Results Supplement

Evaluating markers of epithelial-mesenchymal transition to identify cancer patients at risk for metastatic disease

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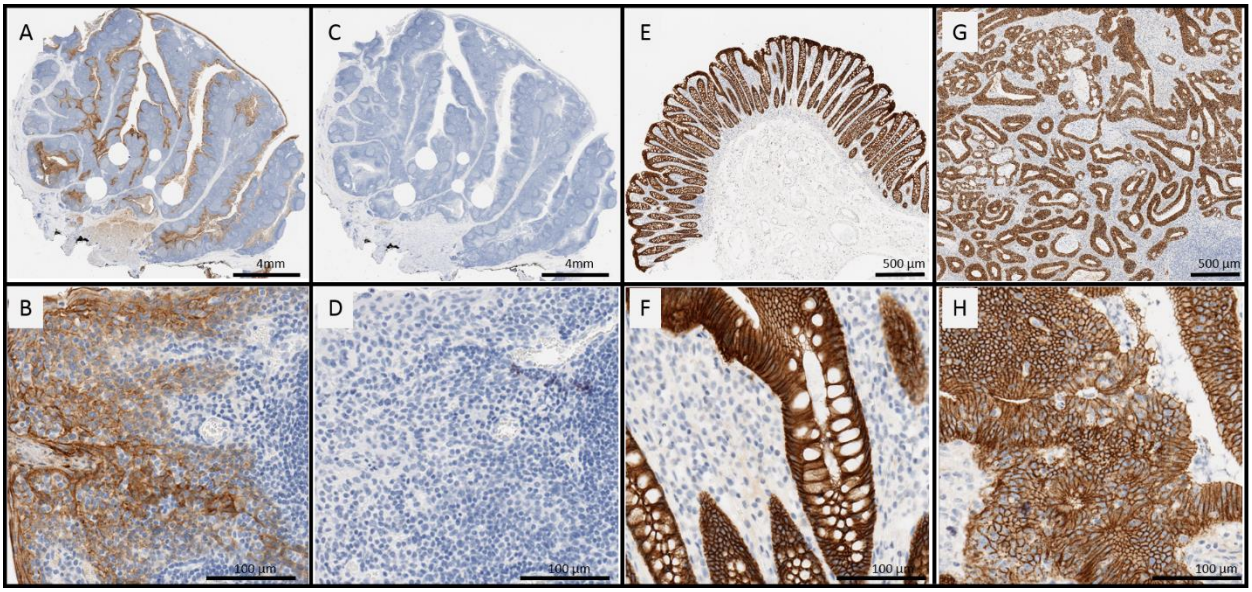
Table S1. Average continuous Snail expression in tumor tissue compared to non-neoplastic adjacent tissue for multiple scoring algorithms

Algorithm	Scale	Tissue Type	N	Marker Expression					
				Weighted Average[a]			Worst Core[b]		
				Mean	SD	P-value	Mean	SD	P-value
First Nuclear	Percent Positive Nuclei (0-100)	Tumor	185	41.7	22.1	0.02	52.3	24.9	0.0004
		Non-neoplastic	173	47.9	27.9		62.1	27.2	
Second Nuclear	Percent Positive Nuclei (0-100)	Tumor	185	44.4	26.5	0.08	57.0	29.5	0.02
		Non-neoplastic	173	49.5	27.7		64.1	28.0	
Whole Cell	Percent Positive Cells (0-100)	Tumor	185	34.3	24.0	0.005	46.6	28.7	0.004
		Non-neoplastic	173	41.9	26.4		55.3	28.3	

[a]Marker expression values assigned as weighted average of cores by tissue type (weighted by number of nuclei or cells as appropriate).

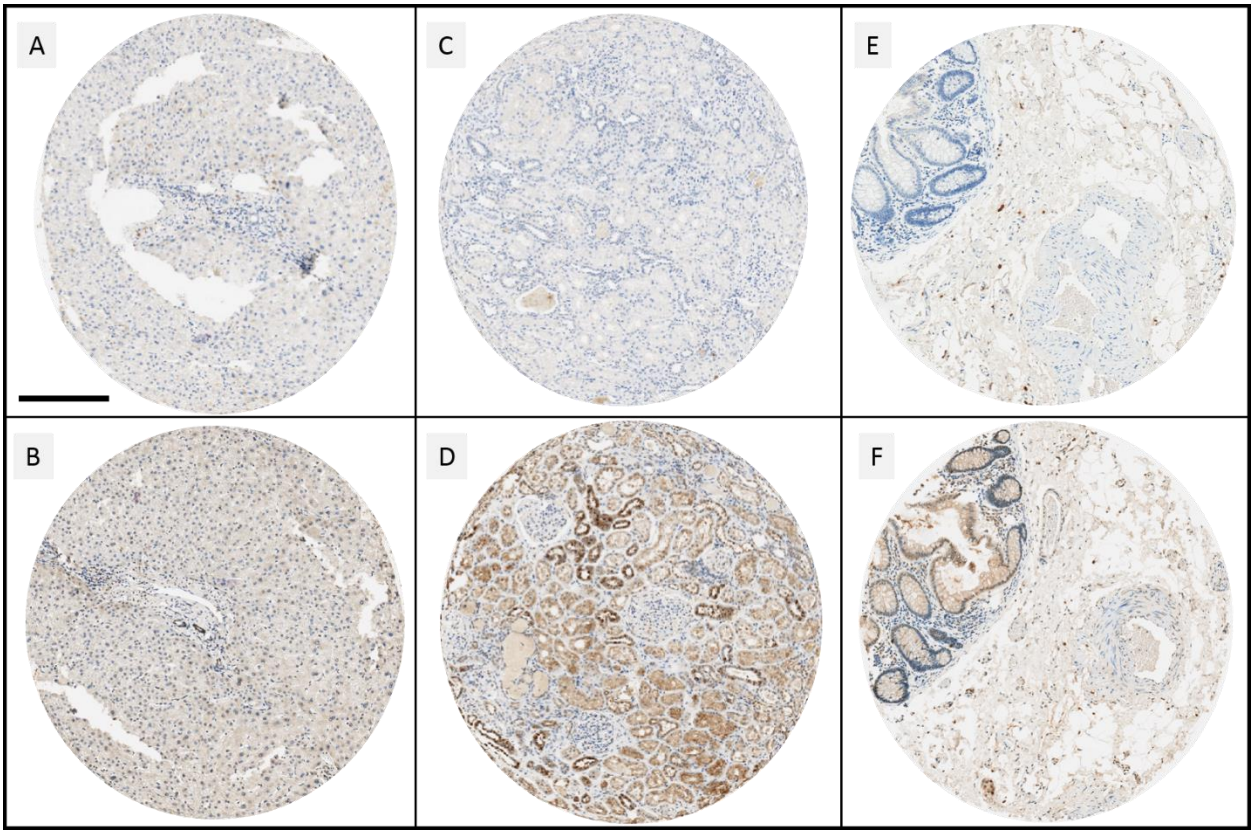
[b]Marker expression values assigned as expression by tissue type of the core with highest Snail expression.

Figure S1. Controls for E-cadherin antibody immunohistochemistry staining specificity



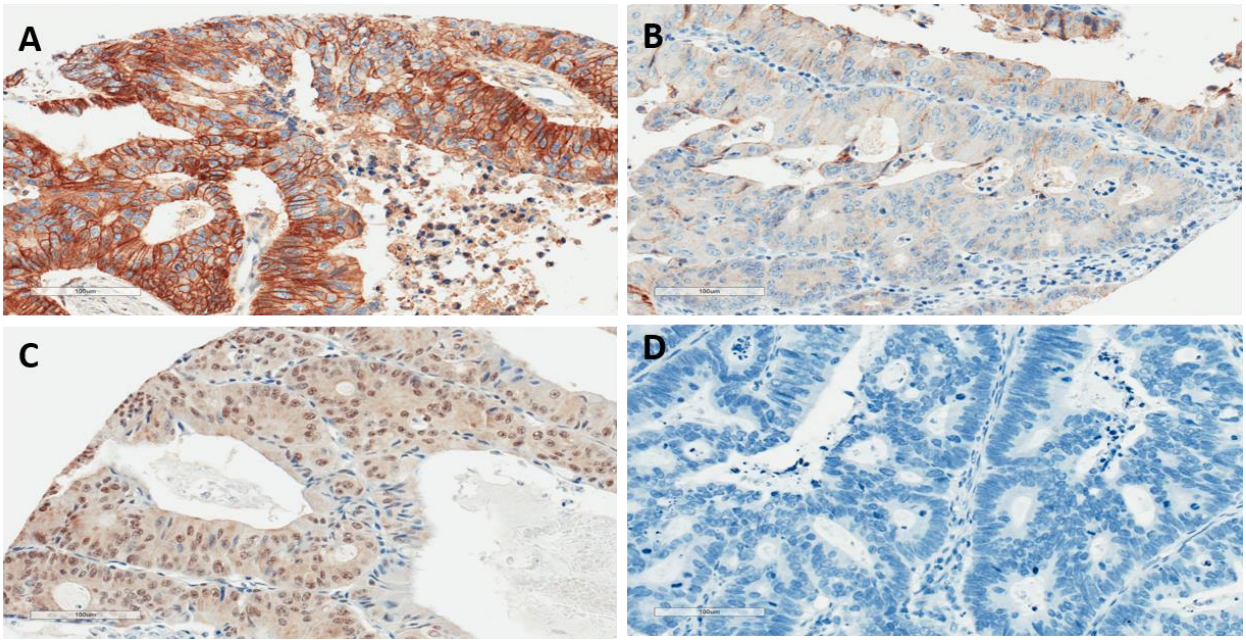
Panels A, C, E and G are low magnification views of control tissues; panels B, D, F and H are corresponding high magnification views. A and B) Positive staining of E-cadherin on human tonsil section. C and D) Human tonsil section that was treated as in panels A and B, but without primary antibody (negative control). E and F) Whole tissue section of non-neoplastic human colon stained for E-cadherin. G and H) Whole tissue section of human colon cancer specimen stained for E-cadherin. Scale bar sizes are shown in each panel.

Figure S2. Controls for Snail antibody immunohistochemistry staining specificity



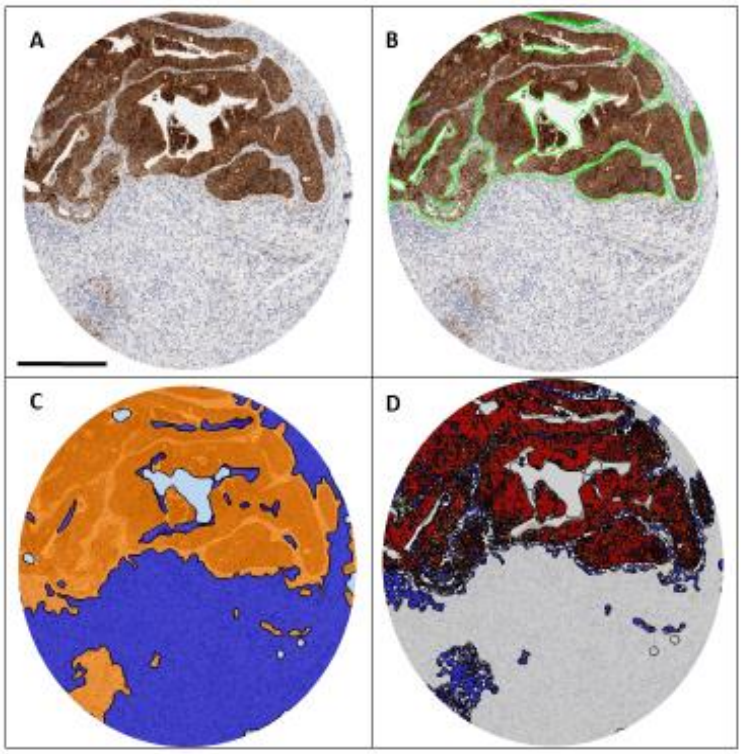
All cores are from a multi-tissue control tissue microarray (TMA). Panels A and B are liver, C and D are kidney, and E and F are colon tissue. Two sections of this TMA were treated identically except that one section was stained with goat IgG instead of the primary antibody to Snail. Equal concentrations of IgG and Snail antibody were used (2.5ug/ml final concentration). TMA cores shown in panels A, C, E are from the section stained with goat IgG (negative control) and cores in panels B, D, E are from the section stained with Snail antibody (positive control). Except for some light background and a few immune cells there was no DAB signal with the goat IgG. Liver tissue was negative and kidney and colon tissue stained positive with the Snail antibody. Bar represents 250 µm.

Figure S3. Immunohistochemistry examples for positive and negative core staining for E-cadherin and Snail



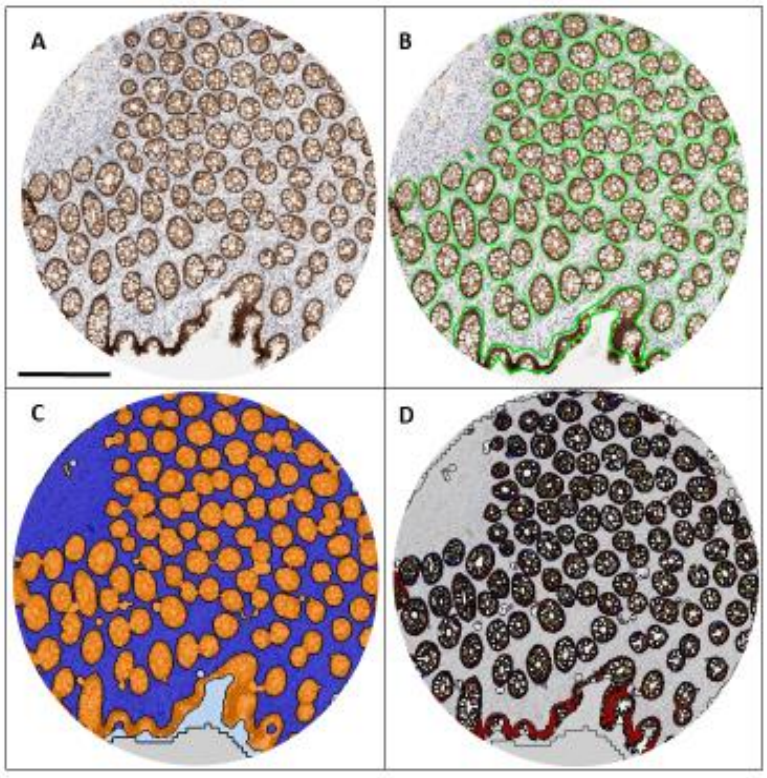
A) E-cadherin positive, B) E-cadherin negative, C) Snail positive, D) Snail negative

Figure S4. Illustration of Tissue Studio analysis of colon tumor tissue



(A) Original image of TMA core stained for E-Cadherin. Bar = 300um. **(B)** Green lines show manual annotation of tumor areas on image **(C)** Mark-up of image by Tissue Studio Composer. The algorithm was trained to differentiate between epithelial and stromal regions. Orange highlighted areas are enriched in epithelial cells and closely match the regions that were manually annotated in (B). Blue highlighted areas are enriched for stromal cells. Only the epithelial enriched regions were analyzed. **(D)** Mark-up of analysis results for tumor tissue. Blue= IHC negative, yellow = 1+, orange = 2+, red = 3+ (staining intensity). This tumor tissue core has high concentrations of E-Cadherin, with most of the staining classified as 3+ in intensity.

Figure S5. Illustration of Tissue Studio analysis of non-neoplastic colon tissue



(A) Original image of TMA core stained for E-Cadherin. Bar = 300um. **(B)** Green lines show manual annotation of colon crypt areas on image **(C)** Mark-up of image by Tissue Studio Composer. The algorithm was trained to differentiate between epithelial and stromal regions. Orange highlighted areas are enriched in epithelial cells and closely match the regions that were manually annotated in (B). Blue highlighted areas are enriched for stromal cells. Only the epithelial enriched regions were analyzed. **(D)** Mark-up of analysis results for non-neoplastic tissue. Blue= IHC negative, yellow = 1+, orange = 2+, red = 3+ (staining intensity).

Kaplan-Meier survival curves jointly-stratified by expression status of two EMT markers and having a statistically-significant logrank test

Figure S6. Survival by joint dichotomous E-cadherin and Snail weighted average status