to ultraviolet light at three time points during facial motor neurons differentiation (20, 24 and 30 hpf), then imaged at 3 dpf. For each conversion time, neurons older than that time point contain converted (magenta/purple) Dendra, while younger neurons contain only unconverted (green) Dendra. (A) In wild type larvae, older facial motor neurons are located in the ventral-most part of the facial motor nucleus. (B) In pk1b(fh122) mutant larvae, facial motor neurons adopt the same dorsoventral age topography observed in wild type larvae. Note that there is a modest shift in the earliest conversion time point, such that the oldest mutant neurons are found laterally as well as ventrally.

Table S1. Effects of migration phenotype and muscle target on the position of facial motor pools analyzed using a linear mixed effects model. See also Figure 2.

	t statistic	DF	p value	AIC	BIC	Log Likelihood
Rostrocaudal Coordinate				2431.3	2453.3	-1209.6
Migration Phenotype	7.3006	288	$p < 0.000001$			
Muscle Target	0.58814	288	$p = 0.56$			
Interaction	-0.47243	288	$p = 0.64$			
Intercept	-9.6363	288	$p < 0.000001$			
Mediolateral Coordinate				2458.9	2481	-1223.5
Migration Phenotype	0.83606	288	$p = 0.40$			
Muscle Target	4.9507	288	$p < 0.001$			
Interaction	-1.0599	288	$p = 0.29$			
Intercept	-11.932	288	$p < 0.000001$			
Dorsoventral Coordinate				2431.2	2453.3	-1209.6
Migration Phenotype	2.2913	288	$p = 0.023$			
Muscle Target	4.6845	288	$p < 0.001$			
Interaction	-1.1255	288	$p = 0.26$			
Intercept	-3.6827	288	$p < 0.001$			

Table S2. Effects of migration phenotype and response type (as categorized by calcium imaging) on the position of facial motor neurons analyzed using a linear mixed effects model. See also Figure 6.

	t statistic	**DF**	**p value**	**AIC**	**BIC**	**Log Likelihood**
Dorsoventral Coordinate				691.9	707.23	-339.95
Migration Phenotype	2.5037	91	$p = 0.014$			
Response Type	-3.777	91	$p = 0.000283$			
Interaction	1.4951	91	$p = 0.1383$			
Intercept	0.7036	91	$p = 0.4835$			
Dorsoventral Coordinate, WT only				318.01	324.96	-155
Response Type	-5.5794	40	$p < 0.00001$			
Intercept	4.3456	40	$p < 0.0001$			
Dorsoventral Coordinate, llk only				375.54	383.42	-183.77
Response Type	-5.6921	51	$p < 0.000001$			
Intercept	10.466	51	$p < 0.0000001$			

Table S3. Effect of migration phenotype on rhythmic operculum inter-movement interval analyzed using a linear mixed effects model. See also Figure 7.

	t statistic	**DF**	**p value**	**AIC**	**BIC**	**Log Likelihood**
Inter-Mvmt Interval				11522	11552	-5757.1
Migration Phenotype	0.5061	14399	$p = 0.613$			
Intercept	3.4878	14339	$p < 0.001$			

SUPPLEMENTAL REFERENCES

S1. Diogo, R., Hinits, Y., and Hughes, S.M. (2008) Development of mandibular, hyoid and hypobranchial muscles in the zebrafish: homologies and evolution of these muscles within bony fishes and tetrapods. BMC Dev. Biol. *8,* 24.