square test).

Supplementary Fig. 1: Representative images from the IHC analysis of claudin-2 expression using paraffin-embedded normal and colon cancer samples. A colon cancer progression tissue array (normal adjacent colon, n=13; adenomas n=14; and adenocarcinomas n=13) from the Cooperative Human Tissue Network (CHTN, NCI), and IHC-specific mouse anti-claudin-2 antibody (Invitrogen Corp; 32-5600) were used. (i & ii) Representative normal colon, (iii-iv) Representative colon adenocarcinoma. [Magnifications: 100x (i & iii) and 400x (ii & iv)].

Supplementary Fig. 2: *Claudin-2 expression in IBD-associated cancer.* IHC staining for claudin-2 was performed using archived paraffin sections of Crohn's disease- and ulcerative colitis (UC)-associated colon cancers (n=19). **A.** Representative claudin-2 expression in colon with colitis from Crohn's disease patients [A(i) & A(ii)] and associated cancer [A(iii) & A(iv)]. **B.** Similar increase in claudin-2 expression was observed in ulcerative colitis (UC) associated cancer [B(iii) & B(iv)] compared to the colon with colitis only [B(i) & B(ii)]. [Magnifications: 100x [A(i), A(iii), B(i) & B(iii) and 200x [A(ii), A(iv), B(ii) & B(iv)].

Supplementary Fig. 3: *Claudin-2 overexpression does not modulate expression of other claudin proteins*. Total RNA was isolated from ~90 % confluent $HCT116^{Control}$ and $HCT^{Claudin\text{-}2}$ cells and was subjected to real time RT-PCR using gene specific real-time PCR primers. No major change in the expression of claudin-3, claudin-4 or claudin-7 was observed due to the forced expression of claudin-2. Values presented are mean$\pm$SEM.

Supplementary Fig. 4: *5-FU treatment affected cell proliferation and apoptosis.* Exponentially growing $HCT116^{Control}$ and $HCT^{Claudin-2}$ cells were exposed to 5-FU (100 μM) for 48 hours. Cell proliferation and apoptosis were determined using MTT-assay and cell death ELISA assay, as described previously (Dhawan, Singh et al. 2005; Singh, Sugimoto et al. 2007). Values presented are mean±SEM and are presented as % change compared to the respective untreated control cells.

Supplementary Fig. 5: *EGFR-blocking antibody prevented the co-culture dependent increase in claudin-2 expression.* Caco-2 cells were subjected to co-culture in the presence or absence of EGFR blocking antibody (clone#528, 20 μg/ml) and effect on claudin-2 expression was determined.

Supplementary Fig. 6: *EGF stimulation decreased trans-epithelial resistance (TER) and increased paracellular permeability.* A. TER in Caco-2 cells (***$p<0.001$). Confluent cells plated on transwell filters (0.4-μm pore size) were serum starved and then exposed to EGF (100 ng/ml). TER was measured before treatment and 8 & 24 hours after EGF treatment. Results are expressed in Ω x cm^2, and B. paracellular permeability for FITC-dextran (4 kDa) $^{\$\$\$}p<0.001$. Medium containing FITC-dextran (4 kDa) was added to the top (inner) chamber of the transwell. Samples were collected from the bottom (outer) chamber after 1, 4 and 24 hours after EGF-treatment. Data are presented as the total amount of FITC-dextran (4 kDa, Sigma-Aldrich Inc.) collected in the bottom chamber at the indicated time points.

Supplementary Fig. 7: *EGF-dependent increase in claudin-2 expression in Caco-2 cells was specific.* Total RNA was isolated from control and EGF-treated

Caco-2 cells and was subjected to real time RT-PCR using gene specific primers. Among claudin-2, 3, 4 and 8, only claudin-2 expression was significantly increased in the EGF-treated cells compared to the control cells. Values presented are mean±SEM. **P<0.01 *versus* control cells.

Supplementary Table. 1: Demographics and case information for the VMC and MCC patients represented by 10 normal adjacent colon samples, 55 adenocarcinomas and 195 adenocarcinoma patients are presented in table format. The Vanderbilt test set includes 14 patients from the University of Alabama-Birmingham Medical Center (14 tumors provided by Martin J. Heslin, M.D.). All patients were diagnosed with colorectal adenocarcinoma (stages I-IV) according to current American Joint Commission on Cancer (AJCC) guidelines. *Other* in the VMC medical record implies not otherwise specified or Asian and Hispanic or Hispanic NOS.

Supplementary Methods.

Real Time-qPCR: RT-qPCR was done as previously described (Krishnan, Singh et al. 2010). In brief, Total RNA was extracted using RNeasy Mini Kits (Qiagen) and 1 µg of total RNA was converted to cDNA. Following the reverse transcription (RT) reaction, all samples were subjected to real time PCR analysis with SYBR Green PCR Master Mix (Applied Biosystems). For all targets, the cycling conditions were: 95°C for 10 minutes, followed by 40 cycles each consisting of 95°C for 15 seconds, 60°C for 30 seconds and 72°C for 45 seconds. Serial dilutions were performed to generate a standard curve for each

gene target in order to define the efficiency of the qRT-PCR reaction. The integrity and specificity of the amplified PCR products were confirmed by dissociation curve analysis (SDS 2.0 software, Applied Biosystems). β-actin was used as the reference gene.

***Measurements of trans-epithelial resistance (TER) and paracellular permeability*:** Confluent cells were plated on transwell filters (0.4 μm) and TER and paracellular flux were determined as described previously (Singh, Sugimoto et al. 2007). Data for the paracellular flux are presented as the total amount of FITC-dextran (4 kDa, Sigma-Aldrich Inc.) collected in the bottom chamber at the indicated time points.

Cell-death ELISA assay: was done as previously described (Singh, Sugimoto et al. 2007). In brief, a total of 25,000 cells were used to determine apoptosis with the cell death detection enzyme-linked immunosorbent assay kit (Roche Applied Science) according to the manufacturer's instructions. Experiments were carried out at least three times.

Supplementary Acknowledgement: Support for our studies also came from the Society of University Surgeons-Ethicon Scholarship Award (J.J.S.) CA112215 (T.J.Y.), DK052334, CA069457, CA95103 (R.D.B.); R01DK053620, R01AT004821, P01CA116087, (K.T.W.) & P50CA095103 (tissue core; K.W.).