

# RNA-binding protein HuR promotes growth of small intestinal mucosa by activating the Wnt signaling pathway

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**ABSTRACT** Inhibition of growth of the intestinal epithelium, a rapidly self-renewing tissue, is commonly found in various critical disorders. The RNA-binding protein HuR is highly expressed in the gut mucosa and modulates the stability and translation of target mRNAs, but its exact biological function in the intestinal epithelium remains unclear. Here, we investigated the role of HuR in intestinal homeostasis using a genetic model and further defined its target mRNAs. Targeted deletion of HuR in intestinal epithelial cells caused significant mucosal atrophy in the small intestine, as indicated by decreased cell proliferation within the crypts and subsequent shrinkages of crypts and villi. In addition, the HuR-deficient intestinal epithelium also displayed decreased regenerative potential of crypt progenitors after exposure to irradiation. HuR deficiency decreased expression of the Wnt coreceptor LDL receptor-related protein 6 (LRP6) in the mucosal tissues. At the molecular level, HuR was found to bind the *Lrp6* mRNA via its 3'-untranslated region and enhanced LRP6 expression by stabilizing *Lrp6* mRNA and stimulating its translation. These results indicate that HuR is essential for normal mucosal growth in the small intestine by altering Wnt signals through up-regulation of LRP6 expression and highlight a novel role of HuR deficiency in the pathogenesis of intestinal mucosal atrophy under pathological conditions.

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## INTRODUCTION

The mammalian intestinal epithelium is among the most rapidly self-renewing tissues in the body, and its integrity is preserved through strict regulation of cell proliferation, migration, differentiation, and apoptosis (Wang, 2007; Sato and Clevers, 2013; Xiao *et al.*, 2013).

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Abbreviations used: Fzd7, Frizzled-7; IEC, intestinal epithelial cell; IE-HuR<sup>-/-</sup>, intestinal epithelial-specific HuR deletion; IP, immunoprecipitation; LRP6, LDL receptor-related protein 6; RBP, RNA-binding protein; RNP, ribonucleoprotein; UTR, untranslated region; Wnt3a-IECs, Wnt3a-transfected IEC-6 cells.

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Undifferentiated intestinal epithelial cells (IECs) replicate rapidly in the proliferative zone within crypts and differentiate as they migrate up the luminal surface to replace lost cells (Sato and Clevers, 2013). Apoptosis occurs in both the crypt area, where this process maintains the balance in cell number between newly divided and surviving cells, and the luminal surface of the intestine, where differentiated cells are lost (Gunther *et al.*, 2013). The epithelium of the human small intestine undergoes ~10<sup>11</sup> mitoses/d, and this dynamic turnover rate is tightly regulated by numerous factors at multiple levels in order to maintain proper homeostasis (Johnson, 1988; Wang, 2007; Gunther *et al.*, 2013). Inhibition of intestinal mucosal growth occurs commonly in various critical disorders, particularly in patients who undergo massive surgical operations and are then supported with total parenteral nutrition (Wildhaber *et al.*, 2003), leading to mucosal atrophy, delayed healing, and barrier dysfunction (Wildhaber *et al.*, 2003; Puleo *et al.*, 2011). However, the exact mechanisms underlying the control of intestinal epithelial integrity, especially at the posttranscriptional level, remain largely unknown.

The regulation of mRNA stability and translation is a major mechanism by which mammalian cells control gene expression (Garneau *et al.*, 2007). The posttranscriptional fate of a given mRNA is predominantly governed by the interaction of specific mRNA sequences (*cis*-elements) with two major types of *trans*-acting factors: RNA-binding proteins (RBPs) and microRNAs (miRNAs; Keene, 2007; Zhang *et al.*, 2009; Mendell *et al.*, 2012). Ribonucleoprotein (RNP) associations regulate the intracellular transport of the mRNA and its association with the translation and decay machineries (Keene, 2007; Houseley and Tollervey, 2009; Zou *et al.*, 2010). Many labile mRNAs contain relatively long 3'-untranslated regions (UTRs) bearing sequences (e.g., U- and AU-rich elements [AREs]) that function as determinants of mRNA stability and translation (Gherzi *et al.*, 2004; Keene, 2007). Among the RBPs that regulate specific subsets of mRNAs are several RBPs that modulate mRNA turnover (HuR, NF90, AUF1, BRF1, TTP, KSRP) and RBPs that modulate translation (HuR, TIAR, NF90, TIA-1; Gherzi *et al.*, 2004; Keene, 2007; Lee *et al.*, 2010). In cells responding to proliferative, immune, and stress-causing stimuli, RBPs bind to the specific sequences in the 3'-UTRs or coding regions (CRs) of collections of target mRNAs and alter their turnover and translation rates (Liao *et al.*, 2007; Lee *et al.*, 2010; Yu *et al.*, 2013). In addition, RBPs and miRNAs can jointly regulate shared target mRNAs (Srikantan *et al.*, 2012). For example, HuR competes with miR-195 to modulate *Stim1* mRNA stability antagonistically (Zhuang *et al.*, 2013), whereas CUG-binding protein 1 (CUGBP1) and miR-222 repress *Cdk4* mRNA translation synergistically (Xiao *et al.*, 2011).

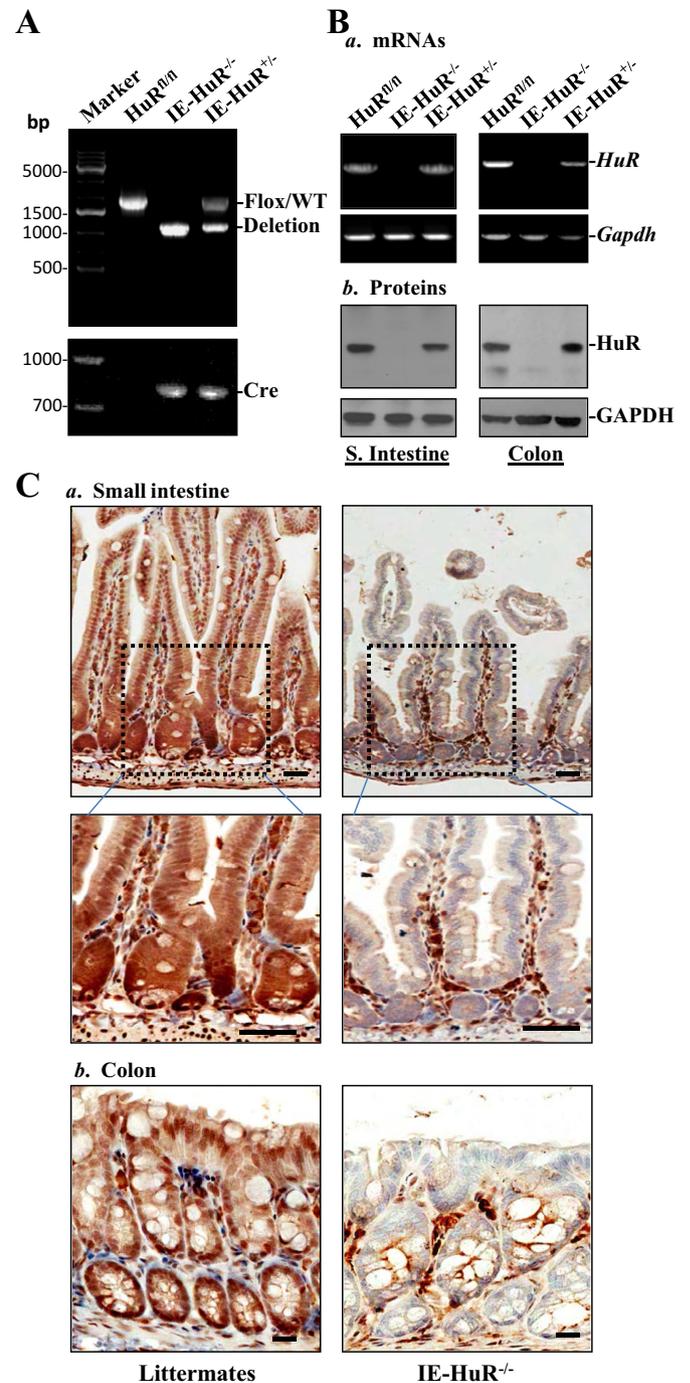
The ubiquitous RBP HuR plays a role in the posttranscriptional control of mRNAs bearing AREs (Abdelmohsen *et al.*, 2007; Papadaki *et al.*, 2009). Because constitutive HuR inactivation *in vivo* is lethal to embryos (Katsanou *et al.*, 2009), most of our knowledge about HuR function comes from studies conducted in cultured cells and/or experimental conditions in which the levels of HuR are artificially increased through the use of transgenic mice. In this regard, overexpression of HuR in mouse macrophages is shown to modulate the translation of selective inflammatory mRNAs (Katsanou *et al.*, 2005), and fertility is compromised in mice overexpressing HuR in testis (Levadoux-Martin *et al.*, 2003). Several studies using conditional HuR-knockout mice further revealed that myeloid deletion of HuR exacerbates the production of proinflammatory cytokines and increases the sensitivity to acute inflammatory reactions such as endotoxemia (Yiakouvakis *et al.*, 2012) and that target deletion of HuR in germ cells leads to male but not female sterility (Chi *et al.*, 2011). Developmental deletion of HuR also indicates that HuR is crucial for cellular differentiation, maturation, and migration (Ghosh *et al.*, 2009; Katsanou *et al.*, 2009; Papadaki *et al.*, 2009). Here we study the role of HuR in intestinal epithelial homeostasis by using a conditional gene-targeting approach and demonstrate that HuR is a positive posttranscriptional modulator in intestinal mucosal growth by activating Wnt signaling pathway through stimulation of Wnt coreceptor low-density-lipoprotein (LDL) receptor-related protein 6 (Lrp6) expression.

## RESULTS

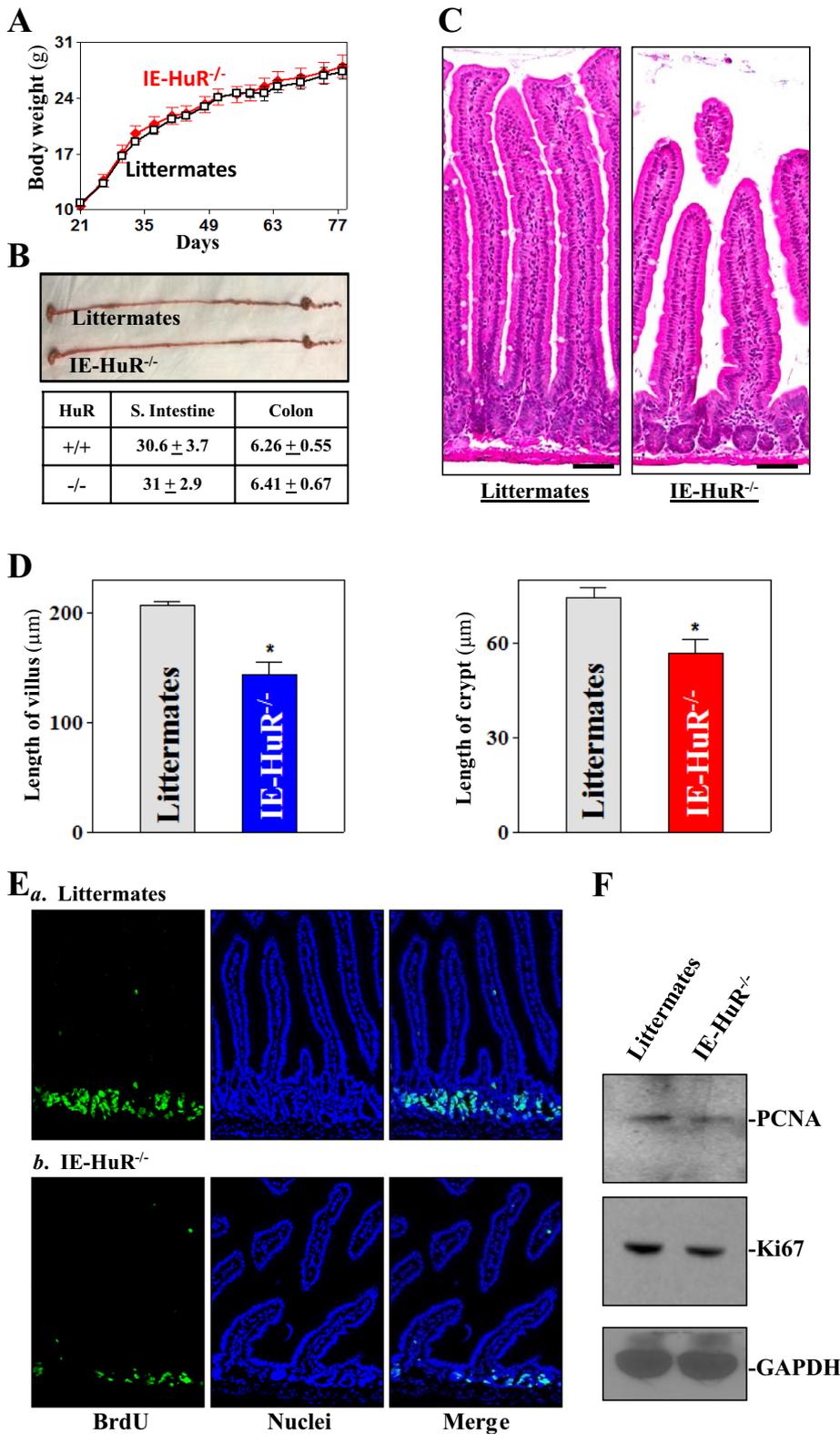
### Tissue-specific HuR deletion results in small intestinal mucosal atrophy

To investigate the *in vivo* function of HuR in intestinal epithelium, we generated intestinal epithelium-specific HuR deletion (IE-HuR<sup>-/-</sup>) mice by crossing HuR<sup>fl/fl</sup> mice with mice carrying Villin-Cre (Supplemental Figure S1A). As described previously (Mosmann *et al.*, 1983; Katsanou *et al.*, 2009), HuR<sup>fl/fl</sup> mice were produced via standard gene-targeting procedures in embryonic stem cells and contained a

fully functional HuR allele. Heterozygous IE-HuR<sup>fl/+</sup> mice appeared phenotypically normal and were subsequently intercrossed for the generation of homozygous IE-HuR<sup>-/-</sup> mice. Age-matched IE-HuR<sup>-/-</sup> mice and littermate control mice (3 or 4 mo old) were used for comparison of phenotype and showed HuR deletion at genomic DNA isolated from the intestinal mucosa of IE-HuR<sup>-/-</sup> animals (Figure 1A).



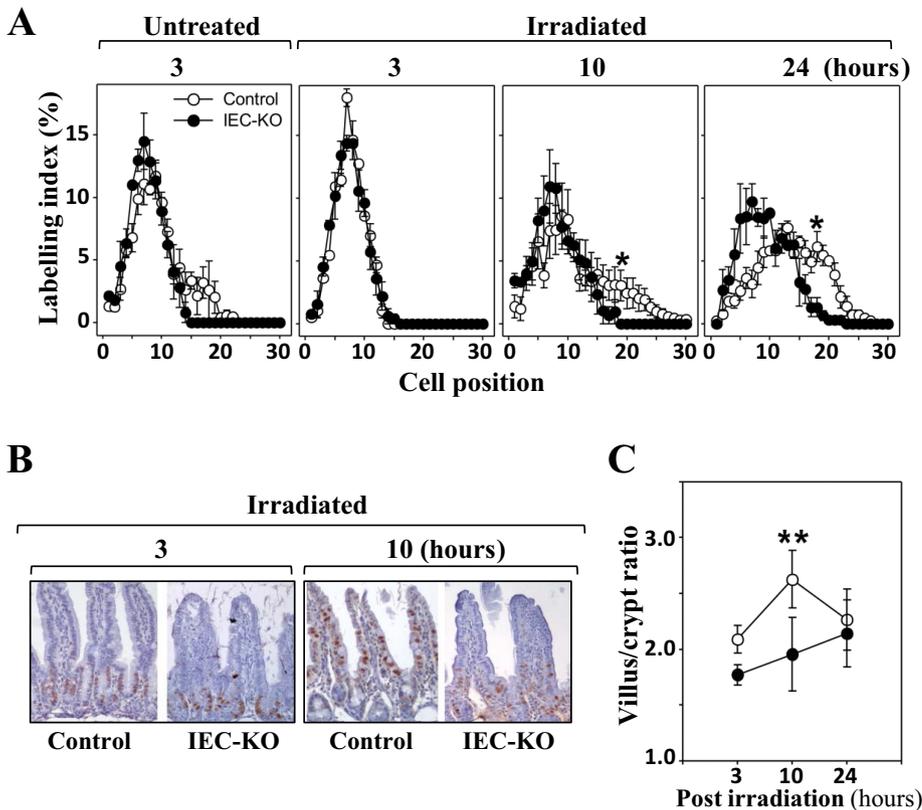
**FIGURE 1:** Characterization of intestinal epithelium-specific HuR deletion (IE-HuR<sup>-/-</sup>) mice. (A) PCR analysis of genomic DNA from small intestinal mucosa indicates floxed, HuR deletion, and Cre bands in HuR<sup>fl/fl</sup>, IE-HuR<sup>fl/+</sup>, and IE-HuR<sup>-/-</sup> mice. (B) Levels of HuR mRNA (a) and protein (b) in small (S) intestinal (left) and colonic (right) mucosa as measured by RT-PCR and Western blotting analyses. (C) Immunohistochemical staining of HuR, shown as in dark brown in small intestinal (a) and colonic (b) mucosa. Scale bar, 50  $\mu$ m.



**FIGURE 2:** HuR deletion in IECs inhibits small intestinal mucosal growth. (A) Body weights of age- and sex-matched littermate and IE-HuR<sup>-/-</sup> mice. Values are the means ± SEM ( $n = 10$ ). (B) Comparison of gastrointestinal gross morphology in littermates and IE-HuR<sup>-/-</sup> mice. (C) Photomicrographs of hematoxylin and eosin stain staining of the small intestine. Scale bar, 50  $\mu\text{m}$ . (D) Changes in the length of villi (left) and crypt (right) of the mucosa described in C ( $n = 6$ ). \* $p < 0.05$  compared with littermates. (E) Proliferating cells in small intestinal crypts as measured by BrdU labeling. BrdU (1 h postinjection, S phase), green. (F) Expression of PCNA and Ki67 proteins in the small intestinal mucosa.

Consistent with this finding, HuR mRNA and protein in the small intestinal and colonic mucosa were undetectable in IE-HuR<sup>-/-</sup> mice (Figure 1B), whereas HuR expression levels in the intestinal mucosa of HuR<sup>fl/fl</sup>-Cre<sup>-</sup> and HuR<sup>fl/+</sup> mice were normal. Immunohistochemical staining assays revealed that HuR levels almost completely disappeared in epithelial cells in the intestinal mucosa of IE-HuR<sup>-/-</sup> mouse, but its expression was unaffected in submucosal connective tissue (Figure 1C). On the other hand, there were no changes in HuR expression levels in stomach mucosa, lung, liver, and pancreas in IE-HuR<sup>-/-</sup> mice compared with those observed in littermates (Supplemental Figure S1, B and C). These findings suggest that the IE-HuR<sup>-/-</sup> mouse is a suitable gene-targeting model of HuR deficiency in the intestinal epithelium.

Generally, IE-HuR<sup>-/-</sup> mice looked normal; there were no significant differences in body weight (Figure 2A), gastrointestinal gross morphology (Figure 2B), reproduction, and general appearances between IE-HuR<sup>-/-</sup> mice and littermate controls. Of interest, IE-HuR<sup>-/-</sup> mice exhibited significant mucosal atrophy in the small intestine, as indicated by a decrease in the lengths of villi and crypts (Figure 2, C and D). The proliferating crypt cell population, marked by bromodeoxyuridine (BrdU; S phase), decreased remarkably in the small intestine of HuR<sup>-/-</sup> mice compared with those from littermates (Figure 2E). Accordingly, the levels of cell proliferation marker proteins proliferating cell nuclear antigen (PCNA) and Ki67 were also decreased in the small intestinal mucosa of IE-HuR<sup>-/-</sup> mice (Figure 2F). Moreover, the loss of HuR in IECs inhibited the regenerative potential of crypt progenitors, since S-phase descendants in the villous regions decreased significantly in IE-HuR<sup>-/-</sup> mice compared with those observed in control littermates after exposure to irradiation (Figure 3, A and B). Consistently, the villus/crypt ratio in IE-HuR<sup>-/-</sup> mice also decreased when measured 10 h after irradiation (Figure 3C). We also examined changes in colonic mucosal growth in IE-HuR<sup>-/-</sup> mice and found that epithelium-specific HuR deletion did not alter mucosal growth in the colon. There were no significant decreases in the lengths of villi and crypts and BrdU-labeled cell proliferation in IE-HuR<sup>-/-</sup> mice compared with littermate controls (unpublished data). In addition, specific HuR deletion in IECs did not affect lineage differentiation in the intestine (Supplemental Figure S2, A and B), gut permeability (Supplemental Figure S2C), or crypt number per tissue area (Supplemental Figure S2D).



**FIGURE 3:** HuR deletion in IECs alters the regenerative potential of crypt progenitors. (A) Histograms depicting the labeling indices of cells at the S phase in resting (untreated) or S-phase descendants in the regenerating mucosa at different times (hours) in littermate (control) IEC-HuR<sup>-/-</sup> (IEC-KO) mice postirradiation as measured by BrdU detection. Values are shown as means  $\pm$  SEM ( $n = 4$ ). \* $p < 0.05$ . (B) Representative photomicrographs of paraffin-embedded small intestine. Sections stained with hematoxylin (blue) and anti-BrdU (brown). (C) Villus/crypt ratio of small intestinal mucosa postregeneration. Data were derived from 12 random villi and/or crypts from three mice per group/per time point; \*\* $p < 0.001$  compared with littermates.

These results indicate that conditional HuR deletion in IECs results in small intestinal mucosal atrophy but does not alter colonic mucosal growth.

#### HuR interacts with the 3'-untranslated region of *Lrp6* mRNA

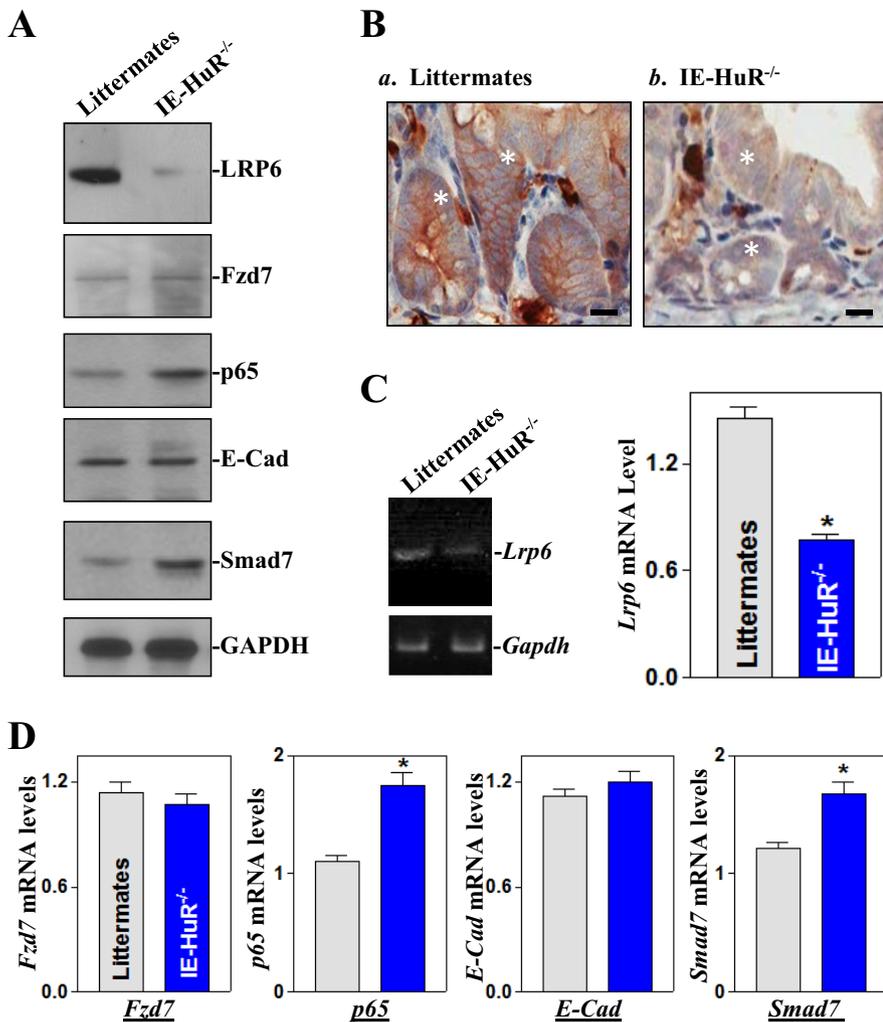
To investigate the mediators of the HuR-elicited effects, we found that the HuR-deficient intestinal epithelium was associated with decreased levels of *Lrp6* mRNA and LDL-receptor-related protein 6 (LRP6; Figure 4, A–C). In contrast, HuR deletion increased expression of p65 and Smad7 in the intestinal mucosa, although it failed to alter the levels of Frizzled-7 (Fzd7) or E-cadherin (Figure 4D). Because there are several potential hits of HuR motif in the *Lrp6* mRNA, we further examined whether HuR directly interacted with the *Lrp6* mRNA in cultured IEC-6 cells by performing ribonucleoprotein immunoprecipitation (RIP) assays using anti-HuR antibody under conditions that preserved RNP integrity (Lal *et al.*, 2004). The interaction of *Lrp6* mRNA with HuR was examined by isolating RNA from the immunoprecipitated material and subjecting it to reverse transcription (RT), followed by either conventional PCR or real-time quantitative PCR (qPCR) analyses. As shown in Figure 5A, the *Lrp6* PCR products were highly enriched in HuR samples compared with control immunoglobulin G (IgG) samples. HuR was also found to bind the p65 mRNA, although it did not preferentially associate with *Fzd7*, *E-cad*, and *Smad7* mRNAs (Supplemental Figure S3). To determine whether HuR binds to specific regions of the *Lrp6* 5'-UTR, CR, and

3'-UTR, we further tested [HuR/*Lrp6* mRNA] associations by using biotinylated transcripts that spanned the *Lrp6* mRNA regions shown (Figure 5B, schematic). After incubation with cytoplasmic lysates, the interaction between the biotinylated *Lrp6* transcripts and HuR was examined by biotin pull-down, followed by Western blot analysis (Abdelmohsen *et al.*, 2007; Chen *et al.*, 2008). As shown, HuR readily associated with the *Lrp6* 3'-UTR, particularly the fragment 3'UTR-F2 (spanning positions 5881–6441), but not with *Lrp6* 5'-UTR and fragments of CR transcripts. Moreover, the abundance of [HuR/*Lrp6* mRNA] complexes was enriched in the intestinal mucosa isolated from littermate control mice but not in the mucosa from IEC-HuR<sup>-/-</sup> mice, as measured by RIP/qPCR analysis (Figure 5C). These results indicate that the *Lrp6* mRNA is a novel target of HuR in the intestinal epithelium.

#### HuR regulates LRP6 expression posttranscriptionally

To determine the functional consequences of [HuR/*Lrp6* mRNA] associations, we reduced HuR levels by small interfering RNA (siRNA) targeting the HuR mRNA (siHuR), as reported previously (Liu *et al.*, 2009). IEC-6 cells transfected with siHuR showed <10% of HuR levels as compared with the levels seen in cells transfected with control siRNA (C-siRNA; Figure 6A, top). Of importance, HuR silencing reduced LRP6 protein by ~85% (Figure 6A, middle), but it decreased *Lrp6* mRNA levels by only ~40% (Figure 6B).

The reduction in *Lrp6* mRNA by HuR silencing was due to the destabilization of *Lrp6* mRNA, since silencing HuR selectively lowered the *Lrp6* mRNA half-life (Figure 6C). To examine whether HuR silencing also alters the translation of *Lrp6* mRNA, we examined changes in the level of new LRP6 protein synthesis after transfection with siHuR and demonstrated that newly synthesized LRP6 protein decreased significantly in HuR-silent cells compared with cells transfected with C-siRNA (Figure 6D). Inhibition of LRP6 protein synthesis by HuR silencing was specific, since there was no change in nascent glyceraldehyde-3-phosphate dehydrogenase (GAPDH) synthesis after transfection with siHuR. To further define the role of HuR in the regulation of LRP6 translation, we examined the relative distribution of *Lrp6* mRNA in individual fractions from polyribosome gradients. Although decreasing the levels of HuR did not affect global polysomal profiles (unpublished data), the abundance of *Lrp6* mRNA associated with actively translating fractions (fractions 9–11) decreased in HuR-silent cells with a shift of *Lrp6* mRNAs to low-translating fractions (fractions 6–8; Figure 6E, top); it is important to note that even small shifts in polysome size can reflect large changes in protein synthesis. In contrast, *Gapdh* mRNA, encoding the housekeeping protein GAPDH, distributed similarly in both groups (Figure 6E, bottom). HuR regulates the stability and translation of *Lrp6* mRNA by interacting with *Lrp6* 3'-UTR, since HuR silencing decreased the levels of *Lrp6*-3'UTR-F2 luciferase reporter activity (Figure 6F) but did not affect the activities of Luc-5'-UTR and Luc-CR-F1 reporter genes. On the other hand, ectopic



**FIGURE 4:** HuR deficiency correlates with reduction in LRP6 levels. (A) Changes in expression of LRP6, Fzd7, p65, E-cad, and Smad7 proteins in small intestinal mucosa in littermates and IE-HuR<sup>-/-</sup> mice. (B) Immunohistochemical staining of LRP6 in mucosal tissues. Scale bar, 50  $\mu$ m. (C) *Lrp6* mRNA levels in the intestinal mucosa as measured by RT-PCR (left) and qPCR (right) analyses. Values are the means  $\pm$  SEM ( $n = 6$ ). \* $p < 0.05$  compared with littermates. (D) Levels of *Fzd7*, *p65*, *E-cad*, and *Smad7* mRNAs as measured by qPCR analysis. \* $p < 0.05$  compared with littermates.

overexpression of HuR by infection with the adenoviral vector containing the corresponding HuR cDNA (AdHuR) increased LRP6 expression by increasing *Lrp6* mRNA stability and translation via interaction with its 3'-UTR (Supplemental Figure S4). These results indicate that HuR increases LRP6 expression by stabilizing the *Lrp6* mRNA and enhancing its translation.

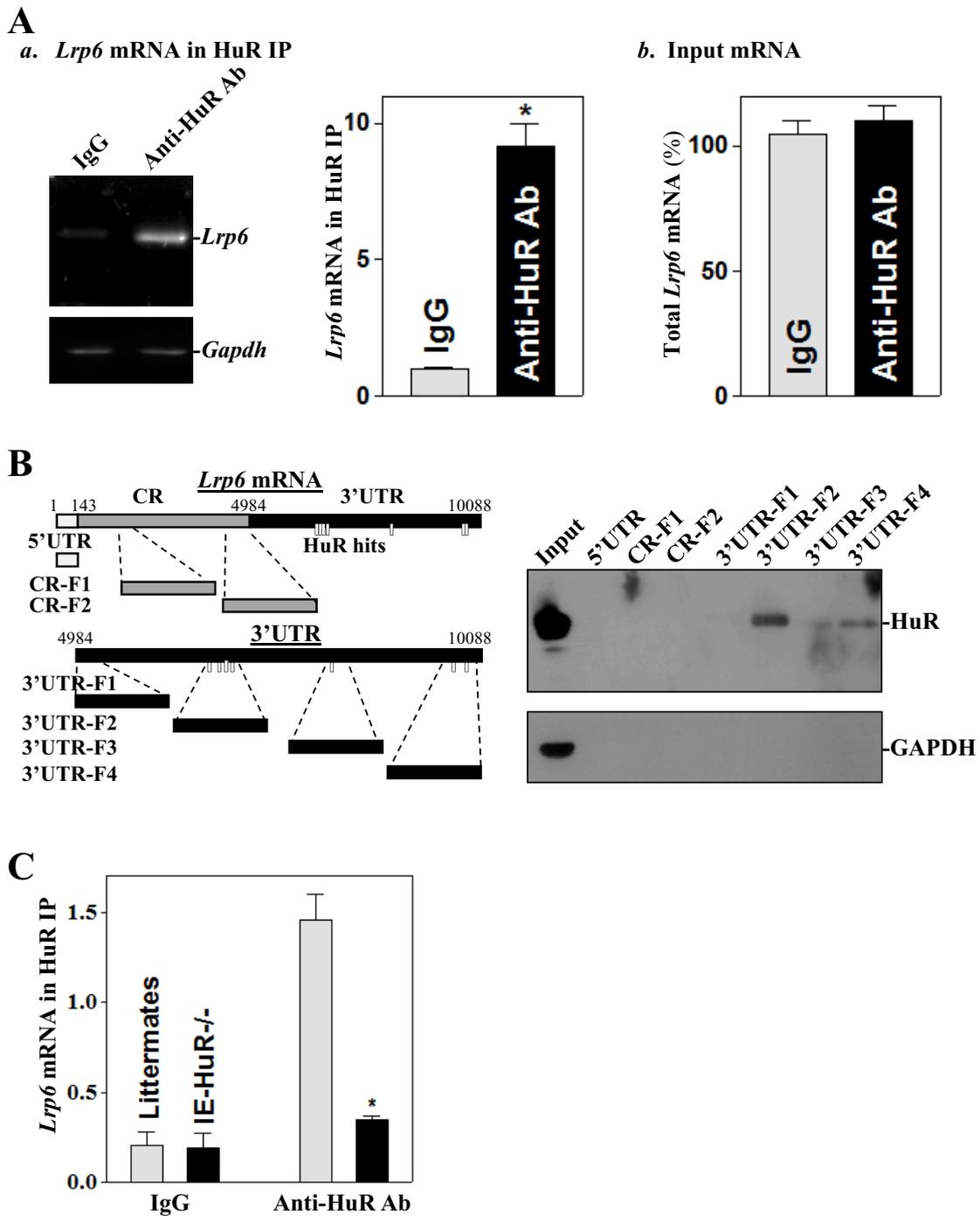
#### HuR silencing inhibits IEC proliferation in vitro

To investigate the consequences of HuR-regulated LRP6 expression upon cell proliferation, we used stable Wnt3a-transfected IEC-6 cells (Wnt3a-IECs) that were recently developed in our laboratory (Liu *et al.*, 2012). Stably transfected Wnt3a-IECs expressed high levels of Wnt3a protein (Figure 7Aa) and exhibited a dramatic activation of Wnt/ $\beta$ -catenin signaling pathway, as indicated by an increase in the levels of the Wnt/ $\beta$ -catenin reporter plasmid superTOPFLASH activity (Figure 7Ab). Consistently, ectopic Wnt3a overexpression increased IEC proliferation (Figure 7Ac), although the levels of HuR and LRP6 were unchanged in Wnt3a-IECs (Figure 7B, left). Cell cycle analysis

indicated that ~35% of parent IECs were in the S phase, whereas the population of S-phase cells significantly increased to ~54% in stable Wnt3a-IECs, along with a decrease in G1-phase cells. Moreover, decreased levels of endogenous LRP6 in stable Wnt3a-IECs by HuR silencing decreased Wnt/ $\beta$ -catenin signaling activity (Figure 7Ca), inhibited cell growth (Figure 7Cb), and returned the cell cycle distribution displayed by parent IECs. When stable Wnt3a-IECs were transfected with siHuR, S-phase cells decreased to ~27% from ~53% in cells transfected with C-siRNA (Figure 7, D and E). The inhibitory effects of decreasing endogenous LRP6 in stable Wnt3a-IECs by HuR silencing were not simply due to clonal variation, since two different clonal populations, Wnt3a-IEC-C1 and Wnt3a-IEC-C2, showed similar responses. LRP6 silencing in stable Wnt3a-IECs by transfection with siLRP6 also resulted in inhibition of the Wnt/ $\beta$ -catenin signaling pathway and cell growth, as indicated by decrease in S-phase cells. Conversely, ectopic overexpression of LRP6 in HuR-silenced cells by transfection with the LRP6 expression vector increased cell growth, along with increase in S-phase cells (Supplemental Figure S5). Marginal inhibition of parental IEC-6 cell proliferation by silencing HuR or LRP6 was also observed (Supplemental Figure S6). On the other hand, silencing HuR or LRP6 alone failed to induce apoptosis in parent IEC-6 cells or Wnt3a-IECs, as measured by assessment of cell viability, annexin-V staining, and levels of cleaved caspase-3. These results indicate that HuR-induced LRP6 expression plays an important role in stimulation of IEC proliferation.

#### DISCUSSION

Our previous studies demonstrated that HuR in IECs regulates expression of several proliferation/apoptosis-associated genes, such as p53 and nucleophosmin (Zou *et al.*, 2006), ATF2 (Xiao *et al.*, 2007), XIAP (Zhang *et al.*, 2009), JunD (Zou *et al.*, 2010), MEK-1 (Wang *et al.*, 2010), c-Myc (Liu *et al.*, 2009), and Stim1 (Zhuang *et al.*, 2013), at the posttranscriptional level, suggesting the involvement of HuR in maintaining intestinal epithelial homeostasis. However, all of these studies were conducted in cultured IECs; therefore the exact function of HuR in the intestinal epithelium in vivo remains to be fully defined. Using a conditional tissue-specific gene-targeting approach, here we provided powerful genetic evidence of the physiological role of HuR in the regulation of intestinal mucosal integrity. Specific deletion of HuR in IECs caused small intestinal mucosal atrophy in mice, although it failed to alter colonic mucosal growth. Experiments aimed at characterizing the aspects of HuR targets in this process suggested that the inhibition of intestinal mucosal growth induced by HuR deletion resulted from the inactivation of Wnt signaling due to the repression of the expression of the Wnt coreceptor LRP6. These findings advance our understanding of the biological function of HuR in the intestinal epithelium and highlight a novel role of HuR deficiency in

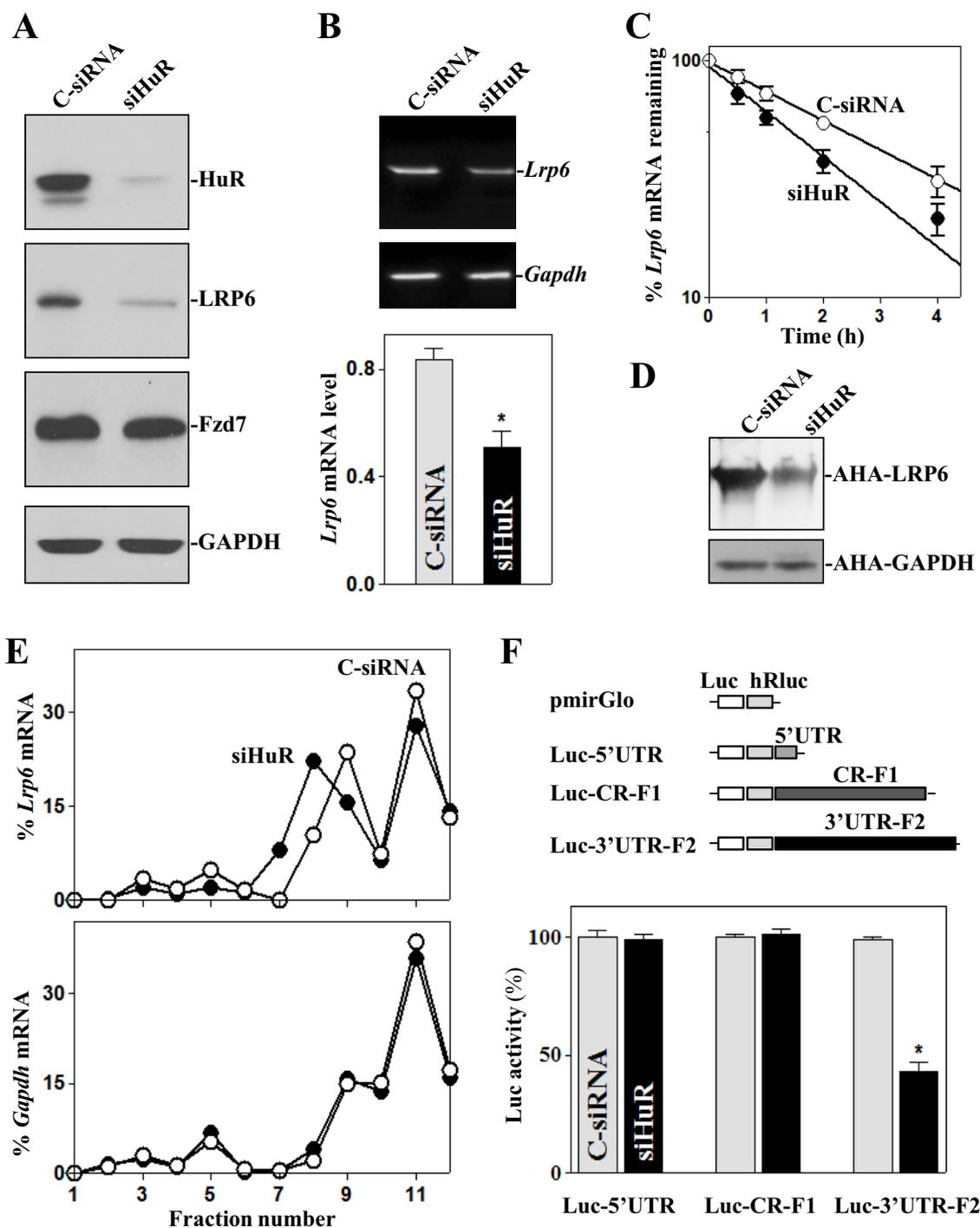


**FIGURE 5:** HuR binds the 3'-UTR of *Lrp6* mRNA. (A) Association of endogenous HuR with endogenous *Lrp6* mRNA in IEC-6 cells as measured by RIP/qPCR analysis using either anti-HuR antibody (Ab) or control IgG: (a) *Lrp6* mRNAs in HuR IP as measured by RT-PCR (left) and qPCR (right) analyses; and (b) levels of total input mRNAs. Values are the means  $\pm$  SEM from triplicate samples. \* $p < 0.05$  compared with IgG IP. (B) HuR immunoblots using the pull-down materials by biotinylated transcripts of *Lrp6* 5'-UTR, CR, and 3'-UTR. Left, schematic representation of various biotinylated *Lrp6* transcripts. (C) Association of HuR with the *Lrp6* mRNA in small intestinal mucosa in littermates and IE-HuR<sup>-/-</sup> mice as measured by RIP/qPCR analysis. Values are the means  $\pm$  SEM ( $n = 5$ ). \* $p < 0.05$  compared with littermates.

the pathogenesis of intestinal mucosal atrophy under pathological conditions.

Several recent mouse HuR gene-deletion studies in diverse tissue/organ cell types have improved our understanding of physiological roles of HuR in mammals that, in some circumstances, contradicts conventional wisdom of previous in vitro cell biology studies (Xiao *et al.*, 2007; Katsanou *et al.*, 2009; Papadaki *et al.*, 2009; Young

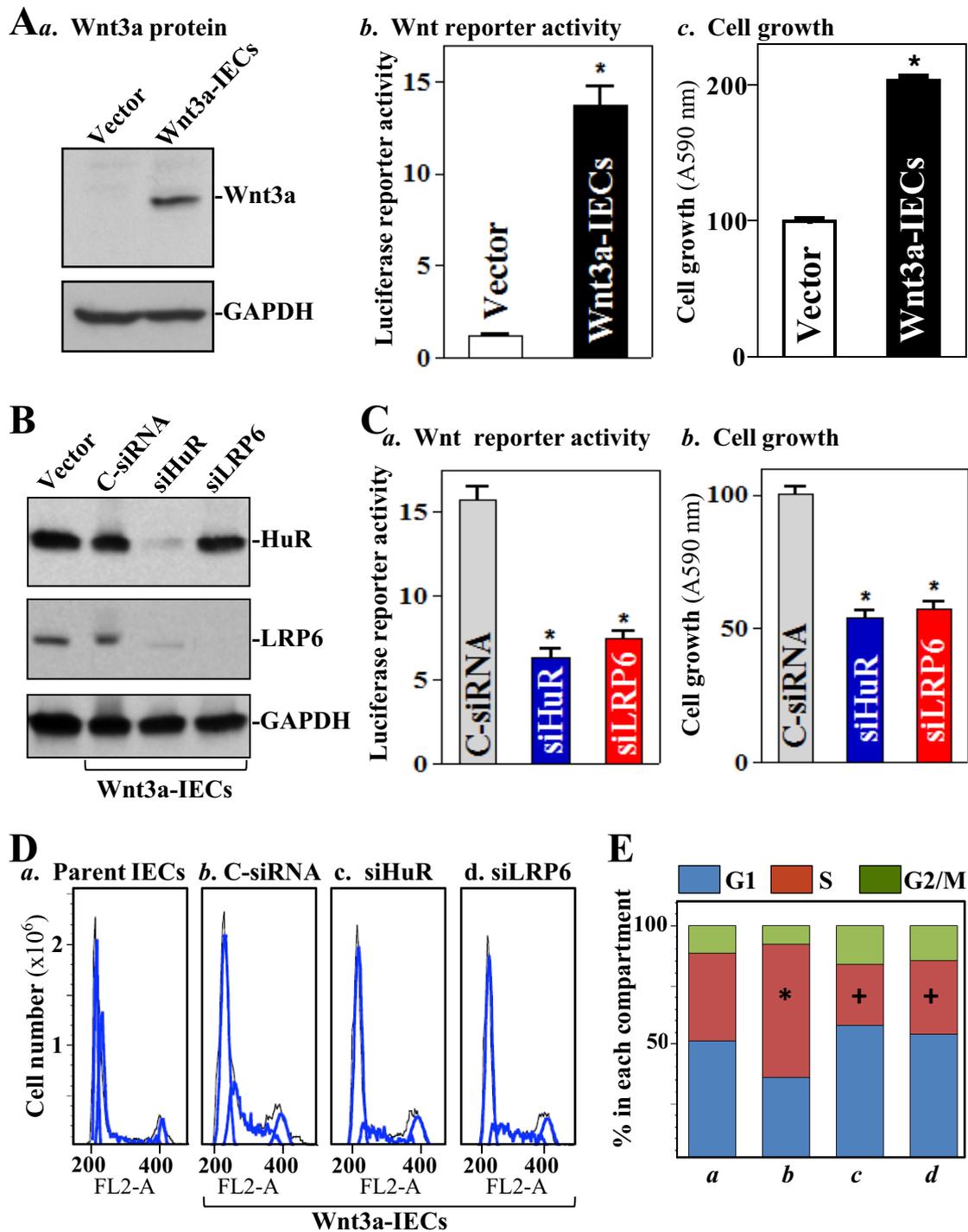
*et al.*, 2009). Results from our in vivo studies demonstrate the essential role of HuR in normal mucosal growth in the small intestine, since significant defects in mucosal renewal occurred in IE-HuR<sup>-/-</sup> mice, as indicated by a decrease in cell proliferation in the crypts and subsequent shrinkages of crypts and villi in the mucosal tissue. This inhibitory phenotype in small intestinal mucosal growth by specific HuR deletion is not surprising, because HuR up-regulates expression of



**FIGURE 6:** HuR silencing destabilizes *Lrp6* mRNA and represses its translation. (A) Immunoblots of HuR and LRP6 in cells transfected with siHuR or C-siRNA for 48 h. (B) Levels of *Lrp6* mRNA as measured by RT-PCR (top) and qPCR (bottom) analyses in cells treated as described in A. Values are the means  $\pm$  SEM ( $n = 3$ ). \* $p < 0.05$  compared with C-siRNA. (C) Half-life of the *Lrp6* mRNA in cells described in A. (D) Newly synthesized LRP6 protein as measured by L-azidohomoalaine (AHA) incorporation assays. (E) Distributions of *Lrp6* (top) and *Gapdh* (bottom) mRNAs in each gradient fraction of polysomal profiles prepared from cells described in A. (F) Levels of reporter activities as measured by analysis of *Lrp6* 5'-UTR, CR, and 3'-UTR luciferase reporters after HuR silencing. Top, schematic of plasmids of different chimeric firefly luciferase *Lrp6* reporters. \* $p < 0.05$  compared with C-siRNA.

several cell proliferation-promoting proteins (Liang *et al.*, 2012; Woodhoo *et al.*, 2012), and small intestinal mucosa is the tissue with the most rapid turnover rate in the body (Johnson, 1988; Sato and Clevers, 2013). Our previous *in vitro* studies (Liu *et al.*, 2009) indicated that HuR stimulates translation of the transcription factor

c-Myc, which is crucial for stimulation of IEC proliferation and enhances gut mucosal healing after injury (Wang and Johnson, 1994; Liu *et al.*, 2005). Consistent with our findings, a recent study (Giammanco *et al.*, 2014) shows that conditional tissue-specific genetic deletion of HuR in IECs reduces intestinal tumor development



**FIGURE 7:** Silencing HuR or LRP6 inhibits cell proliferation in stable Wnt3a-transfected IEC-6 cells. (A) Western blot analysis of the levels of Wnt3a protein (a), Wnt/ $\beta$ -catenin signaling activity as measured by using the TOPFLASH reporter assay (b) and cell growth as examined by MTT assay (c) in stable Wnt3a-transfected cells. Values are the means  $\pm$  SEM ( $n = 6$ ). \* $p < 0.05$  compared with vector. (B) Immunoblots of HuR and LRP6 after transfection with siHuR or C-siRNA for 48 h. (C) Changes in Wnt/ $\beta$ -catenin signaling activity and cell growth. Values are the means  $\pm$  SEM ( $n = 3$ ). \* $p < 0.05$  compared with C-siRNA. (D) Flow cytometric analysis of cell cycle distribution. Black line, area; blue line, curve fit; FL2-A, DNA content. (E) The relative G1, S, and G2/M compartments calculated from data described in D. Values are the means from three separate experiments. \*+ $p < 0.05$  compared with parent IECs and Wnt3a-IECs transfected with C-siRNA, respectively.

by altering distinct signaling pathways of proliferation and apoptosis. Although the present study is tightly focused on the role of HuR in regulating intestinal mucosal growth, HuR-modulated apoptosis in IECs is also implicated in intestinal epithelial homeostasis. Because

HuR can act as a proapoptotic or antiapoptotic factor, depending on cell type, magnitude of damage, type of apoptotic inducers, and the presence or absence of other factors, the effect of HuR deletion on IEC apoptosis in vivo will be investigated separately.

Although HuR levels were undetectable in the colonic epithelium of IE-HuR<sup>-/-</sup> mice, there were no significant differences in the rates of colonic mucosal growth between HuR-deficient mice and littermates. The exact reasons for which HuR depletion failed to alter colonic mucosal growth remain unknown, but basal mucosal turnover rate in the colon is lower than that observed in the small intestine (Johnson, 1988). In addition, the basal level of LRP6 (a major target of HuR in the small intestinal epithelium) was markedly lower in the colonic mucosa than in the small intestinal mucosa (unpublished data). Because limited information is available on the specific mechanisms underlying the control of mucosal growth throughout different sections of the intestinal tract, the relative importance of Wnt signaling, particularly possible alternatives for LRP6 in colonic mucosa, are unclear. Adenomatous polyposis coli (APC) protein was recently shown to function as an RNA-binding protein, and the collection of interacting transcripts provides a link between Wnt pathway proteins and RNA networks (Preitner *et al.*, 2014). However, the exact role of APC in colonic mucosal homeostasis in HuR-deficient mice remains to be investigated.

Another significant finding from this study is that the *Lrp6* mRNA is a novel target of HuR and that [HuR/*Lrp6* mRNA] association not only stabilizes *Lrp6* mRNA but also enhances its translation. Through the use of various ectopic reporters bearing partial transcripts spanning the *Lrp6* 5'-UTR, CR, and 3'-UTR with or without the predicted HuR-hit motif, our results further show that HuR interacted predominantly with the *Lrp6* 3'-UTR F2 element (spanning positions 5881–6441) but not with the 5'-UTR or CR elements. Although we did not characterize the specific nucleotides with which HuR interacts and increases *Lrp6* mRNA stability and translation, there are four predicted HuR-hit motifs within the *Lrp6* 3'-UTR F2 region. Moreover, the F2 sequence of the *Lrp6* 3'-UTR was found to be functional, because both repression of LRP6 by HuR silencing and stimulation of reporter activity by HuR overexpression occurred only when cells were transfected with the *Lrp6*-3'-UTR-F2 luciferase reporter constructs but not with the *Lrp6*-5'-UTR or *Lrp6* CR-F1 reporter constructs. These observations are consistent with other results, which demonstrated that HuR associates with its target mRNAs via their 3'-UTRs, thus stabilizing mRNAs and/or increasing their translation (Lal *et al.*, 2004; Xiao *et al.*, 2007; Wang *et al.*, 2010). Although HuR commonly interacts with the 3'-UTRs of target transcripts, in some instances it also associates with the CRs of target mRNAs for its regulatory actions. In this regard, we reported that HuR stabilizes *Xiap* mRNA by directly interacting with the *Xiap* CR (Zhang *et al.*, 2009).

Intestinal epithelium-specific HuR deletion inhibits small intestinal mucosal growth by inactivating Wnt signaling as a result of repression of LRP6 expression. Wnt signaling is critically required for gut development and acts as a key regulator of intestinal epithelial renewal (van Es *et al.*, 2005; Liu *et al.*, 2012). Central to this signaling pathway is the stabilization of  $\beta$ -catenin and its interaction with DNA-binding factors of the T-cell factor (TCF) family in the nucleus (Clevers, 2006). In response to stress, released Wnt proteins in the extracellular milieu bind to serpentine receptors of the Fzd family and to coreceptors LRP5/6, which leads to accumulation of dephosphorylated  $\beta$ -catenin and its stabilization (He *et al.*, 2004; Clevers, 2006). Subsequently, the stabilized  $\beta$ -catenin undergoes nuclear translocation and association with TCF transcription factors, enabling transactivation of their target genes. Conditional Wnt gene deletion or overexpression of the Wnt natural inhibitor Dickkopf1 disrupts gut development, represses mucosal growth, and delays healing of damaged mucosa (van Es *et al.*, 2005; Koch *et al.*, 2009). Activation of Wnt signaling also modulates the sensitivity of cells to apoptosis by altering c-Myc expression (You *et al.*, 2002). In this study, LRP6

expression levels decreased dramatically in the intestinal mucosal tissue in IE-HuR<sup>-/-</sup> mice and in an HuR-silent population of IEC-6 cells. Moreover, LRP6 silencing or decreased levels of LRP6 by HuR silencing inhibited Wnt/ $\beta$ -catenin signaling pathway and repressed cell proliferation in stable Wnt3a-IECs, whereas ectopic overexpression of LRP6 in HuR-silenced cells promoted cell proliferation. In sum, our results indicate that HuR is essential for the maintenance of intestinal mucosal homeostasis by altering Wnt signaling activity through posttranscriptional regulation of LRP6 expression.

## MATERIALS AND METHODS

### Animal studies

All experiments were approved according to animal experimental ethics committee guidelines by the University of Maryland Baltimore Institutional Animal Care and Use Committee. Mice were housed and handled in a specific pathogen-free breeding barrier and cared for by trained technicians and veterinarians. The strategy to generate and genotype IE-HuR<sup>-/-</sup> mice is provided in Supplemental Figure S1A. The HuR floxed mouse has been described elsewhere (Katsanou *et al.*, 2009; Chi *et al.*, 2011; Yiakouvakaki *et al.*, 2012), and HuR<sup>fllox/fllox</sup> (HuR<sup>fl/fl</sup>) was crossed with Villin-Cre mice (kindly provided by Deborah Gumucio, University of Michigan, Ann Arbor, MI) to yield IE-HuR<sup>-/-</sup> mice. HuR<sup>fl/fl</sup>-Cre<sup>-</sup> mice served as littermate control. Animals were killed by pentobarbital overdose. BrdU was incorporated in intestinal mucosa by intraperitoneal injection of 2 mg of BrdU (Sigma-Aldrich, St. Louis, MO) in phosphate-buffered saline. A 4-cm small intestinal segment taken from 0.5 cm distal to the ligament of Treitz and the segment of middle colon were collected 1 h after injection. The mucosa was scraped from the underlying smooth muscle with a glass microscope slide and used for measurements of the levels of various mRNA and protein expression and HuR association with given mRNAs.

### Cell cultures and plasmid construction

The IEC-6 cell line, derived from normal rat intestinal crypt cells, was used at passages 15–20 in experiments (Liu *et al.*, 2003). Antibodies recognizing HuR, LRP6, Frizzled-7 (Fzd7), Smad7, and GAPDH were obtained from Santa Cruz Biotechnology (Santa Cruz, CA) and BD Biosciences (Sparks, MD), and the secondary antibody was obtained from Sigma-Aldrich. Stable Wnt3a-transfected IEC-6 cells (Wnt3a-IECs) were developed and maintained as described in our previous studies (Liu *et al.*, 2012).

Recombinant adenoviral plasmids containing human HuR (Ad-HuR) were constructed by using the Adeno-X Expression System (Clontech, Mountain View, CA; Zou *et al.*, 2006). The chimeric firefly luciferase reporter construct containing *Lrp6* mRNA was described previously (Liu *et al.*, 2009). The full-length *Lrp6* 5'-UTR, two fragments of CR, and different 3'-UTR fragments were subcloned into the pmirGLO Dual-Luciferase miRNA Target Expression Vector (Promega, Madison, WI) to generate the pmirGLO-Luc-*Lrp6*-5'-UTR, pmirGLO-Luc-*Lrp6*-CR, and pmirGLO-*Lrp6*-3'-UTR reporter constructs. The Wnt/ $\beta$ -catenin signaling luciferase reporter plasmid superTOPFLASH was purchased from Addgene (Cambridge, MA). Transient transfections were performed using 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 S1.

### Western blot analysis

Whole-cell lysates were prepared using 2% SDS, sonicated, and centrifuged at 4°C for 15 min. The supernatants were boiled

and size-fractionated by SDS-PAGE. After the blots were incubated with primary antibody and then secondary antibodies, immunocomplexes were developed by using chemiluminescence.

### RT followed by PCR and real-time qPCR analyses

Total RNA was isolated from cells after different treatments by using RNeasy Mini Kit (Qiagen, Valencia, CA) and used in reverse transcription and PCR amplification reactions as described (Xiao *et al.*, 2011). The levels of *Gapdh* PCR product were assessed to monitor the even RNA input in RT or qPCR samples. qPCR was performed using 7500-Fast Real-Time PCR Systems (Applied Biosystems, Foster City, CA) with specific primers, probes, and software (Applied Biosystems).

### Biotin pull-down assays and RIP analysis

The synthesis of biotinylated transcripts and analysis of HuR bound to biotinylated RNA were carried out as previously described (Xiao *et al.*, 2007; Yu *et al.*, 2011). cDNA from IEC-6 cells was used as a template for PCR amplification of 5'-UTR, CR, and 3'-UTR of *Lrp6* mRNA. The 5' primers contained the T7 RNA polymerase promoter sequence (T7; CCAAGCT-TCTAATACGAC-TCACTATAGGGAGA). All sequences of oligonucleotides for preparation of full-length *Lrp6* 5'-UTR and various fragments of CR or 3'-UTR are described in Supplemental Table S1. 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 (Zhang *et al.*, 2009). Biotinylated transcripts were incubated with cytoplasmic lysates for 30 min at room temperature. Complexes were isolated with paramagnetic streptavidin-conjugated Dynabeads (Dyna, Oslo, Norway) and analyzed by Western blot analysis.

To assess the association of endogenous HuR with endogenous *Lrp6* mRNA, immunoprecipitation (IP) of RNP complexes was performed as described (Xiao *et al.*, 2007; Cui *et al.*, 2012). 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  $\mu$ g) IP antibody (IgG, anti-HuR). RNA in IP materials was used in RT followed by PCR and qPCR analysis to detect the presence of *Lrp6* and *Gapdh* mRNAs.

### Cell proliferation assays

Cell proliferation was determined by the 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyletetrazolium bromide (MTT) assay kit (Roche, Indianapolis, IN) and flow cytometry analysis (Mosmann *et al.*, 1983; Xiao *et al.*, 2013). For MTT assays, cells were seeded into 96-well plates, and after 48 h, MTT was added to each well. For cell-cycle analysis, isolating and staining of nuclei from cells were performed with a CycleTestPlus DNA Reagent Kit (BD Biosciences, San Jose, CA). After propidium iodide was stoichiometrically bound to the nuclei, the samples were run on a flow cytometer.

### Statistics

Results are expressed as the means  $\pm$  SEM from three to six samples. The significance of the difference between means was determined by Student's *t* test;  $p < 0.05$  was considered significant.

### Additional methods

Additional methods, including the procedures for irradiation, histological analysis, assays for in vivo gut permeability, analysis of newly translated protein, and polysome analysis, are given in the Supplementary Methods.

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## REFERENCES

- Abdelmohsen K, Pullmann R Jr, Lal A, Kim HH, Galban S, Yang X, Blethrow JD, Walker M, Shubert J, Gillespie DA, *et al.* (2007). Phosphorylation of HuR by Chk2 regulates SIRT1 expression. *Mol Cell* 25, 543–557.
- Chen J, Xiao L, Rao JN, Zou T, Liu L, Bellavance E, Gorospe M, Wang JY (2008). JunD represses transcription and translation of the tight junction protein zona occludens-1 modulating intestinal epithelial barrier function. *Mol Biol Cell* 19, 3701–3712.
- Chi MN, Auriol J, Jegou B, Kontoyiannis DL, Turner JM, de Rooij DG, Morello D (2011). The RNA-binding protein ELAVL1/HuR is essential for mouse spermatogenesis, acting both at meiotic and postmeiotic stages. *Mol Biol Cell* 22, 2875–2885.
- Clevers H (2006). Wnt/b-catenin signaling in development and disease. *Cell* 127, 469–480.
- Cui YH, Xiao L, Rao JN, Zou T, Liu L, Chen Y, Turner DJ, Gorospe M, Wang JY (2012). miR-503 represses CUG-binding protein 1 translation by recruiting CUGBP1 mRNA to processing bodies. *Mol Biol Cell* 23, 151–162.
- Garneau NL, Wilusz J, Wilusz CJ (2007). The highways and byways of mRNA decay. *Nat Rev Mol Cell Biol* 8, 113–126.
- Gherzi R, Lee KY, Briata P, Wegmuller D, Moroni C, Karin M, Chen CY (2004). A KH domain RNA binding protein, KSRP, promotes ARE-directed mRNA turnover by recruiting the degradation machinery. *Mol Cell* 14, 571–583.
- Ghosh M, Aguila HL, Michaud J, Ai Y, Wu MT, Hemmes A, Ristimaki A, Guo C, Furneaux H, Hla T (2009). Essential role of the RNA-binding protein HuR in progenitor cell survival in mice. *J Clin Invest* 119, 3530–3543.
- Giammanco A, Blanc V, Montenegro G, Klos C, Xie Y, Kennedy S, Luo J, Chang SH, Hla T, Nalbantoglu I, *et al.* (2014). Intestinal epithelial HuR modulates distinct pathways of proliferation and apoptosis and attenuates small intestinal and colonic tumor development. *Cancer Res* 74, 5322–5335.
- Gunther C, Neumann H, Neurath MF, Becker C (2013). Apoptosis, necrosis and necroptosis: cell death regulation in the intestinal epithelium. *Gut* 62, 1062–1071.
- He X, Semenov M, Tamai K, Zeng X (2004). LDL receptor-related proteins 5 and 6 in Wnt/beta-catenin signaling: arrows point the way. *Development* 131, 1663–1677.
- Houseley J, Tollervey D (2009). The many pathways of RNA degradation. *Cell* 136, 763–776.
- Johnson LR (1988). Regulation of gastrointestinal mucosal growth. *Physiol Rev* 68, 456–502.
- Katsanou V, Milatos S, Yiakouvakis A, Sgantzis N, Kotsioni A, Alexiou M, Harokopos V, Aidinis V, Hemberger M, Kontoyiannis DL (2009). The RNA-binding protein Elav1/HuR is essential for placental branching morphogenesis and embryonic development. *Mol Cell Biol* 29, 2762–2776.
- Katsanou V, Papadaki O, Milatos S, Blackshear PJ, Anderson P, Kollias G, Kontoyiannis DL (2005). HuR as a negative posttranscriptional modulator in inflammation. *Mol Cell* 19, 777–789.
- Keene JD (2007). RNA regulons: coordination of post-transcriptional events. *Nat Rev Genet* 8, 533–543.
- Koch S, Capaldo CT, Samarin S, Nava P, Neumaier I, Skerra A, Sacks DB, Parkos CA, Nusrat A (2009). Dkk-1 inhibits intestinal epithelial cell migration by attenuating directional polarization of leading edge cells. *Mol Biol Cell* 20, 4816–4825.
- Lal A, Mazan-Mamczarz K, Kawai T, Yang X, Martindale JL, Gorospe M (2004). Concurrent versus individual binding of HuR and AUF1 to common labile target mRNAs. *EMBO J* 23, 3092–3102.
- Lee EK, Kim HH, Kuwano Y, Abdelmohsen K, Srikantan S, Subaran SS, Gleichmann M, Mughal MR, Martindale JL, Yang X, *et al.* (2010). hnRNP C promotes APP translation by competing with FMRP for APP mRNA recruitment to P bodies. *Nat Struct Mol Biol* 17, 732–739.

- Levadoux-Martin M, Gouble A, Jegou B, Vallet-Erdtmann V, Aurioi J, Mercier P, Morello D (2003). Impaired gametogenesis in mice that over-express the RNA-binding protein HuR. *EMBO Rep* 4, 394–399.
- Liang PI, Li WM, Wang YH, Wu TF, Wu WR, Liao AC, Shen KH, Wei YC, Hsing CH, Shiue YL, et al. (2012). HuR cytoplasmic expression is associated with increased cyclin A expression and poor outcome with upper urinary tract urothelial carcinoma. *BMC Cancer* 12, 611.
- Liao B, Hu Y, Brewer G (2007). Competitive binding of AUF1 and TIAR to MYC mRNA controls its translation. *Nat Struct Mol Biol* 14, 511–518.
- Liu L, Li L, Rao JN, Zou T, Zhang HM, Boneva D, Bernard MS, Wang JY (2005). Polyamine-modulated expression of c-myc plays a critical role in stimulation of normal intestinal epithelial cell proliferation. *Am J Physiol Cell Physiol* 288, C89–C99.
- Liu L, Rao JN, Zou T, Xiao L, Smith A, Zhuang R, Turner DJ, Wang JY (2012). Activation of Wnt3a signaling stimulates intestinal epithelial repair by promoting c-Myc-regulated gene expression. *Am J Physiol Cell Physiol* 302, C277–C285.
- Liu L, Rao JN, Zou T, Xiao L, Wang PY, Turner DJ, Gorospe M, Wang JY (2009). Polyamines regulate c-Myc translation through Chk2-dependent HuR phosphorylation. *Mol Biol Cell* 20, 4885–4898.
- Liu L, Santora R, Rao JN, Guo X, Zou T, Zhang HM, Turner DJ, Wang JY (2003). Activation of TGF- $\beta$ -Smad signaling pathway following polyamine depletion in intestinal epithelial cells. *Am J Physiol Gastrointest Liver Physiol* 285, G1056–G1067.
- Mendell JT, Olson EN (2012). MicroRNAs in stress signaling and human disease. *Cell* 148, 1172–1187.
- Mosmann T (1983). Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J Immunol Methods* 65, 55–63.
- Papadaki O, Milatos S, Grammenoudi S, Mukherjee N, Keene JD, Kontoyiannis DL (2009). Control of thymic T cell maturation, deletion and egress by the RNA-binding protein HuR. *J Immunol* 182, 6779–6788.
- Preitner N, Quan J, Nowakowski DW, Hancock ML, Shi J, Tcherkezian J, Young-Pearse TL, Flanagan JG (2014). APC is an RNA-binding protein, and its interactome provides a link to neural development and microtubule assembly. *Cell* 158, 368–382.
- Puleo F, Arvanitakis M, Van Gossum A, Preiser JC (2011). Gut failure in the ICU. *Semin Respir Crit Care Med* 32, 626–638.
- Sato T, Clevers H (2013). Growing self-organizing mini-guts from a single intestinal stem cell: mechanism and applications. *Science* 340, 1190–1194.
- Srikantan S, Tominaga K, Gorospe M (2012). Functional interplay between RNA-binding protein HuR and microRNAs. *Curr Protein Peptide Sci* 13, 372–379.
- van Es JH, Jay P, Gregorieff A, van Gijn ME, Jonkheer S, Hatzis P, Thiele A, van den Born M, Begthel H, Brabletz T, et al. (2005). Wnt signalling induces maturation of Paneth cells in intestinal crypts. *Nat Cell Biol* 7, 381–386.
- Wang JY (2007). Polyamines and mRNA stability in regulation of intestinal mucosal growth. *Amino Acids* 33, 241–252.
- Wang JY, Johnson LR (1994). Expression of protooncogenes c-fos and c-myc in healing of gastric mucosal stress ulcers. *Am J Physiol Gastrointest Liver Physiol* 266, G878–G886.
- Wang PY, Rao JN, Zou T, Liu L, Xiao L, Yu TX, Turner DJ, Gorospe M, Wang JY (2010). Post-transcriptional regulation of MEK-1 by polyamines through the RNA-binding protein HuR modulating intestinal epithelial apoptosis. *Biochem J* 426, 293–306.
- Wildhaber BE, Yang H, Teitelbaum DH (2003). Total parenteral nutrition-induced apoptosis in mouse intestinal epithelium: modulation by keratinocyte growth factor. *J Surg Res* 112, 144–151.
- Woodhoo A, Iruarizaga-Lejarreta M, Beraza N, Garcia-Rodriguez JL, Embade N, Fernandez-Ramos D, Martinez-Lopez N, Gutierrez-De Juan V, Arteta B, Caballeria J, et al. (2012). Human antigen R contributes to hepatic stellate cell activation and liver fibrosis. *Hepatology* 56, 1870–1882.
- Xiao L, Cui YH, Rao JN, Zou T, Liu L, Smith A, Turner DJ, Gorospe M, Wang JY (2011). Regulation of cyclin-dependent kinase 4 translation through CUG-binding protein 1 and microRNA-222 by polyamines. *Mol Biol Cell* 22, 3055–3069.
- Xiao L, Rao JN, Zou T, Liu L, Cao S, Martindale JL, Su W, Chung HK, Gorospe M, Wang JY (2013). miR-29b represses intestinal mucosal growth by inhibiting translation of cyclin-dependent kinase 2. *Mol Biol Cell* 24, 3038–3046.
- Xiao L, Rao JN, Zou T, Liu L, Marasa BS, Chen J, Turner DJ, Zhou H, Gorospe M, Wang JY (2007). Polyamines regulate the stability of activating transcription factor-2 mRNA through RNA-binding protein HuR in intestinal epithelial cells. *Mol Biol Cell* 18, 4579–4590.
- Yiakouvakis A, Dimitriou M, Karakasiliotis I, Eftychi C, Theocharis S, Kontoyiannis DL (2012). Myeloid cell expression of the RNA-binding protein HuR protects mice from pathologic inflammation and colorectal carcinogenesis. *J Clin Invest* 122, 48–61.
- You Z, Saims D, Chen S, Zhang Z, Guttridge DC, Guan KL, MacDougald OA, Brown AM, Evan G, Kitajewski J, et al. (2002). Wnt signaling promotes oncogenic transformation by inhibiting c-Myc-induced apoptosis. *J Cell Biol* 157, 429–440.
- Young LE, Sanduja S, Bemis-Standoli K, Pena EA, Price RL, Dixon DA (2009). The mRNA binding proteins HuR and tristetraprolin regulate cyclooxygenase 2 expression during colon carcinogenesis. *Gastroenterology* 136, 1669–1679.
- Yu TX, Rao JN, Zou T, Liu L, Xiao L, Ouyang M, Cao S, Gorospe M, Wang JY (2013). Competitive binding of CUGBP1 and HuR to occludin mRNA controls its translation and modulates epithelial barrier function. *Mol Biol Cell* 24, 85–99.
- Yu TX, Wang PY, Rao JN, Zou T, Liu L, Xiao L, Gorospe M, Wang JY (2011). Chk2-dependent HuR phosphorylation regulates occludin mRNA translation and epithelial barrier function. *Nucleic Acids Res* 39, 8472–8487.
- Zhang X, Zou T, Rao JN, Liu L, Xiao L, Wang PY, Cui YH, Gorospe M, Wang JY (2009). Stabilization of XIAP mRNA through the RNA binding protein HuR regulated by cellular polyamines. *Nucleic Acids Res* 37, 7623–7637.
- Zhuang R, Rao JN, Zou T, Liu L, Xiao L, Cao S, Hansraj NZ, Gorospe M, Wang JY (2013). miR-195 competes with HuR to modulate stim1 mRNA stability and regulate cell migration. *Nucleic Acids Res* 41, 7905–7919.
- Zou T, Mazan-Mamczarz K, Rao JN, Liu L, Marasa BS, Zhang AH, Xiao L, Pullmann R, Gorospe M, Wang JY (2006). Polyamine depletion increases cytoplasmic levels of RNA-binding protein HuR leading to stabilization of nucleophosmin and p53 mRNAs. *J Biol Chem* 281, 19387–19394.
- Zou T, Rao JN, Liu L, Xiao L, Yu TX, Jiang P, Gorospe M, Wang JY (2010). Polyamines regulate the stability of JunD mRNA by modulating the competitive binding of its 3' untranslated region to HuR and AUF1. *Mol Cell Biol* 30, 5021–5032.