Data S1. Lines 220, 243 and 244. We generated three *Pax6-DF4:mGFP* lines with the same transgenic construct. They all showed similar GFP expression patterns. All three express GFP in a subset of ACs. The number of GFP^+ cells is similar in 243 and 244, but is greater in 220. The GFP^+ cells in all three lines project to two major IPL sublaminae. In addition, line 220 shows sparsely distributed GFP^+ neurites in sublaminae 1 and 5 (Fig. 1C,D,I). Lines 243 (Fig. 1E-G) and 244 (not shown) did not have obvious differences in IPL projection patterns. The variability between lines is probably due to positional effects at the (unknown) genomic integration sites.

We were interested whether *Pax6-DF4*-directed expression labeled discreet subtypes or random populations of ACs. The diversity of ACs in the larval zebrafish retina is unknown. A systematic Golgi study in adult roach, another cyprinid teleost and relative of the zebrafish, reported 70 distinct morphological classes of ACs (Wagner and Wagner, 1988). Although our analysis of single-cell morphology was not exhaustive, we repeatedly found the same three morphologically distinct AC types labeled in line 220 (each of which could project to either the ON or the OFF IPL, making a total of at least six AC subtypes in line 220). An example of each of the major morphological types is shown in Fig 7A-C. As the same types of cells were consistently labeled across individuals, it seems that GFP expression is confined to a fixed subset of ACs. We also observed similar morphological classes labeled across individuals in line 243.

Our analysis of line 220 with ChAT double-staining (Fig. 1I-K) and other molecular markers such as GAD67 (not shown) indicate that the GFP^+ population does not overlap with a known molecularly defined AC subclass. However, as the ACs are labeled genetically, it seems likely that they share certain (as yet unknown) gene expression patterns that would define them as a molecularly homogenous subclass. One possibility is that the DF4 element only drives transgene expression efficiently in a subset of the $Pax6^+$ ACs. In that case, the ACs labeled in our lines would constitute a subset of the $Pax6^+$ population.

Reference

Wagner, H. J. and Wagner, E. (1988). Amacrine cells in the retina of a teleost fish, the roach (Rutilus rutilus): a Golgi study on differentiation and layering. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **321**, 263-324.