Supplementary Material and Methods

Tissue in the Discovery Cohort

Only adjacent samples confirmed not to contain neoplastic tissue on review of histology were included in the analysis. Of the patients with neoplasia, nine had both the neoplastic lesions and the adjacent tissue analyzed. One patient included in the UCN group was found to have dysplasia in the surrounding mucosa on review of histology and the adjacent mucosa was not included in the final analysis. One patient included in the nUCaN group had poor RNA retrieval from the adjacent cancer and the neoplastic lesion was not analyzed. Hematoxylin and eosin stained sections were reviewed by an expert GI pathologist (JH). All tissue from UC patients used in the study had either quiescent disease or minimally active colitis. Control patients were excluded if they had infectious colitis or a neoplastic lesion as their indication for a colectomy. Mucosa from control patients was confirmed as normal histologically. Tissue blocks among normal controls, UC patients without neoplasia, and patients with UC-associated neoplasia were matched by location in the colon to give similar proportions of tissues from the right and left colon in each group.

RNA and DNA Extraction

For FFPET, four 10 micron sections were cut with a sterile blade and placed in RNase-free PCR tubes. Deparaffinization was performed by vortexing the tissue in 1.5 ml octane followed by addition of 150 µL methanol. Following repeat vortexing and centrifuging the sample, the octane and methanol were removed and the tissue was dried at room temperature for 1 hr. Seven µl proteinase K was added to 200 µl digestion buffer and the sample incubated at 55^{o}C for 16 hrs. RNA was then extracted using the Ambion RecoverAll Kit (Life Technologies, Carlsbad, CA) per manufacturer's directions. An RNA cleanup step was then performed using the RNeasy MinElute Cleanup Kit (Qiagen, Hilden, Germany). DNA extraction was performed using the QIAamp DNA FFPE Tissue Kit according to manufacturer's directions (Qiagen). For frozen samples, tissue was

homogenized using the bullet blender (Next Advance, Averill Park, NY) and extraction performed using the AllPrep DNA/RNA/miRNA kit (Qiagen).

Nanostring Analysis

100ng of total RNA were prepared by hybridizing specific biotin-labeled reporter and capture probes to each miRNA according to manufacturer's instructions (NanoString Technologies, Seattle, Wa). Using the nCounter Prep Station, resulting probes were purified and immobilized on a streptavidin-coated cartridge. Individual reporter probe barcodes were counted with a high-density scanner (1155 fields of view per flow cell) using the nCounter Digital Analyzer.

Nanostring data collected from 4 separate arrays were pooled and normalized to detect batch effects. A batch effect was identified in the 4^{th} array from the heat map generated by unsupervised hierarchical clustering. We, therefore, combined and normalized the first 3 arrays using quantile normalization, while the 4th array was normalized separately. Hierarchical clustering was performed using dChip software with the following parameters: Distance matric="1-correlation", Linkage method="Centroid", Smoothing method="By cluster tightness". To identify differentially expressed genes, two-group comparisons were performed using the R/Bioconductor package 'limma'. Multi-dimensional scaling (MDS) was performed using R language. The significant distance between samples was tested using Adonis (non-parametric permutation MANOVA) implemented in R(18).

Real Time PCR

cDNA was prepared from 100 ng total RNA. Primers and probes for miR-31 (assay ID: 002279; catalog #: 4427975) , miR-34a (000426; 4427975), miR-106b (000442, 4427975) miR-193a-3p (002250; 4427975), miR-376c (002122; 4427975), miR-497, (002368; 4427975) pri-miR-193a (Hs03303307_pri; 4427012) and RNU48 (001006; 4427975) were synthesized by Life

Technologies. To synthesize cDNA, the TaqMan miRNA reverse transcriptase kit or the High Capacity RNA-to-cDNA kit (Life Technologies) were used for mature miRNAs and pri-miRNA, respectively. TaqMan quantitative real time reverse transcription-polymerase chain reaction (qPCR) was performed with the Roche 480 Light Cycler (Roche, Indianapolis, IN) using the TaqMan Universal Master Mix II, no UNG (Life Technologies); qPCR thermal profile included 95°C for 10 min followed by 45 cycles of 95°C for 15s, 60°C for 60s, and 72^{o}C for 1s and a final hold of 40^{o}C for 30s. To measure expression of *IL17RD*, primers were synthesized (F: ACCAAGTGGCAGTCACCATT, R: ACTGTTGAGCTGCTTCGGAT), and qPCR was performed following cDNA synthesis using the Fast Mix SYBR Green Master Mix in the Roche 480 Light Cycler. Thermal profile included pre-incubation (95°C for 5 min), 45 amplification cycles (95°C for 10s, 60°C for 15s and 72°C for 15s), a melt curve (95°C for 5s, 65°C for 1min and 97°C continuous acquisition) and cooling (40°C for 30s).

Genomic Bisulfite Sequencing

PCR was performed on bisulfite-treated DNA using a thermal profile which included activation at 95°C for 10 min, amplification for 45 cycles at 95°C for 30s, 51°C to 47°C with 0.25°C decrease/cycle touch down for 30s, followed by 72°C for 60s, a final extension of 62°C for 7 min, and a final hold at 37°C for 10 min in the Roche Light Cycler 480. PCR amplicons were purified using the QIAquick pcr purification kit (Qiagen) and products subcloned into pCR™4-TOPO® vector using the TOPO® TA cloning kit (Life Technologies). Sequencing was carried out with the Applied Biosystems 3730XL.

Colonocyte Isolation

Following washing with transport media, 10 ml of chelating buffer (transport media containing 1 mM EDTA and 1 mM EGTA) was added to the samples and incubated at +4^{0}C overnight. The following day, the sample was centrifuged at 200g for 5 min at +4^{0}C. The supernatant was

discarded and pellet was resuspended in 0.5 ml PBS. 10 ml PBS was added to the biopsies and shaken vigorously to release the colonocytes into suspension. The colonocytes in suspension were transferred to a new tube and centrifuged for 5 min at $+4^0$C. The supernatant was discarded and pelleted colonocytes resuspended in 0.5 ml PBS and combined with the colonocyte isolate from the previous step. Three to five centrifugation steps were carried out for 5 min at $+4^0$C at gradually increasing speeds for up to 7000 rcf, and successive pelleted colonocyte fractions were combined. The tissue remaining after suspension of colonocytes in EDTA and EGTA media constitutes the stromal fraction of the isolate. RNA was then isolated from the colonocyte and stromal fractions using AllPrep DNA/RNA/miRNA kit (Qiagen).

Samples Analyzed by Immunohistochemistry

Five micron sections from FFPET were cut and mounted on Vectabond-coated Superfrost Plus slides from three patients in each group in the validation cohort. A tissue microarray was also prepared with punch biopsies from 27 FFPE UC-associated cancers. Staining was evaluated on 24 of these cancers as three cancers from the array were excluded as they did not have sufficient tissue for analysis.

Cell Culture Conditions

Cells were maintained at 37°C in a humidified atmosphere of 5%CO2-95% air as recommended by ATCC. HCT116 and HT29 cells were cultured in McCoy's 5A Modified Medium containing 10% fetal bovine serum. DLD-1 cells were cultured in RPMI 1640 containing 10% heat-inactivated fetal bovine serum, and LoVo cells were cultured in Ham's F-12K medium containing 10% fetal bovine serum.

Wild Type and Mutant IL17RD 3'UTR Oligonucleotide Sequences

<u>Wild Type 3'UTR</u>

CTGAGGTCTCATACAAGGATATTTGGAGTGAAATGCTGGCCAGTACTTGTTCTCC
CTTGCCCCAACCCTTTACCGGATATCTTGACAAACTCTCCAATTTTCTAAAATGA
TATGGAGCTCTGAAAGGCATGTCCATAAGGTCTGACAACAGCTTGCCAAATTTGG
TTAGTCCTTGGATCAGAGCCTGTTGTGGGAGGTAGGGAGGAAATATGTAAAGAAA
AACAGGAAGATACCTGCACTAATCATTCAGACTTCATTGAGCTCTGCAAACTTTG
CCTGTTTGCTATTGGCTACCTTGATTTGAAATGCTTTGTGAAAAAAGGCACTTTT
AACATCATAGCCACAGAAATCAAGTGCCAGTCTATCTGGAATCCATGTTGTATTG
CAGATAATGTTCTCATTTATTTTTGATGTAGAATTTACATTGCCATGGGTGTTAA
ATAAGCTTTGAGTCAAAAGTCAAGAAAGTGACTGAATATACAGTCACCTTTTATG
AAATGAGTCTCTGTGTTACTGGGTGGCATGACTGATTGAGGTGAAGCTCACGGGG
CCAGGCTGACCGTCTTGACCGTTCCACTTGAGATAGGTTGGTCATCGTGCAGAAG
GCCCCAGGACCTCAGCACACACAGCCTCCTCTTGGTCTGAGTAGGCATCATGTGG
GGGCCAGATCTGCCTGCTGTTTCCATGGGTTACATTTACTGTGCTGTATCTCAGA
TGTTGGTGTCTGGAAGTTTATTCTTAAGAGACTGCTACCCAGCTGGTCTGTATTA
TTGGAAGTTGCAGTTCGTGCTTTGGTTGGCCTTCTGGTCTAAAGCTGTGTCCTGA
ATATTAGGGATCACAATTCACTGAAATACAGCAGTGTGTGGAGGTGATGGCCAGT
TAATCTGCTGAACTGGTTTTGACTAATGAC

Mutated 3’ UTR

CTGAGGTCTCATACAAGGATATTTGGAGTGAAATGCTCTTGTTCTCCCTTGCCCC
AACCCTTTACCGGATATCTTGACAAACTCTCCAATTTTCTAAAATGATATGGAGC
TCTGAAAGGCATGTCCATAAGGTCTGACAACAGCTTGCCAAATTTGGTTAGTCCT
TGGATCAGAGCCTGTTGTGGGAGGTAGGGAGGAAATATGTAAAGAAAAACAGGAA
GATACCTGCACTAATCATTCAGACTTCATTGAGCTCTGCAAACTTTGCCTGTTTG
CTATTGGCTACCTTGATTTGAAATGCTTTGTGAAAAAAGGCACTTTTAACATCAT
AGCCACAGAAATCAAGTGCCAGTCTATCTGGAATCCATGTTGTATTGCAGATAAT
GTTCTCATTTATTTTTGATGTAGAATTTACATTGCCATGGGTGTTAAATAAGCTT
TGAGTCAAAAGTCAAGAAAGTGACTGAATATACAGTCACCTTTTATGAAATGAGT
CTCTGTGTTACTGGGTGGCATGACTGATTGAGGTGAAGCTCACGGGGCCAGGCTG
ACCGTCTTGACCGTTCCACTTGAGATAGGTTGGTCATCGTGCAGAAGGCCCCAGG
ACCTCAGCACACACAGCCTCCTCTTGGTCTGAGTAGGCATCATGTGGGGGCCAGA
TCTGCCTGCTGTTTCCATGGGTTACATTTACTGTGCTGTATCTCAGATGTTGGTG
TCTGGAAGTTTATTCTTAAGAGACTGCTACCCAGCTGGTCTGTATTATTGGAAGT
TGCAGTTCGTGCTTTGGTTGGCCTTCTGGTCTAAAGCTGTGTCCTGAATATTAGG
GATCACAATTCACTGAAATACAGCAGTGTGTGGAGGTGATTAATCTGCTGAACTG
GTTTTGACTAATGAC

Supplementary Figure Legend

Supplementary Figure 1. IL17RD potentiates EGF-induced EGFR signaling. A) Cellular proliferation of HT29 and HCT116 cells 96 hours after transfection with a plasmid encoding *IL-17RD* compared to an EV plasmid. B) Cellular proliferation of HT29 and HCT116 cells treated with EGF for 96 hrs following transfection with a plasmid encoding IL-17RD or EV plasmid. C) pEGFR, EGFR, pAKT, and pERK expression in HT29 cells 15 minutes after treatment with EGF (+) or media without EGF (-) and transfected with Flag-tagged *IL17RD*, EV plasmid, or no transfection (vehicle) (representative images of two blots containing 3 samples per group). D) pAKT expression in HT29 cells 30 minutes after treatment with media with or without EGF and transfected with a Flag-tagged *IL17RD* plasmid or EV plasmid. E) EGFR expression in untreated cells 96 hours after transfection with *IL17RD*.