SUPPLEMENTARY FIGURE LEGENDS

Supplementary FIG. 1: Targeted deletion of HuR in mice fails to affect Goblet cell function or enterocyte differentiation in the small intestinal mucosa. (***A***) Goblet cells as examined by alcian blue staining (blue, *left* panel) and mucin-2 immunochemical staining (red, *right*-panel). (***B***) Enterocyte differentiation as measured by villin immunostaining assays (Green).

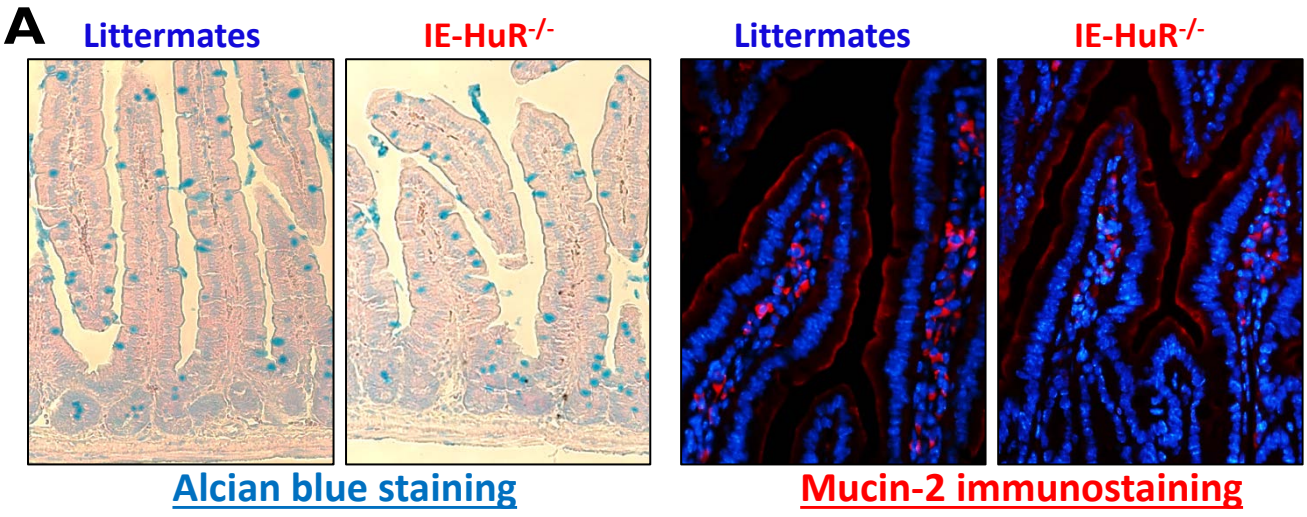
Supplementary FIG. 2: HuR deletion in IECs induces microbiota dysbiosis in mice. (***A***) Altered richness and diversity of colonic mucosal microbiome in IE-HuR$^{-/-}$ mice compared with those observed in control littermates (n = 3). *P < 0.05 compared with control littermates. (***B***) Changes in the abundance of the top 30 genera in colonic mucosa between IE-HuR$^{-/-}$ and control littermate mice.

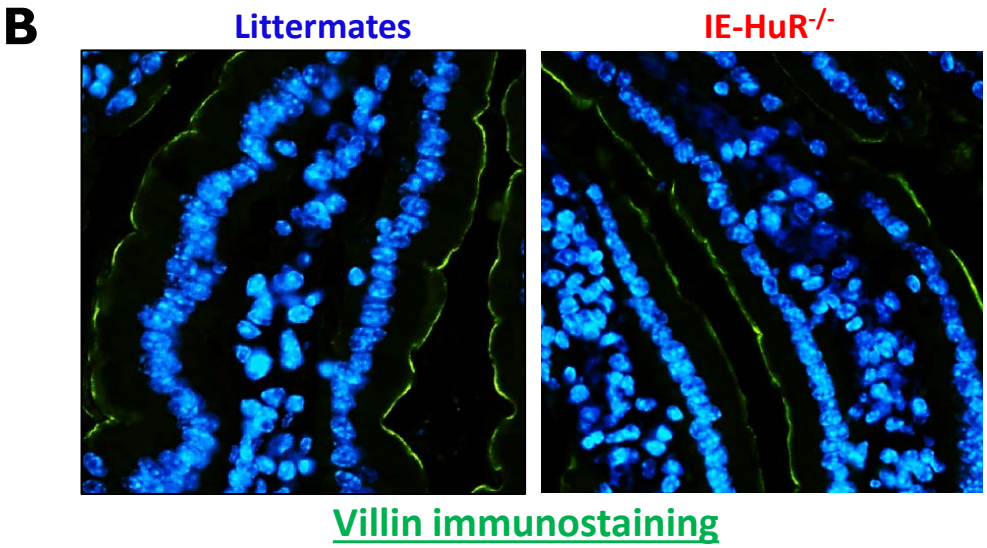
Supplementary FIG. 3: Representative H&E staining sections of ileal and colonic mucosal tissues from control individuals and patients with of IBD.

Supplementary FIG. 4: Distribution of TLR2 in the colonic mucosa from control littermate and IE-HuR$^{-/-}$ mice. Red, TLR2; green, E-cadherin (E-cad); blue, nucleus stained by DAPI.

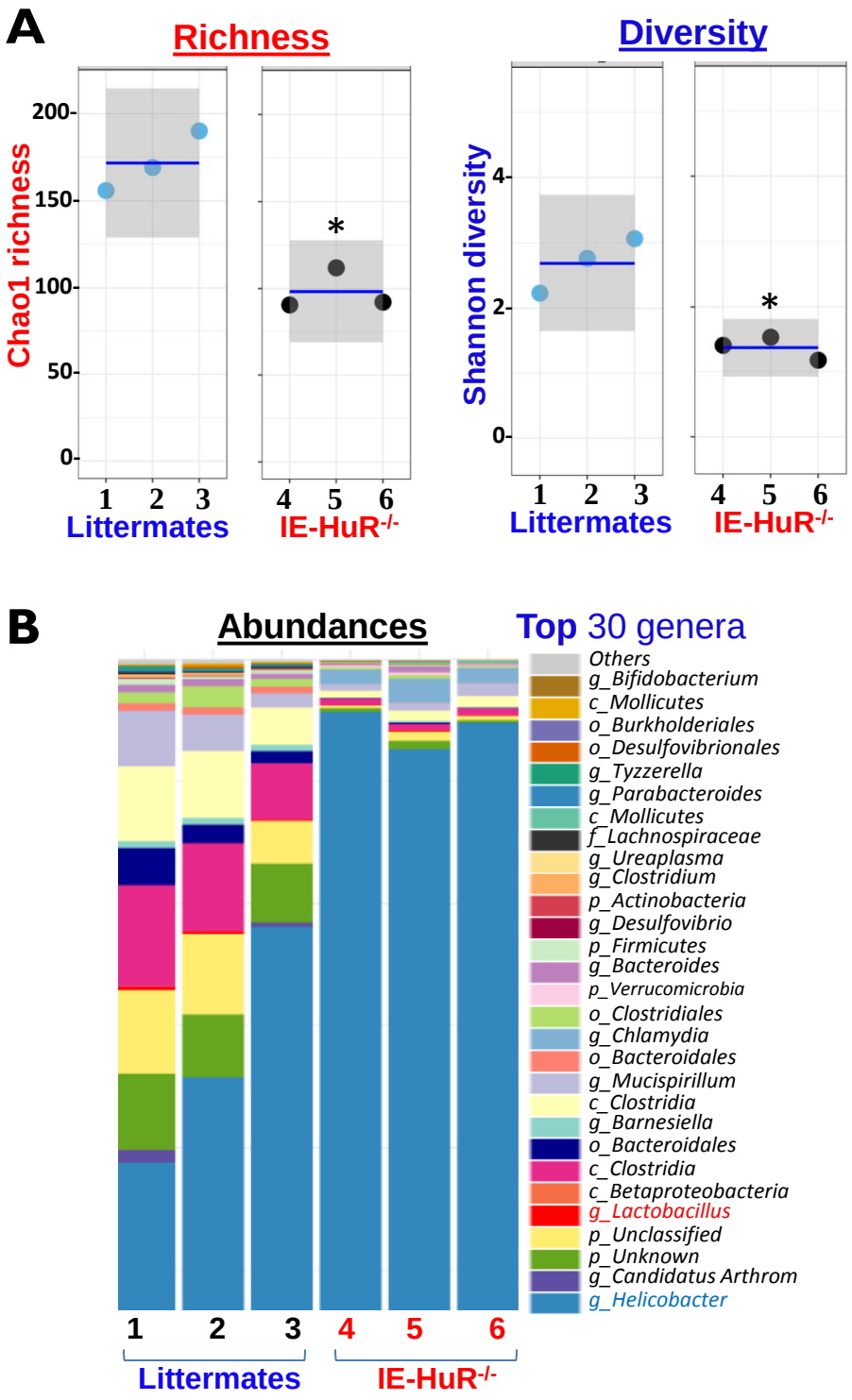
Supplementary FIG. 5: Distribution of TLR4 in the small intestinal mucosa from IE-HuR$^{-/-}$ mice and control littermates. Red, TLR4; green, E-cadherin; blue, nucleus stained by DAPI.

Supplementary FIG. 6: Subcellular distribution of TLR2 after CNPY3 overexpression. (***A***) Immunoblots of CNPY3, HuR, and TLR2 48 h after transfection with CNPY3 expression vector in the colon carcinoma cell line HCT116. (***B***) Immunoblots of membrane and cytoplasmic TLR2 in cells described in ***A***. (***C***) Quantitative analysis derived from densitometric scans of immunoblots of TLR2 in cells described in ***B*** (n = 3).

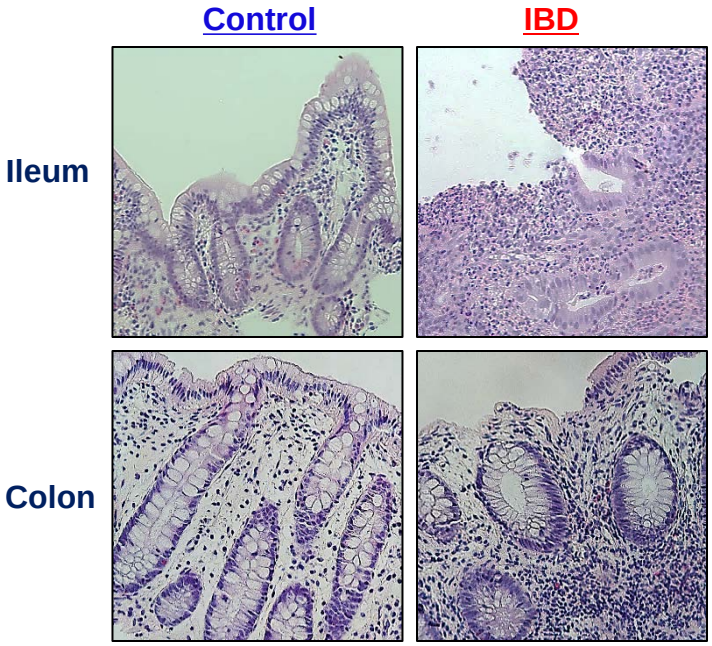




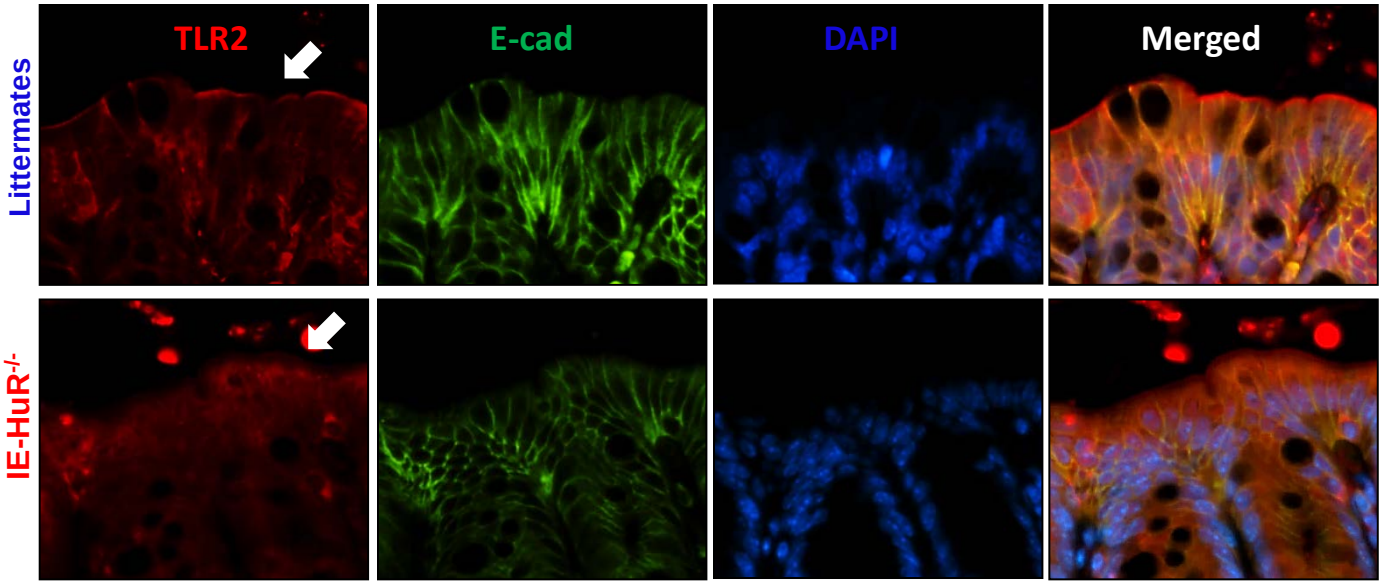
Supplementary Figure 1



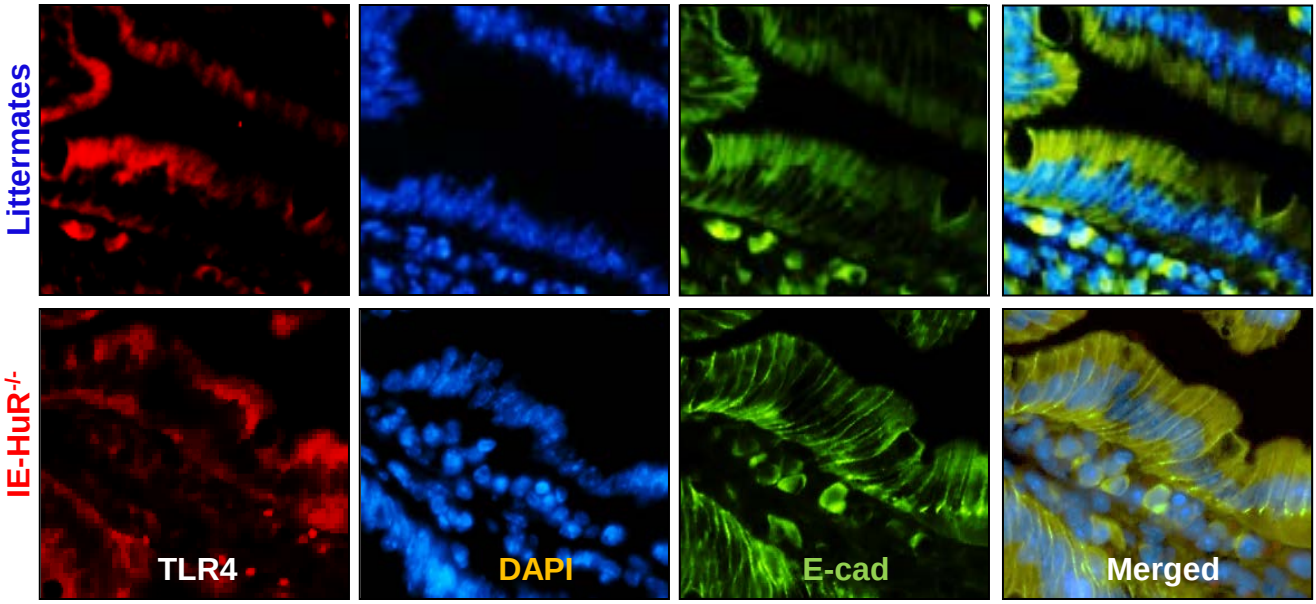
Supplementary Figure 2



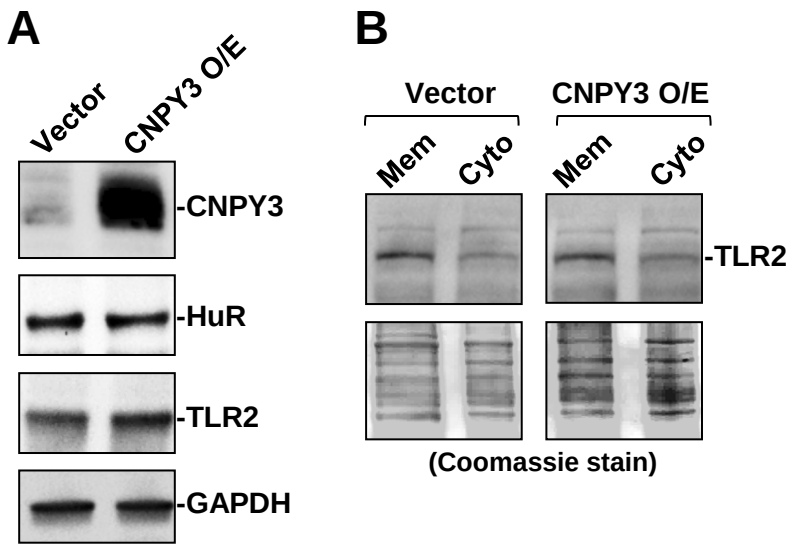
Supplementary Figure 3



Supplementary Figure 4



Supplementary Figure 5



Supplementary Figure 6

Supplementary Table 1. Primers

F-CNPY3-T7-5’	TAATACGACTCACTATAGGGCACATCTTCCAGCACCTG
R-CNPY3-T7-5’	CTCAGGCATTGAATCCATG
F-CNPY-T7-CR	TAATACGACTCACTATAGGGCATGGATTCAATGCCTGAG
R-CNPY-T7-CR	GTGGGCTCAGAGCTCATCAG
F-CNPY-T7-3’	TAATACGACTCACTATAGGGCTGATGAGCTCTGAGCCCAC
R-CNPY-T7-3’	GGACTCAGAAATCAGAAC
pmiR-F-CNPY3-5’	GAGCTCCACATCTTCCAGCACCTG
pmiR-R-CNPY3-5’	TCTAGACTCAGGCATTGAATCCATG
pmiR-F-CNPY3-CR	GAGCTCCATGGATTCAATGCCTGAG
pmiR-R-CNPY3-CR	TCTAGAGTGGGCTCAGAGCTCATCAG
pmiR-F-CNPY3-3’	GAGCTCCTGATGAGCTCTGAGCCCAC
pmiR-R-CNPY3-3’	TCTAGAGGACTCAGAAATCAGAAC

Supplementary Methods

Plasmid Construction

Recombinant adenoviral plasmids containing human HuR cDNA (AdHuR) were constructed by using the Adeno-X Expression System (Clontech) as described previously.[30] The chimeric firefly luciferase reporter construct containing the *Cnpy3* mRNA was generated as described.[31] The full-length *Cnpy3* 5'-UTR, CR, and 3'-UTR fragments were subcloned into the pmirGLO Dual-Luciferase miRNA Target Expression Vector (Promega, Madison, WI) to generate the pmirGLO-Luc-CNPY3-5′UTR, pmirGLO-Luc-CNPY3-CR, and pmirGLO-CNPY3-3′UTR reporter constructs as described previously (30,34). The NF-κB signaling luciferase reporter plasmid was purchased from Addgene (Cambridge, MA). Transient transfections were conducted using the Lipofectamine Reagent as recommended by the manufacturer, and the levels of firefly luciferase activity were normalized to *Renilla* luciferase activity. All of the primer sequences for generating these constructs are provided in Supplemental Table 1.

Intestinal Organoid Culture

Isolation and culture of primary enterocytes were conducted following the method described previously.[5,29] Briefly, primary crypts were released from the small intestinal mucosa in mice; then, the isolated crypts were mixed with matrigel and cultured in Advanced Dulbecco's modified Eagle medium /F12 medium. The growth of organoids was examined under phase-contrast microscopy.

Isolation of Membrane and Cytoplasmic Protein

Membrane and cytoplasmic protein fractions from IEC cells were isolated and purified with Mem-PER Plus membrane protein extraction kit (Thermoscientific, Rockford, IL). Briefly, the cells were permeabilized with a mild detergent to release soluble cytosolic proteins before a second detergent was used to solubilize membrane proteins. The quality and quantity of protein from each fraction were monitored by Coomassie blue staining.

Assays of Newly Translated Protein and Polysome Analysis

New synthesis of nascent CNPY3 protein was detected by Click-iT protein analysis detection kit (Life technologies, Grand Island, NY) and conducted following the company's instructions.[32] Briefly, cells were incubated in methionine-free medium and then exposed to L-azidohomoalanine (AHA). After mixing cell lysates with the reaction buffer for 20 min, the biotin-alkyne/azide-modified protein complex was pulled down using paramagnetic Streptavidin-conjugated Dynabeads. The pulldown material was resolved by 10% SDS-PAGE and analyzed by Western immunoblotting analysis using antibodies against CNPY3 or gp96.

Polysome analysis was carried out as described previously.[27] Briefly, cells at ~70% confluence were incubated in 0.1 mg/ml cycloheximide and then lifted by scraping in polysome extraction lysis buffer. Nuclei were pelleted, and the resulting supernatant was centrifuged through a 15-60% linear sucrose gradient to fractionate cytoplasmic components according to their molecular weight. The eluted fractions were prepared with a fraction collector (Brandel, Gaithersburg, MD), and their quality was monitored at 254 nm using a UV-6 detector (ISCO, Louisville, KY). After RNA in each fraction was extracted, the levels of each individual mRNA were quantified by reverse transcription (RT) followed by quantitative real-time PCR (Q-PCR) analysis in each of the fractions.

Biotin Pull-down Assays and RIP Analysis

The synthesis of biotinylated transcripts and measurement of HuR bound to biotinylated RNA were performed as previously described.[33] Complementary DNA from HCT-116 cells was used as a template for PCR amplification of 5'-UTR, CR and 3'-UTR segments of *Cnpy3* mRNA. The 5′ primers contained the T7 RNA polymerase promoter sequence (T7; CCAAGCTTCTAATACGAC-TCACTATAGGGAGA). All sequences of oligonucleotides for synthesizing full-length *Cnpy3* 5′-UTR, CR, or 3′-UTR are described in Supplemental Table 1. PCR-amplified products were used as templates to transcribe biotinylated RNAs by using T7 RNA polymerase in the presence of biotin-cytidine 5′-triphosphate as described.[32] Biotinylated transcripts were incubated with cytoplasmic lysates for 30 min at room temperature. Complexes were isolated with paramagnetic streptavidin-conjugated Dynabeads (Dynal, Oslo, Norway) and analyzed by Western blot analysis using anti-HuR antibody.

To assess the association of endogenous HuR with endogenous *Cnpy3* mRNA, immunoprecipitation (IP) of RNP complexes was performed as described.[19,30] Twenty million cells were collected per sample, and lysates were used for IP for 4 h at room temperature in the presence of excess (30 μg) IP antibody (IgG, anti-HuR). RNA in IP materials was used in RT reactions followed by PCR and Q-PCR analysis to detect the levels of *Cnpy3* and *Gapdh* mRNAs.

Q-PCR and Immunoblotting Analyses

Total RNA was isolated by using the RNeasy mini kit (Qiagen, Valencia, CA) and used in reverse transcription (RT) and polymerase chain reaction (PCR) amplification reactions as

described.[25] Q-PCR analysis was performed using Step-one-plus Systems with specific primers, probes, and software (Applied Biosystems, Foster City, CA).

To examine protein levels, whole-cell lysates were prepared using 2% sodium dodecyl sulfate, sonicated, and centrifuged. The supernatants were boiled and size-fractionated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). After transferring proteins onto nitrocellulose filters, the blots were incubated with primary antibody; following incubations with secondary antibody.

Immunohistochemistry

After the slides were fixed in 3.7% formaldehyde in phosphate-buffered saline and rehydrated, they were incubated with the primary antibody against HuR, lysozyme or TLR2 in blocking buffer overnight and then incubated with secondary antibody conjugated with Alexa Fluor-594 (Molecular Probes, Eugene, OR). Finally, the slides were washed, mounted, and viewed through a Zeiss confocal microscope (model LSM410). Images were processed using Photoshop software (Adobe, San Jose, CA). Slides were examined in a blinded fashion by coding them, and only after examination was complete were they decoded.

Chemicals and Cell Culture

The culture medium and fetal bovine serum were purchased from Invitrogen (Carlsbad, CA) and biochemicals were from Sigma (St. Louis, MO). Antibodies recognizing HuR, lysozyme, TLR2, and CNPY3 were purchased from ThermoFisher Scientific (Waltham, MA) and Santa Cruz Biotechnology (Santa Cruz, CA). The secondary antibody conjugated to horseradish peroxidase was obtained from Sigma. The siRNAs targeting CNPY3 and HuR were made by Santa Cruz

Biotechnology. The vector expressing DDK-tagged CNPY3 was from Origene (Rockville, MD). HCT-116 and IEC-6 cells were purchased from the American Type Culture Collection (Manassas, VA) and were maintained in standard culture conditions.[24]