

Boxed inset area from A'' shows single confocal slice of individual cells expressing either GFP or RFP but not both. (C) Single confocal slice of Tg(*otpb.A:Gal4*)^{z67}; Tg(*UAS:GFP*) embryo and inset (C'-C'') shows full complement of cells express GFP in absence of Gal80.

Figure S1. Schematic of positive and negative intersectional methods for labeling sub-groups of cells. (A) Positive intersectional method: a group of cells is labeled by partially overlapping expression domains of two different enhancers. Expression occurs only in cells in which both components (for example, of split Gal4) are present. (B) Negative intersectional method: a group of cells is labeled by two different enhancers; expression occurs in the cells in which only the positive activator is present.

Figure S2. Subgroups of neurons can be distinguished by inhibiting Gal4-driven expression with a fluorescently-tagged Gal80. Single slice confocal images, lateral views, anterior to left, dorsal up, of 72hpf eyes in Tg(*isl2b.3:Gal4*)^{z65}; Tg(*UAS:GFP*) embryos. Scale bar, 50µm. Immunostaining for GFP, green; TagRFP, red; Topro3 nuclear stain, magenta. (A-A'') Embryos injected with hsp70l:TagRFP (no Gal80 expression). (B-B'') Embryos injected with hsp70l:Gal80-2A-TagRFP and heat-shocked at 48hpf show expression of TagRFP (RFP) and inhibition of Gal4-dependent expression. (C-C'') Embryos injected with hsp70l:Gal80-TagRFP and heat-shocked at 48hpf show RFP expression and concomitant inhibition of Gal4-dependent expression. (A'''-C''') shows magnified view of the insets in (A''-C''), demonstrating disjoint GFP and RFP expression.