SUPPLEMENTAL MATERIAL

Balbinot et al., https://doi.org/10.1084/jem.20170934

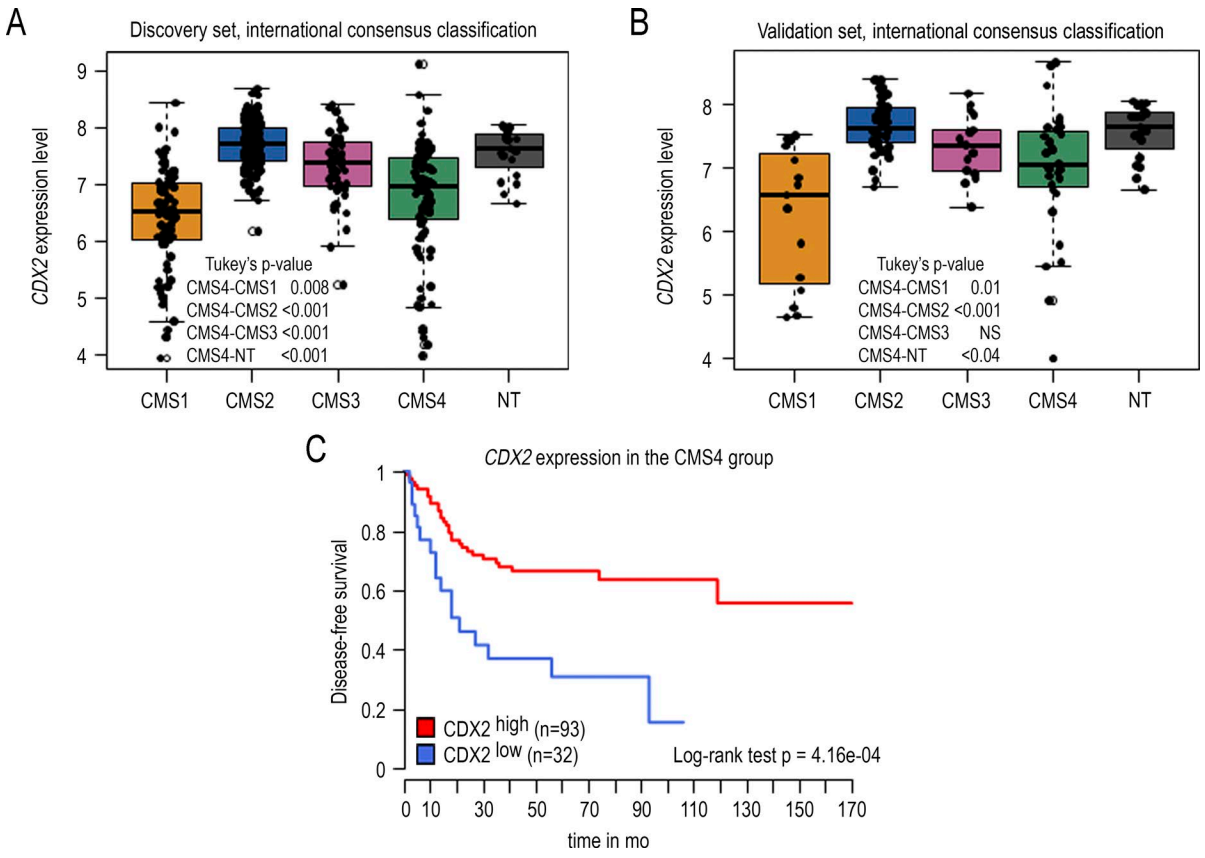

Figure S1. ***CDX2* gene expression level in 566 human colon cancers and 19 nontumoral samples of the GSE39582 dataset. (A)** Boxplot of the level of *CDX2* expression in the 443 samples of the discovery set organized in the four subtypes of the consensus molecular classification of CRC (Guinney et al., 2015). Data are given ±SD. *CDX2* is down-regulated in the mesenchymal subtype (CMS4) and significantly different from the other subtypes ($P = 9.77 \times 10^{-7}$). **(B)** Same as A in the 123 samples of the validation set. **(C)** Disease-free survival comparing $CDX2^{high}$ versus $CDX2^{low}$ CRC in the CMS4 subtype. $CDX2^{low}$ patients exhibit a significantly reduced disease-free survival (P = 0.0004). P-values were calculated with the log-rank test.

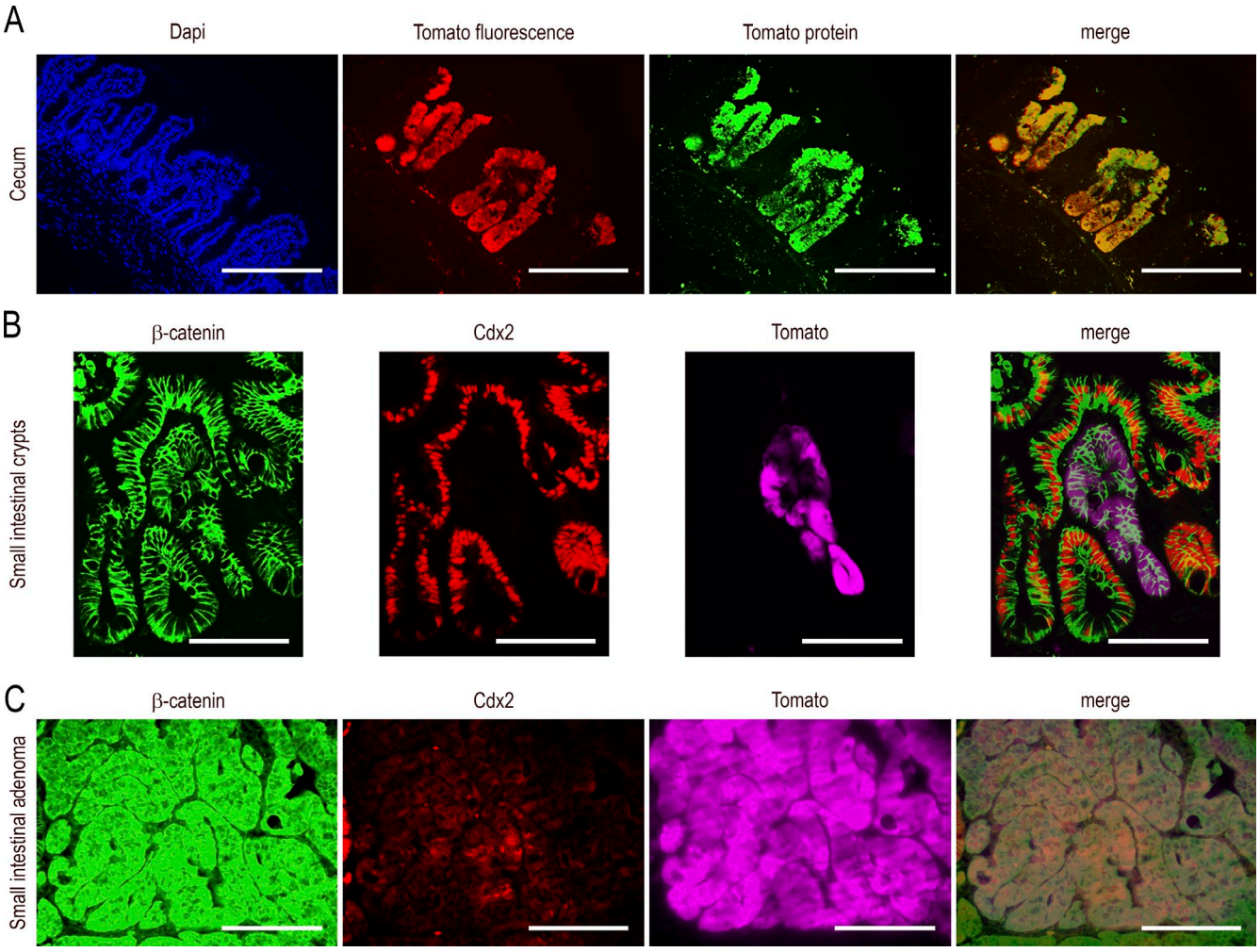

Figure S2. **Validation of the lineage tracing approach and implementation to mixed tumors analysis. (A)** Identical pattern of Tomato in a cecal section of an *AhCre*^{ERT}*::RosaCAG*^{tdTomato} mouse, detected first by direct fluorescence emission after the deparaffinization step, and second by indirect fluorescence after subsequent immunolabeling with Tomato antibody. Bars, 100 µm. **(B)** Mutually exclusive patterns of Tomato and Cdx2 in the small intestine of *AhCre*^{ERT}*::Cdx2*$^{f/f}$*::RosaCAG*^{tdTomato} mice treated with βNF+Tam. Tomato was detected by direct fluorescence emission, and Cdx2 and β-catenin, by indirect immunofluorescence labeling with Cdx2 and β-catenin antibodies. Bars, 50 µm. **(C)** Demonstration of the maintenance of transcriptional activity at the *Rosa26* locus in the tumor cells of small intestinal adenomas of *Apc*$^{+/\Delta 14}$*::AhCre*^{ERT}*::RosaCAG*^{tdTomato} mice. Tomato was detected by direct fluorescence emission, and Cdx2 and β-catenin, by indirect immunofluorescence labeling with Cdx2 and β-catenin antibodies. The cytoplasmic and nuclear localization of β-catenin demonstrates the transformed nature of the cells. Bars, 100 µm. Results were obtained in $n = 3$, 4, and 4 mice in A, B, and C, respectively.

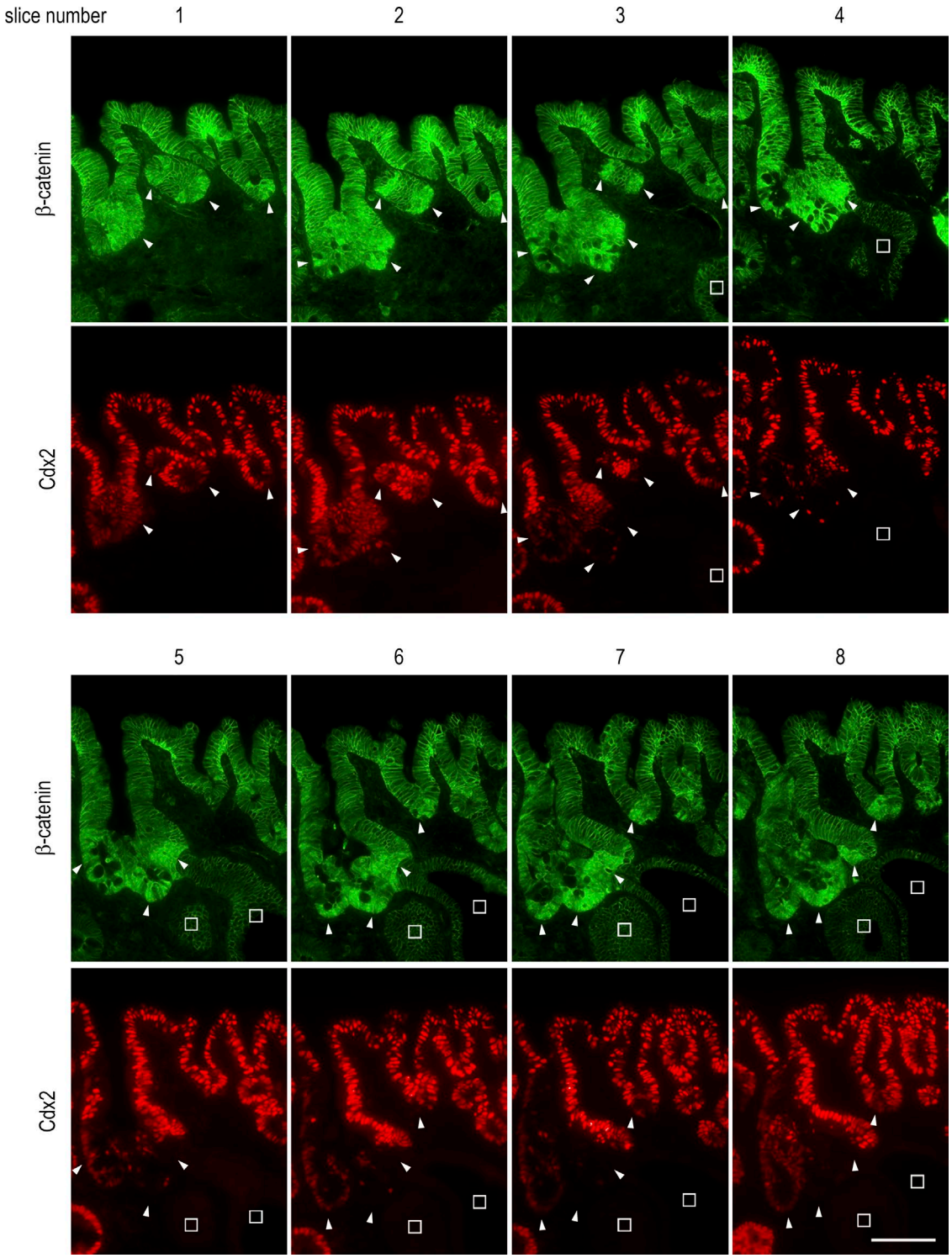

Figure S3. **Surface localization of cells undergoing constitutive activation of the Wnt pathway in mixed tumors.** Immunofluorescence staining of β-catenin and Cdx2 in 8 consecutive sections (6 µm each) at the surface of one cecal mixed tumor of an *Apc*$^{+/\Delta 14}$*::AhCre*^{ERT}*:: Cdx*$^{f/f}$ mouse treated with βNF+Tam. The surface sheet of the epithelium shows nontransformed cells identified by membranous localization of β-catenin, as well as groups of cells with constitutive activation of the Wnt pathway labeled by cytoplasmic/nuclear β-catenin. In the consecutive sections, the transformed cells are located in invaginated structures of the surface sheet (arrowheads); in these cells, Cdx2 levels are reduced compared with the levels in adjacent cells with membranous β-catenin. Open squares point to *Cdx2*-depleted glands. Bar, 50 µm. Data were obtained in $n = 3$ mice.

Tables S1 and S2 are provided as Word files. Table S1 shows genes differentially expressed by RNA sequencing in the cecal lesions of *AhCre^{ERT}::Cdx2$^{f/f}$* mice compared with the cecum of wild-type mice; fold change (FC) > 2; $q < 0.01$. Sheet 1 (pages 1–59) is the total list of differentially expressed genes. Sheet 2 (page 60) lists examples of intestinal- versus gastric-type genes differentially expressed in the cecal lesions of *AhCre^{ERT}::Cdx2$^{f/f}$* mice compared with normal cecum; the cecal lesions of *AhCre^{ERT}::Cdx2$^{f/f}$* mice compared with normal stomach; the cecal heteroplasia of *Cdx2$^{+/-}$* mice compared with normal cecum; and the cecal heteroplasia of *Cdx2$^{+/-}$* mice compared with normal stomach (Het vs. Sto). Sheet 3 (pages 61 and 62) list genes of the stem cell signature, according to Muñoz et al. (2012), which are differentially expressed in the cecal lesions of *AhCre^{ERT}::Cdx2$^{f/f}$* mice compared with normal cecum. Sheet 4 (page 63) lists genes encoding extracellular matrix proteins differentially expressed in the cecal lesions of *AhCre^{ERT}::Cdx2$^{f/f}$* mice compared with normal cecum. Sheet 5 (page 64) lists genes encoding cytokines differentially expressed in the cecal lesions of *AhCre^{ERT}::Cdx2$^{f/f}$* mice compared with normal cecum.

Table S2 lists genes differentially expressed by RNA sequencing; fold change (FC) > 2; $q < 0.05$. Sheet 1 (pages 1–4) show cecal mixed tumors of *Apc$^{+/\Delta 14}$::AhCre^{ERT}::Cdx2$^{f/f}$* mice compared with cecal lesions of *AhCre^{ERT}::Cdx2$^{f/f}$* mice. Sheet 2 (pages 5–17) list small intestinal mixed tumors of *Apc$^{+/\Delta 14}$::AhCre^{ERT}::Cdx2$^{f/f}$* mice compared with adenomas of *Apc$^{+/\Delta 14}$* mice.

REFERENCES

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Muñoz, J., D.E. Stange, A.G. Schepers, M. van de Wetering, B.-K. Koo, S. Itzkovitz, R. Volckmann, K.S. Kung, J. Koster, S. Radulescu, et al. 2012. The Lgr5 intestinal stem cell signature: Robust expression of proposed quiescent '+4' cell markers. *EMBO J.* 31:3079–3091. https://doi.org/10.1038/emboj.2012.166