Supplemental Figure S1.

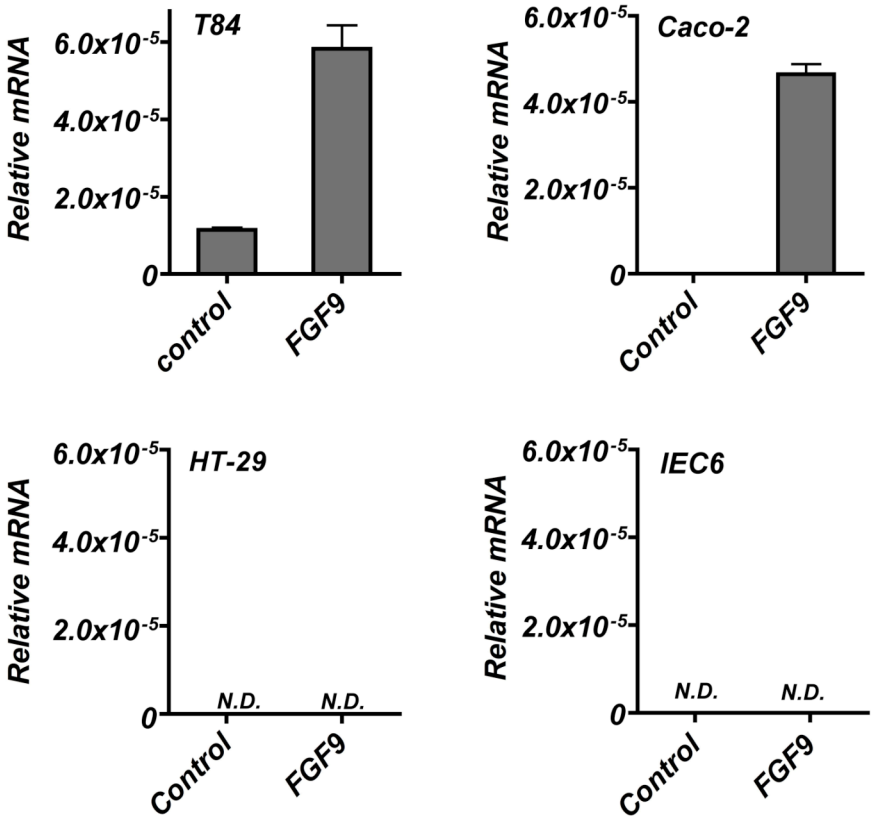

Figure S1. FGF9 Stimulation of α-defensin mRNA expression in various intestinal epithelial cell lines. All celllines were plated at about 40-50% sub-confluence as given in Methods and FGF9 (R&D Systems, Minneapolis, MN) was added daily starting at 24 hours after plating. Caco2, HT-29 and IEC cells were given 50 ng/ml of FGF9 while the optimal dose for T84 cells was determined to be 1 ng/ml. Cells were harvested at ~confluence which occurred by 3 to 4 days of growth in the presence of FGF9. Where possible monolayer resistance, measured daily with the EVOM2 ohm meter (World Precision Instruments, Sarasota, Florida) was used as a measure of culture confluence. Expression levels of HD5 mRNA in total RNA samples from T84, Caco2 and HT-29 cells and rat α-defensin 5 in total RNA from IEC6 cells were determined by quantitative real-time PCR and expressed relative to 18S rRNA. Rat α-defensin 5 primer-probe set was from Applied Biosystems (catalogue number Rn02607254). Values are the mean +SD of n=3 (Caco2, HT-29 and IEC6) and n=4 (T84). N.D.: Not detectable.

Supplemental Figure S2.

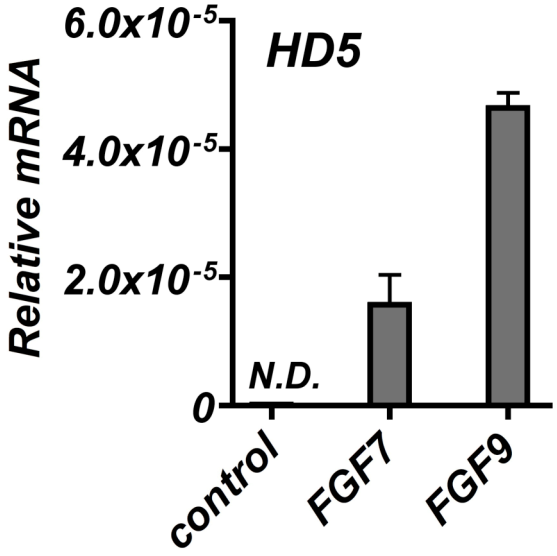

Figure S2. Stimulation of HD5 mRNA expression shows FGF receptor selectivity. Caco2 cells plated as given in Methods and Supplemental Fig 1 were treated with daily additions of optimal doses of FGF7 (10 ng/ml) (R&D Systems, Minneapolis, MN) or FGF9 (50 ng/ml) starting at 24 hours after plating. HD5 mRNA in total RNA samples was determined by quantitative real-time PCR and expressed relative to 18S rRNA. Values are the mean +SD of n=3. N.D.: Not detectable.

Supplemental Figure S3.

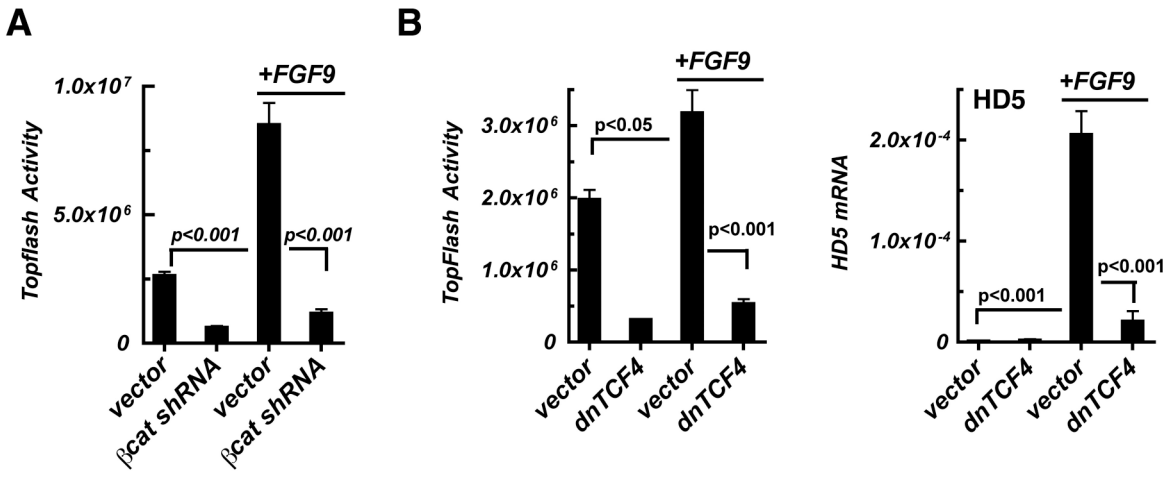

Figure S3. Tcf4/β-catenin activity is required for HD5 expression in response to FGFR-3 signaling. Caco2 cells grown as described in Methods were trypsinized, recovered by centrifugation, resuspended in Opti-MEM I (Invitrogen, Carlsbad, CA) and transfected using siLentFect reagent (BioRad, Hercules, CA). A plasmid expressing Renilla-luciferase was included as an internal control for transfection efficiency. Cells were transfected with 3 μg of the Tcf4 reporter plasmid, pTOPFlash, and co-transfected with 3 μg of plasmids expressing dominant negative Tcf4 (dnTcf4) or shRNA for β-catenin (plasmids kindly provided by Dr. van de Wetering) (29). Control cells were transfected with pUC18, the empty vector. Some cultures received daily additions of FGF9 (final concentration 50 ng/ml) (R&D Systems, Minneapolis, MN) starting at 24 h post-transfection and ending at 96 h post-transfection (2 days post confluence). Luciferase activity was assayed with the Dual Glo Luciferase Assay System (Promega, Madison, WI) Samples were read using the Veritas™ Microplate Luminometer (Turner Biosystems, Sunnyvale, CA). Replicate cultures were analyzed for HD5 mRNA expression as described in methods. Values are expressed as the mean +SD.

Supplemental figure S4.

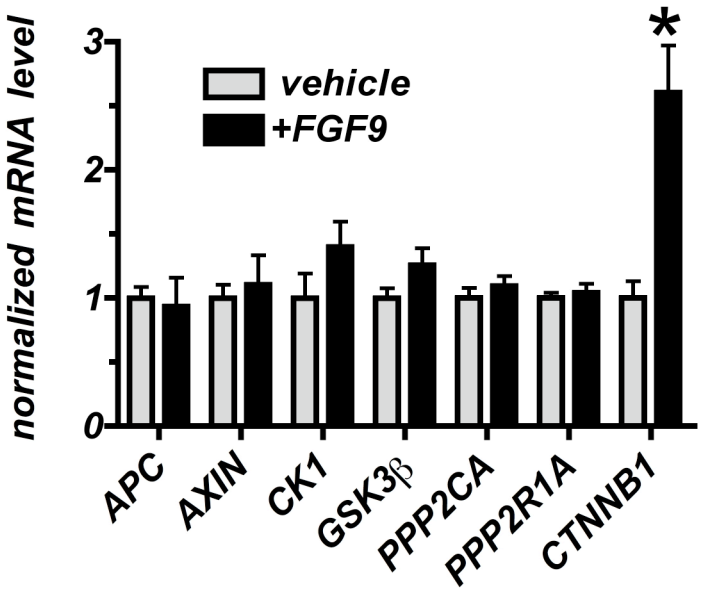

Figure S4. FGFR-3 signaling does not affect mRNA expression levels of components of the β-catenin destruction complex but does augments β-catenin mRNA levels. Caco2 cells were treated with FGF9 or vehicle daily for 3 days. Cells were subsequently harvested and total RNA was isolated. The RT^2 Profiler Human Wnt Signaling PCR Array (PAHS-043: SA Biosciences, QIAGEN, Valencia, CA) was used to assess the mRNA expression level of components of the β-catenin destruction complex as well as β-catenin. mRNA levels are expressed relative to the housekeeping gene ribosomal protein L13a. Values were normalized relative to the vehicle control for each sample. Values are the mean +SD of n=3. * $p<0.001$ vs untreated control.