Studying the Cellular Basis of Cancer Development Using Genetic Mouse Models

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Abstract

Imaging-based lineage tracing is a widely used method to trace progeny of particular cells and it is often done by broadly or tissue-specifically inducing Cre recombinase which can excise or invert LoxP sites. Using the Rosa26-Confetti allele, which is a conditional multicolor reporter (containing GFP, YFP, RFP and CFP), it allows simultaneously labeling of different clones and the subsequent clonal competition over time. Although the confetti allele allows simultaneous tracing of multiple cells, it remains at the observatory level. To advance the technology, we have generated confetti variant series called Red2cDNA that harbor ectopic gene expression in the red clone-specific manner. We have now generated red clone-specific expression of various oncogenes (such as *Wnt*, *Notch*, *Kras*, *PI3K* and *Yap1*) and red clone-specific loss of functions via *FLPe* recombinase and a dox-inducible system for *Cas9* endonuclease. Upon Cre activation in the tissue-specific manner in adult mice, only the red clones ectopically express oncogenes or harbor additional mutations in an otherwise genetically normal microenvironment. We envision that Red2cDNA series as a genetic mosaic system will allow precise modeling of clone-clone competition in various dynamic processes such as early development, regeneration or diseases such as cancer.

1. Red2cDNA Design

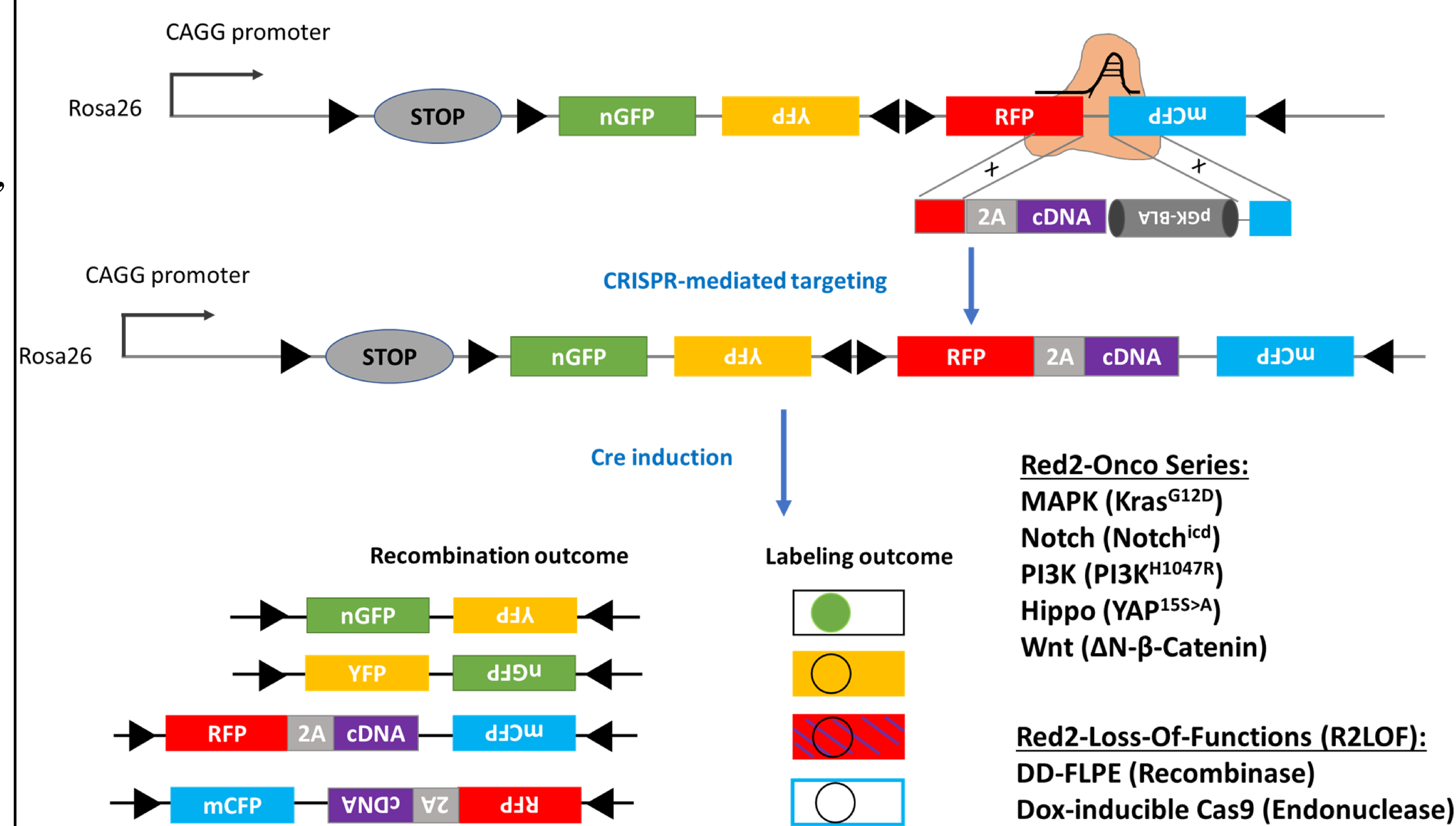

Figure 1. CRISPR-mediated targeting to insert gene of interest linked to the RFP. As a result, upon Cre induction, only the red clones express the gene of interest while all other colors remain genetically unaltered.

2. Red2-Loss-Of-Function (R2LOF) Design Strategy

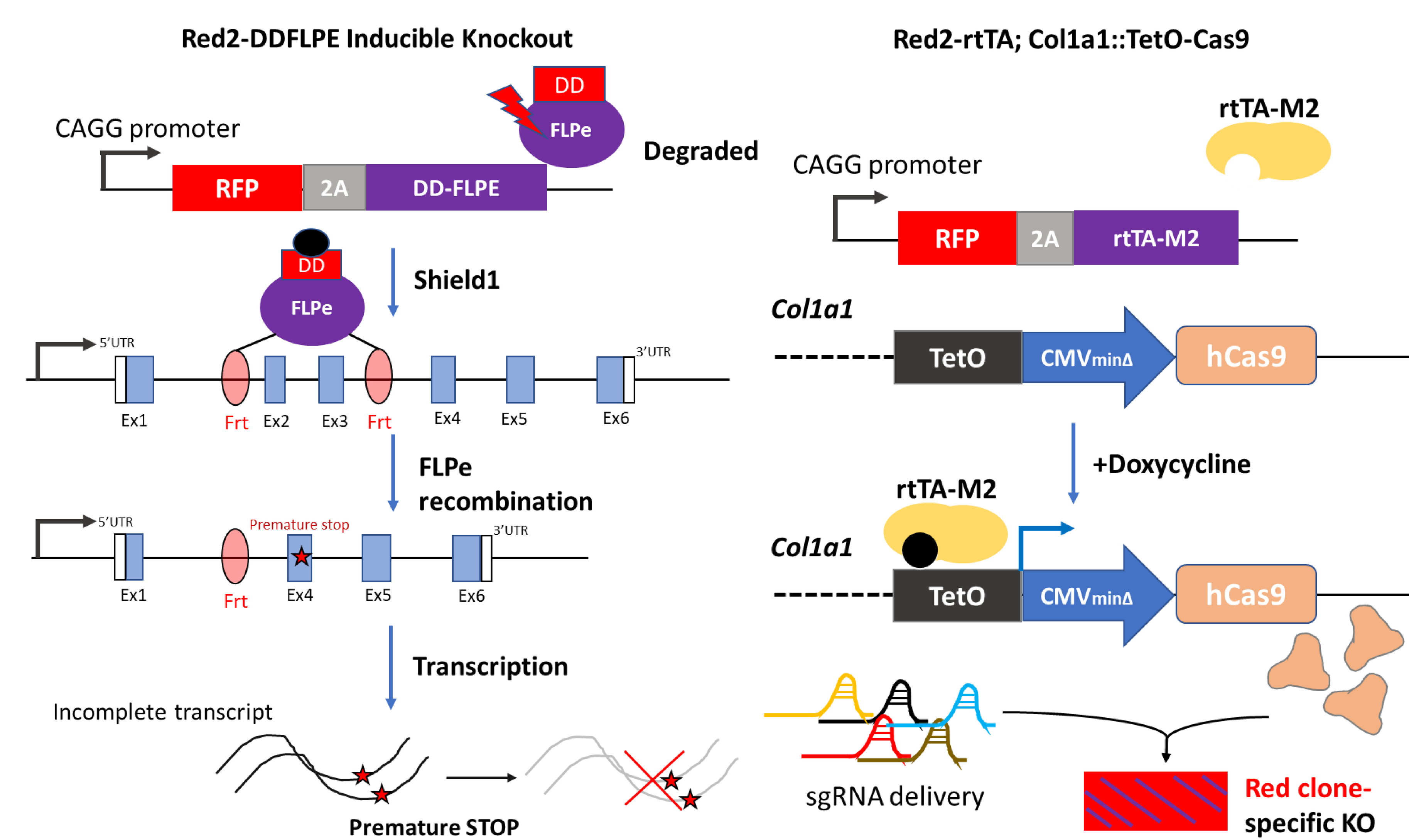

Figure 2. Loss of function in the red clone specific manner with an additional layer of expression control

3. Genotype Confirmation Via Long PCR Amplication

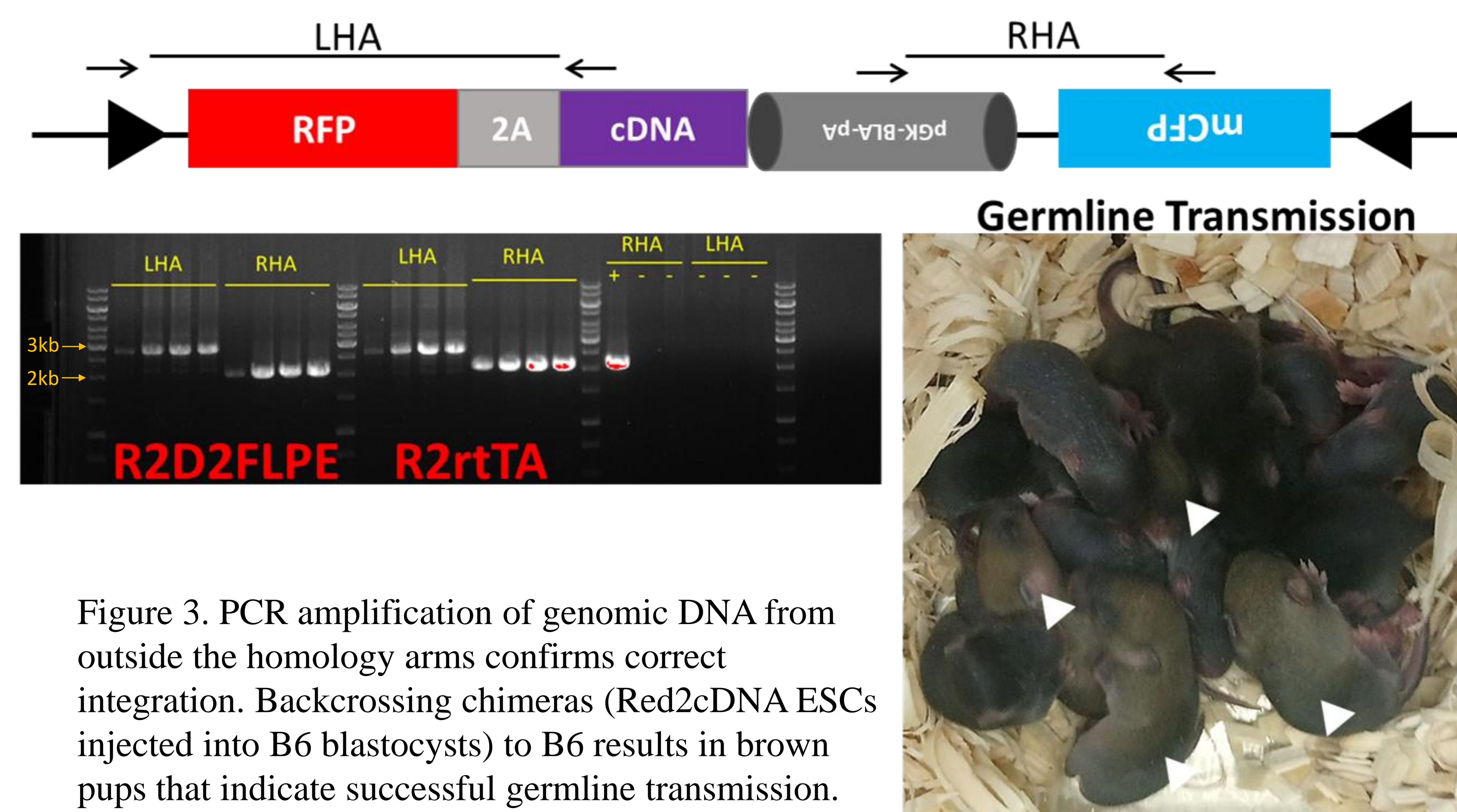

Figure 3. PCR amplification of genomic DNA from outside the homology arms confirms correct integration. Backcrossing chimeras (Red2cDNA ESCs injected into B6 blastocysts) to B6 results in brown pups that indicate successful germline transmission.

4. Using Confetti reporter to visualize clonality in the intestine

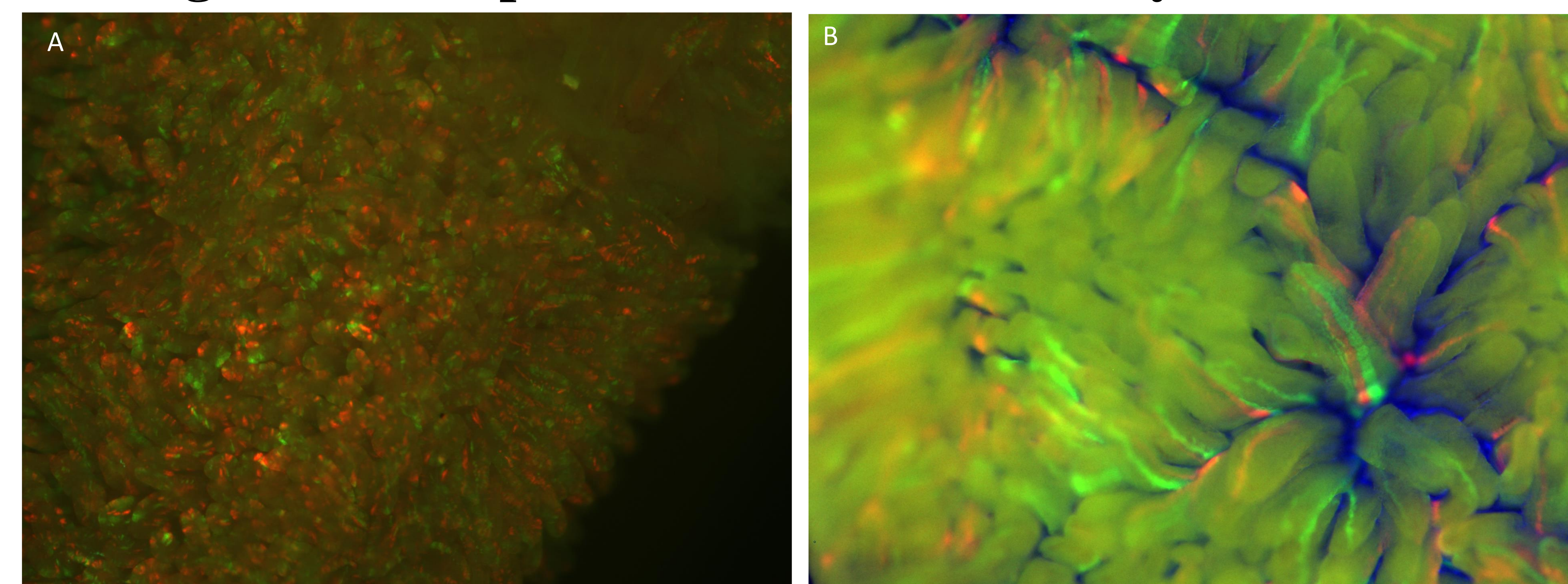

Figure 4. Confetti reporter induction with intestine specific Villin-CreERT2 and analyzed at early time point, 5 days (A) and 1 month (B). Cells are randomly labelled (with any one of the confetti colors) upon induction (A). Overtime, with tissue turnover, the subunit of tissues such as crypt and villus of the small intestine becomes progeny of a single stem cell clone, as indicated with one color stripes (B).

5. Main questions to address with Red2cDNA

a) How do different oncogenes or tumor suppressors alter clonal fitness in adult tissues?
b) Is clonal expansion of mutant cells context-dependent (such as upon injury/ageing)?
c) How do neighboring wildtype cells respond to oncogenic clones?
d) What are the molecular (eg. gene expression) changes in oncogenic and neighboring wildtype cells?