

NEW ANTIBODY REAGENTS TO STUDY STEM CELL-MEDIATED DEVELOPMENT AND REGENERATION OF THE MAMMALIAN INTESTINAL EPITHELIUM

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ABSTRACT

Growth and regeneration of the mammalian intestinal epithelium is driven primarily by a discrete population of stem cells, known as LGR5+ crypt base columnar (CBC) cells, resident at the base of the intestinal crypt. Despite their fundamental importance for intestinal development and regeneration, there is a lack of validated reagents that can discretely identify LGR5+ CBC cells and their direct progeny. This is likely due to the low abundance and/or accessibility of the LGR5 epitope. To address this problem, Cell Signaling Technology, Inc. (CST) has developed highly specific rabbit antibodies targeting Olfactomedin-4 (OLFM4), a glycoprotein that exhibits distinct expression in LGR5+ CBC cells in both human and mouse. These antibodies are able to detect human OLFM4, and mouse Olfm4, respectively, by Western blot in cell and tissue lysates, and by immunohistochemistry (IHC) in formalin-fixed paraffin-embedded tissue samples. Antibody specificity was confirmed by validation on cell types and/or tissues in which the presence/absence or relative abundance of Olfactomedin-4 protein has been described in the published literature. These antibody reagents provide a means to visualize CBC cells with exquisite sensitivity and specificity without the need for transgenic models. This will allow for greater insights into the role and regulation of CBC cells during development, regeneration and oncogenic transformation of the intestinal epithelium.

METHODS

Monoclonal Antibody Generation

OLFM4 (D1E4M) XP® Rabbit mAb # 14369 and Olfm4 (D6Y5A) XP® Rabbit mAb (Mouse Specific) #39141 are recombinant rabbit monoclonal antibodies, generated at Cell Signaling Technology, Inc. using patented XMT® Technology.

Western Blot

Western blot analyses were performed using extracts from cell or tissue extracts, as described in the figure legends. Western blot protocol details can be found at <http://www.cellsignal.com/common/content/content.jsp?id=western>

Immunohistochemistry

Paraffin-embedded tissue sections were deparaffinized and rehydrated, then subjected to antigen retrieval in sodium citrate, pH 6.0. Primary antibodies were incubated overnight at 4°C. Detection was performed using SignalStain® Boost IHC Detection Reagent (HRP, Rabbit) #8114 and SignalStain® DAB Substrate Kit #8059.

Immunofluorescence

Perfusion-fixed mouse small intestine was cryopreserved in 30% sucrose and embedded in OCT medium. 12 µm sections rinsed in PBS prior to blocking and permeabilization with 5% goat serum/PBS containing 0.3% Triton X-100 (PBST). Primary antibodies were diluted in PSBT containing 1% BSA and incubated overnight at 4°C. Detection was performed with Anti-rabbit IgG (H+L), F(ab')₂ Fragment (Alexa Fluor® 488 Conjugate) #4412 for 1 hour at 25°C, and sections were counterstained with DyLight™ 554 Phalloidin #13054 and DRAQ5® #4084.

CONCLUSIONS

OLFM4 (D1E4M) XP® Rabbit mAb #14369 is a recombinant rabbit monoclonal antibody that detects human OLFM4 protein in western blot and immunohistochemistry with high specificity and sensitivity.

Olfm4 (D6Y5A) XP® Rabbit mAb (Mouse Specific) #39141 is a recombinant rabbit monoclonal antibody that detects mouse Olfm4 protein in western blot, immunoprecipitation, immunofluorescence and immunohistochemistry with exceptional specificity.

These antibodies may be used to detect or visualize OLFM4-expressing cells, including intestinal stem/progenitor cells and neutrophils, in normal tissues, intestinal adenoma / carcinoma, and in organoids derived from normal or cancerous tissues [1, 2].

For stem cell research, these tools provide a means to identify intestinal stem/progenitor cells without the obligatory use of transgenic mice models [3].

Antibody Validation

OLFM4 (D1E4M) XP® Rabbit mAb # 14369

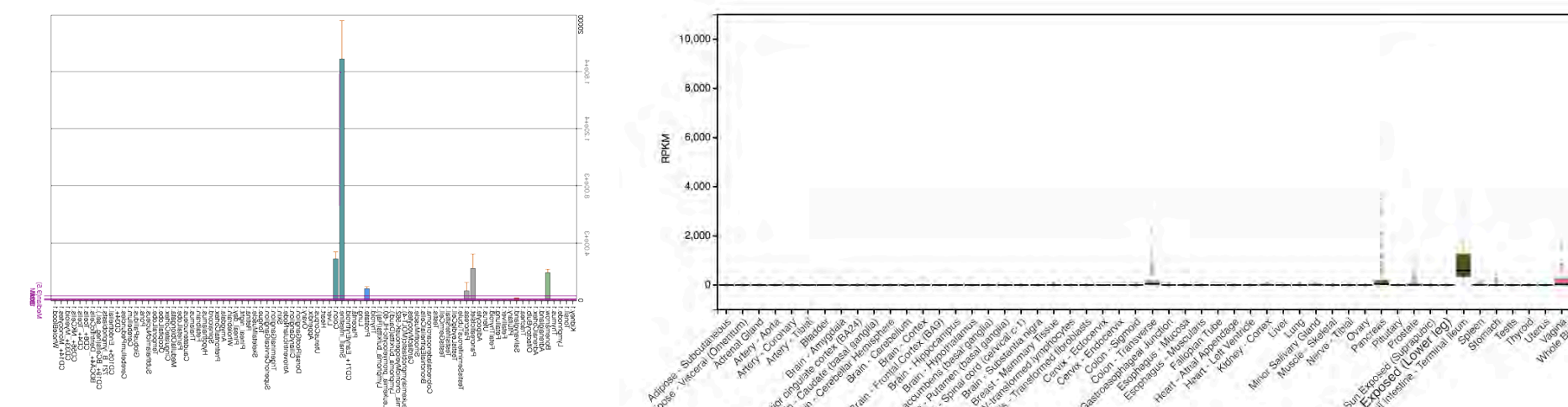


Fig. 1. Messenger RNA expression pattern of human *OLFM4* as reported in BioGPS (left) and GTex Portal (right).

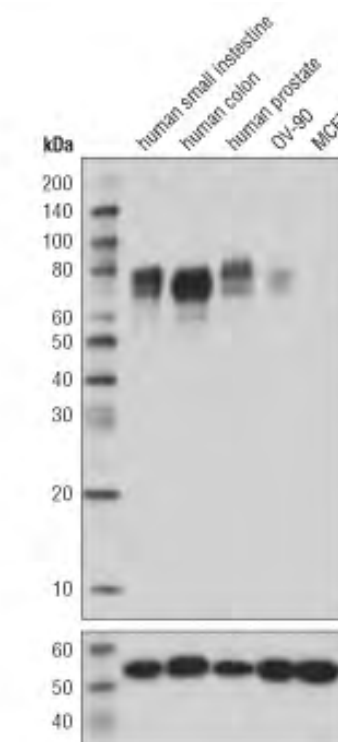


Fig. 2. Western blot analysis of extracts from human small intestine, colon, prostate, and selected cancer cell lines (OV-90, MCF7) using OLFM4 (D1E4M) XP® Rabbit mAb #14369 (upper) and β-actin (D6A8) Rabbit mAb #8457 (lower). Observed protein expression is consistent with mRNA expression levels as reported in bioinformatic databases (BioGPS, GTex Portal, Cancer Cell Line Encyclopedia).

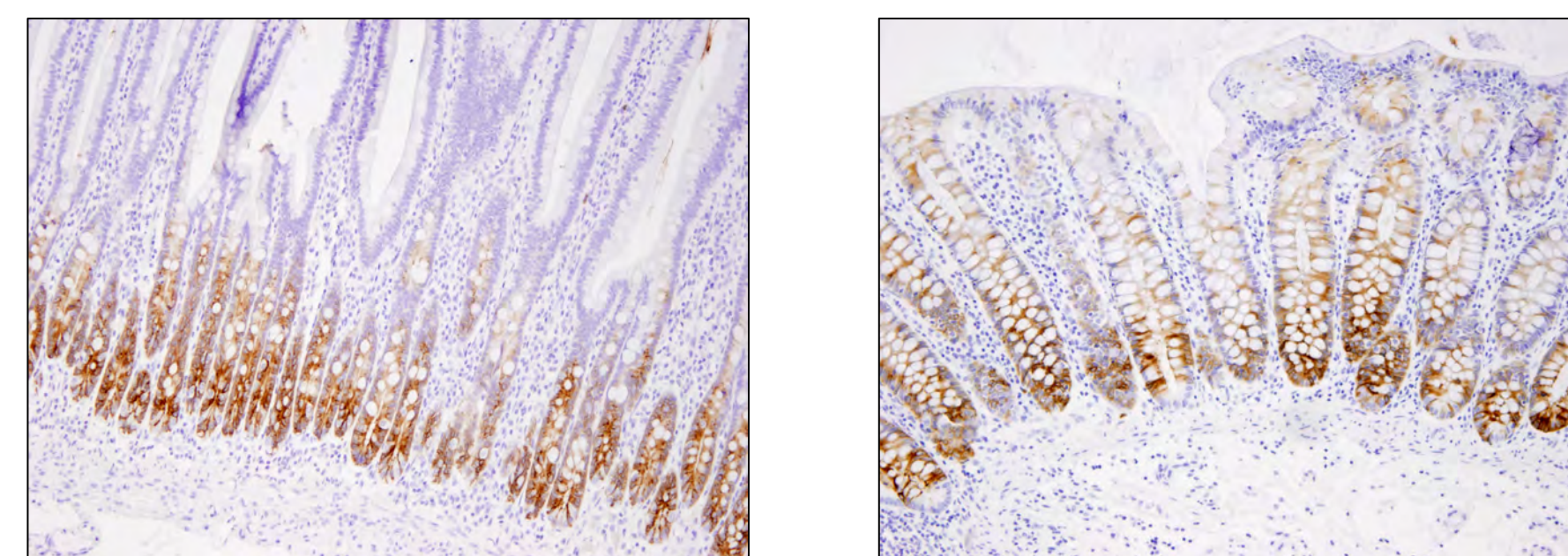


Fig. 3. Immunohistochemical analysis of paraffin-embedded human small intestine (left) and human colon (right) using OLFM4 (D1E4M) XP® Rabbit mAb #14369. Note the robust expression in the crypt component of the small intestine.

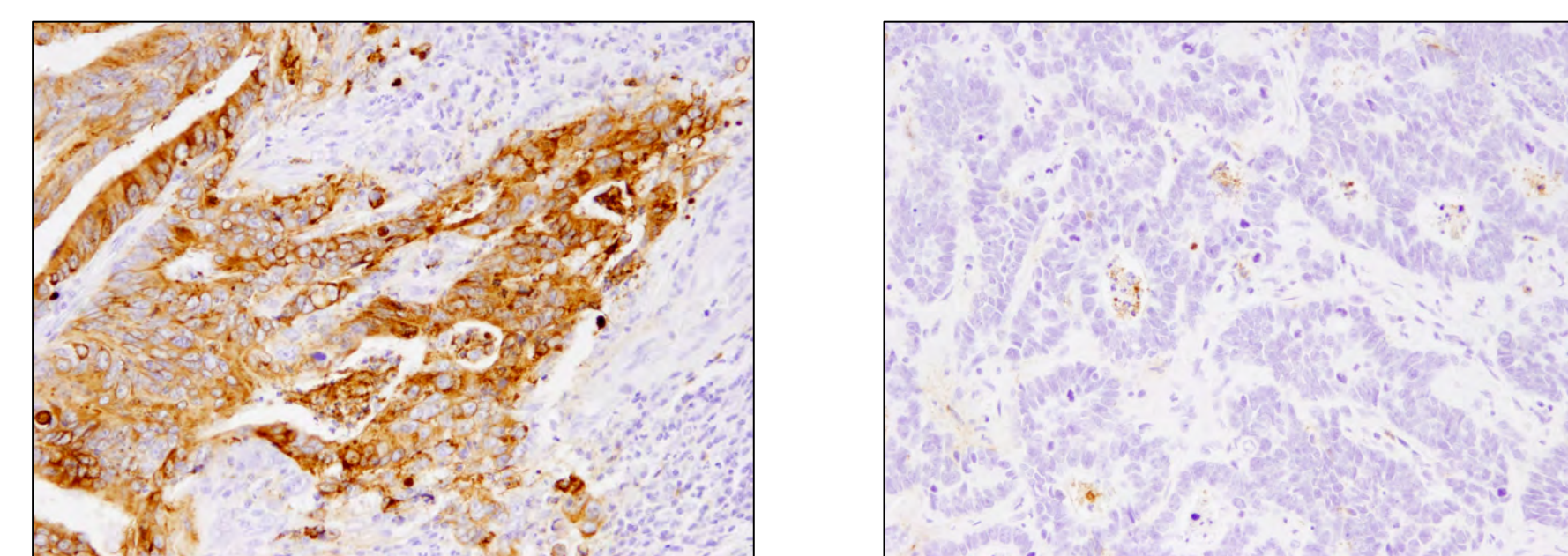


Fig. 4. Immunohistochemical analysis of paraffin-embedded human colon carcinoma, Stage I (left) and Stage IV (right) using OLFM4 (D1E4M) XP® Rabbit mAb #14369. Note the stronger expression in the tumor of the Stage I case versus the Stage IV case, as reported [4].

Antibody Validation

OLFM4 (D6Y5A) XP® Rabbit mAb (Mouse Specific) #39141

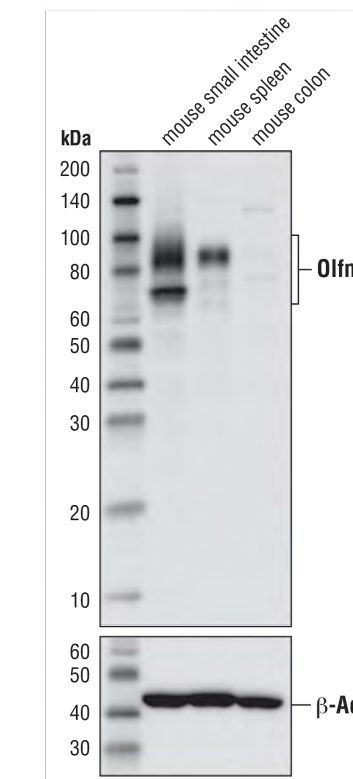


Fig. 5. Western blot analysis of extracts from mouse small intestine, spleen, and colon using Olfm4 (D6Y5A) XP® Rabbit mAb #39141 (upper) and β-actin (D6A8) Rabbit mAb #8457 (lower).

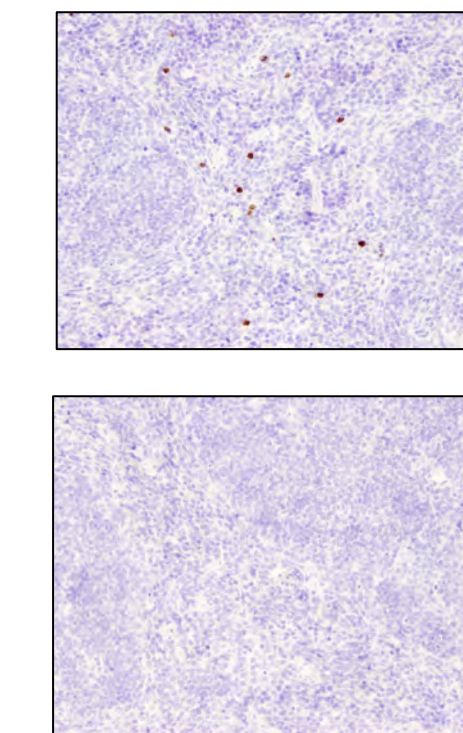


Fig. 6. Immunohistochemical analysis of paraffin-embedded spleen from wild-type mouse (upper) and *Olfm4*^{-/-} mouse (lower), using Olfm4 (D6Y5A) XP® Rabbit mAb (Mouse Specific) #39141.

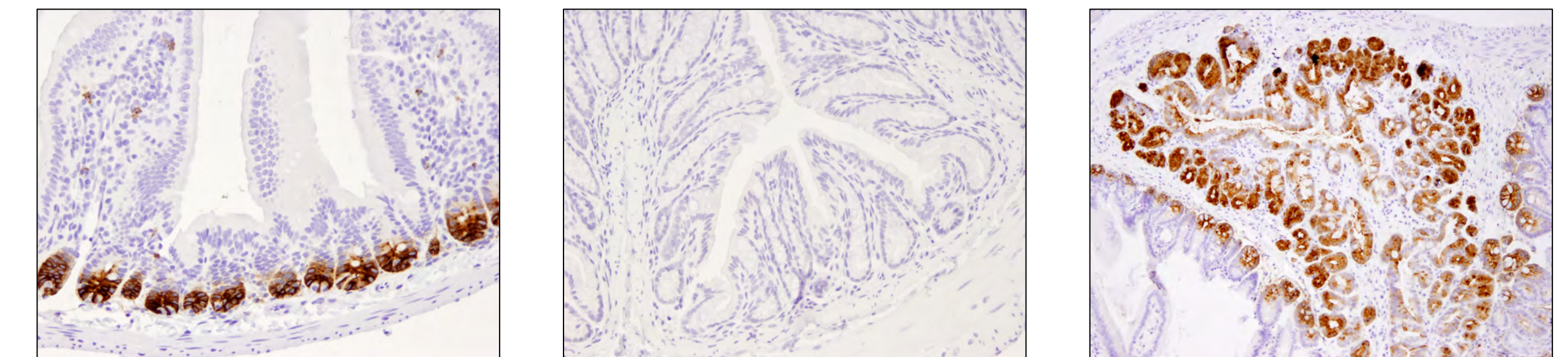


Fig. 7. Immunohistochemical analysis of paraffin-embedded mouse normal small intestine (left), mouse normal colon (middle) and *Apc* (min/+) mouse intestinal adenoma, using Olfm4 (D6Y5A) XP® Rabbit mAb #39141. Note the presence of strong crypt base staining in small intestine, absence of staining in colon, and pervasive staining in adenoma (adjacent to normal regions with crypt base-only staining).

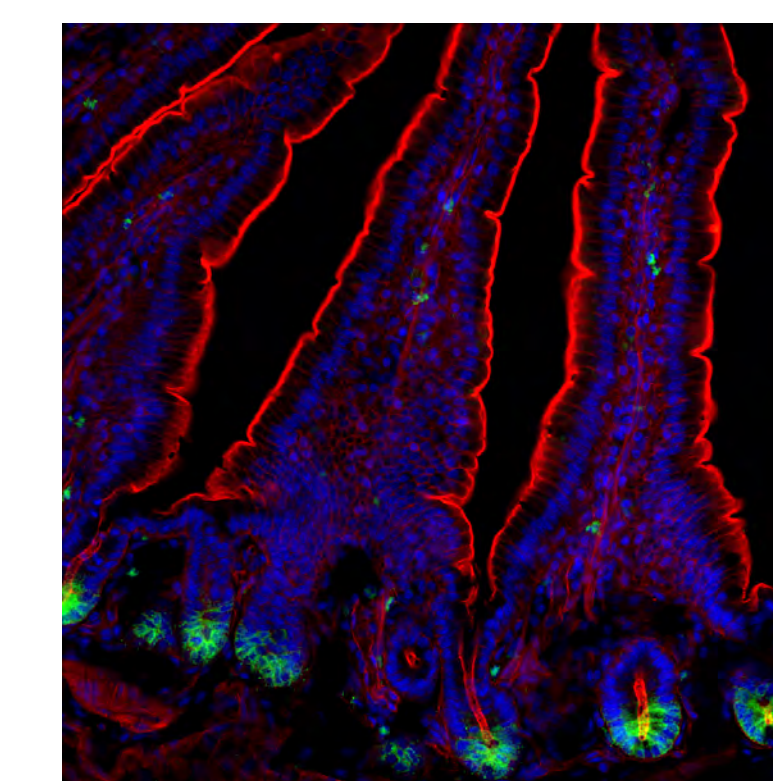


Fig. 8. Confocal immunofluorescent analysis of frozen mouse small intestine, using Olfm4 (D6Y5A) XP® Rabbit mAb (Mouse Specific) #39141 (green). Actin filaments were labeled with DyLight™ 554 Phalloidin #13054 (red). Blue pseudocolor = DRAQ5® #4084 (fluorescent dye).

References

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4. Besson et al. 2011. A quantitative proteomic approach of the different stages of colorectal cancer establishes OLFM4 as a new nonmetastatic tumor marker. *Mol. Cell. Proteomics* 10 (12): M111.009712 DOI:10.1074/mcp.M111.009712.

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