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A Versatile Strategy for Isolating a Highly Enriched Population of Intestinal Stem Cells

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SUMMARY

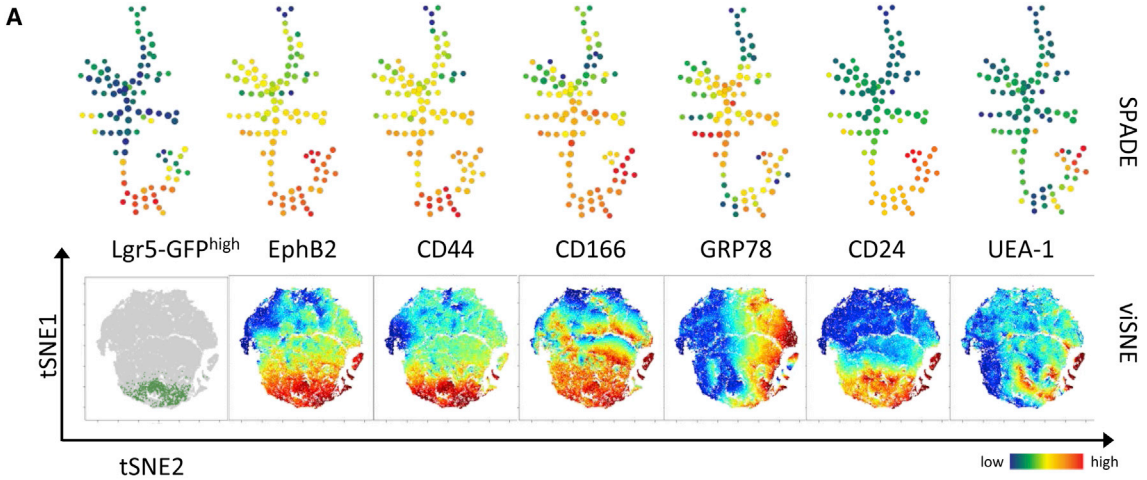
The isolation of pure populations of mouse intestinal stem cells (ISCs) is essential to facilitate functional studies of tissue homeostasis, tissue regeneration, and intestinal diseases. However, the purification of ISCs has relied predominantly on the use of transgenic reporter alleles in mice. Here, we introduce a combinational cell surface marker-mediated strategy that allows the isolation of an ISC population transcriptionally and functionally equivalent to the gold standard Lgr5-GFP ISCs. Used on reporter-free mice, this strategy allows the isolation of functional, transcriptionally distinct ISCs uncompromised by *Lgr5* haploinsufficiency.

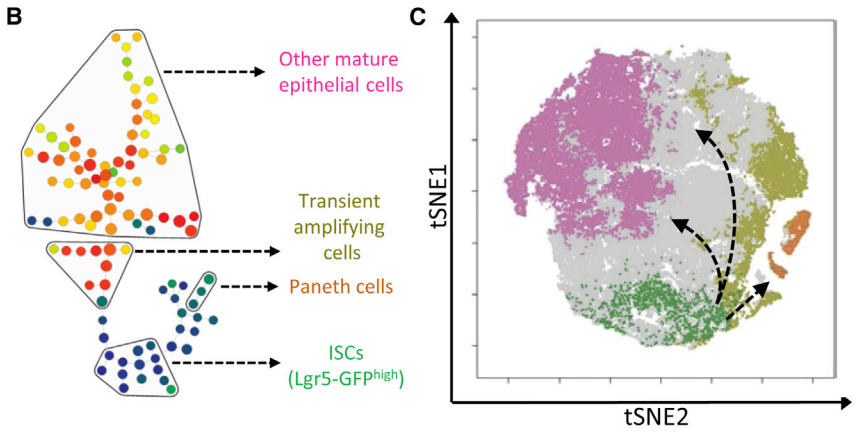
INTRODUCTION

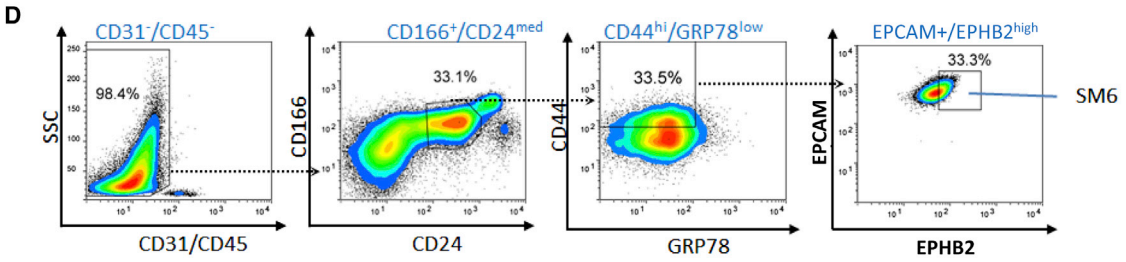
The intestinal epithelium is a dynamic tissue that relies on integration of cell division, differentiation, migration, and apoptosis. Intestinal tissue homeostasis and regeneration are facilitated by multipotent tissue stem cells that have the ability to differentiate into multiple mature cell types. Two types of stem cells are currently proposed to reside in small intestinal crypts: cycling crypt base columnar (CBC) cells and +4 reserve cells (Barker, 2014; Clevers, 2013). CBC stem cells maintain daily homeostasis, while their reserve equivalents have been postulated to play a role in tissue regeneration upon injury (Barker, 2014; Clevers, 2013). The functional study of ISCs has been made possible by the recent characterization of ISC markers such as *Lgr5*, *Olfm4*, or *Sox9*^{low} for CBC cells, and *Bmi1*, *Hopx*, *Lrig1*, or *Sox9*^{high} for their presumed quiescent counterparts (Barker et al., 2007; Gracz and Magness, 2014; Gracz et al., 2010; Powell et al., 2012; Sangiorgi and Capecchi, 2008; Takeda et al., 2011).

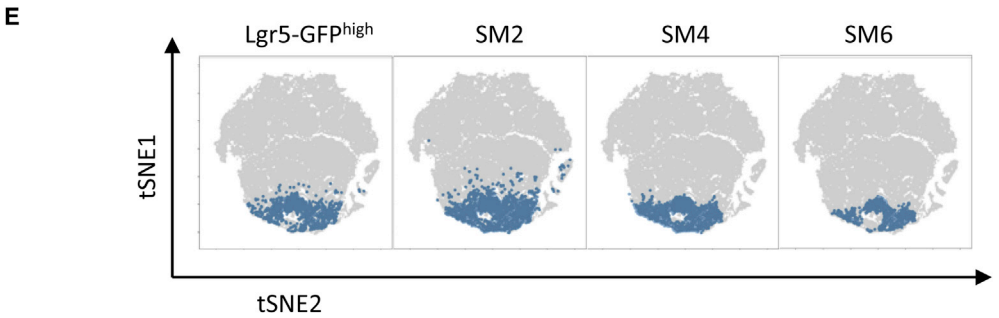
Currently, the isolation of pure ISCs is primarily restricted to the use of targeted murine reporter alleles of ISC markers. However, the fidelity and specificity of these genes to mark ISCs is still controversial (Munoz et al., 2012; Tan and Barker, 2014). The most widely used reporter for CBC cell isolation is the *Lgr5-Gfp* knockin mouse model (Barker et al., 2007), which has facilitated the isolation and characterization of CBC stem cells in many studies (van der Flier et al., 2009). However, this transgenic mouse model has several limitations: (1) the reporter cassette is prone to being silenced in over two-thirds of all crypts resulting in mosaic expression of the *Gfp* allele (Barker et al., 2007; Munoz et al., 2012); (2) LGR5 constitutes the receptor for R-SPONDINS (Carmon et al., 2011; de Lau et al., 2011; Glinka et al., 2011), potent WNT signal enhancers and stem cell growth factors, and the potential haploinsufficiency induced by the loss of one *Lgr5* allele (replaced by the *Gfp* reporter cassette) cannot be excluded; and (3) the extensive breeding required to cross genetically modified mouse models with the *Lgr5-Gfp* reporter strain.

Several strategies have been recently developed for CBC cell isolation via cell surface markers and fluorescence-activated cell sorting (FACS; Gracz et al., 2013; King et al., 2012; Merlos-Suarez et al., 2011; Wang et al., 2013). Although they represent considerable advances in the isolation of CBC cells independently of transgenic reporter alleles, these methodologies are suggested to be contaminated with other cell types and have not been fully characterized at the molecular level. The approach by Merlos-Suarez et al. (2011) mainly relies on extracting a subset of EPHB2 high cells from EPCAM+ epithelial cells (named SM2 in our study). However, the EPHB2 receptor is not only expressed at high levels in CBC cells but also in committed progenitor cells (Merlos-Suarez et al., 2011). In another study, Wang et al. (2013) used three crypt base markers (CD24/CD166/CD44) while depleting for GRP78+ progenitor cells (named SM4 in our study). Nonetheless, the resultant









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