Endogenous Tagging at the *cdh1* Locus for Live Visualization of E-Cadherin Dynamics

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FIG. 1. E-cadherin insertion alleles enable visualization of epithelial adherens junctions *in vivo*. **(A)** Sequence of the endogenous *cdh1* locus as well as the *cdh1-mlanYFP^{x17}* and *cdh1-tomato^{x18}* alleles. Arrow indicates the site cleaved by *cdh1*-targeted Cas9 and used to induce NHEJ. Fused fluorescent protein sequence is indicated in the light and dark shaded sequences. **(B)** Image of a *cdh1-tomato^{x18}* animal. Image is a composite of three maximum projection images covering the entire animal. Scale bar—200 μ m. **(C)** Images of distinct epithelial tissues within the zebrafish larva. Images are maximum projections of z-stacks (otic vesicle, pronephros and intestine, and tail epithelium) or a single confocal image (pharyngeal arches). Scale bar—50 μ m (otic vesicle and pharyngeal pouches); 100 μ m (pronephros and intestine and tail epithelium).

E-CADHERIN IS AN important mediator of epithelial function and is widely used as a marker of epithelial tissues.¹ We found that, during mycobacterial infections, macrophages are reprogrammed to express a broad range of canonical epithelial molecules, including E-cadherin.^{2,3} To generate animals in which this process could be visualized live, we inserted DNA encoding fluorescent proteins directly at the 3' end of the endogenous *cdh1* locus (Fig. 1).³ Nonhomologous end joining was used to insert these fluorescent proteins using the approach of Auer *et al.*⁴ These E-cadherin alleles retain all but the antepenultimate and penultimate amino acids of the E-cadherin coding sequence and directly link E-cadherin to either tdTomato or mlanYFP fluorescent proteins. Although E-cadherin is an essential gene,⁵ animals homozygous for the E-cadherin insertion alleles are viable, indicating that E-cadherin function is largely uncompromised by fluorescent tagging and the two deleted amino acids. These lines, which will be deposited at the Zebrafish International Resource Center, are freely available from the authors and will facilitate the direct visualization of E-cadherin distribution, localization, and dynamics during normal development as well as in disease.

References

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