Expanded View Figures

Figure EV1. No apparent phenotype was observed in *Islr* cKO mice at homeostasis.

A X-gal staining showing the *Twist2*$^{+/Cre}$ activity in stromal cells using *Twist2*$^{+/Cre}$;*R26R*^{LacZ} mice. Scale bar: 50 μm.
B Schematics of Islr genomic locus and strategy for generating loxP-targeted alleles.
C qRT–PCR for *Islr* in intestinal and colonic tissues from control and cKO mice; $n = 4$.
D Western blotting for Islr in colonic tissues from control and cKO mice. α-Tubulin was used as a loading control.
E *In situ* hybridization for *Islr* with RNAscope probe in colons from control and cKO mice, showing that *Islr* is deleted in stromal cells, while not in the epithelial cells. Dashed lines marked the border of epithelium; $n = 3$. Scale bar: 25 μm.
F H&E staining of colons from control and cKO mice, which is identical to panels with non-DSS in Fig 3C. Scale bar: 100 μm.
G Double immunofluorescence for Ki67 and β-catenin, immunohistochemistry for cleaved caspase3 (Casp3), and PAS staining in colons from control and cKO mice; $n = 3$. Scale bar: 50 μm.
H Quantification for crypt number, Ki67$^+$ cells, and Casp3$^+$ and PAS$^+$ cells in (G); $n = 4$.
I Immunohistochemistry for CD45 and immunofluorescence for F4/80 in colons from control and cKO mice after 5-day DSS treatment; $n = 3$. Scale bar: 100 μm.

Data information: In (C and H), data are presented as mean ± SD. $^{**}P < 0.01$ (Student's *t*-test).
Source data are available online for this figure.

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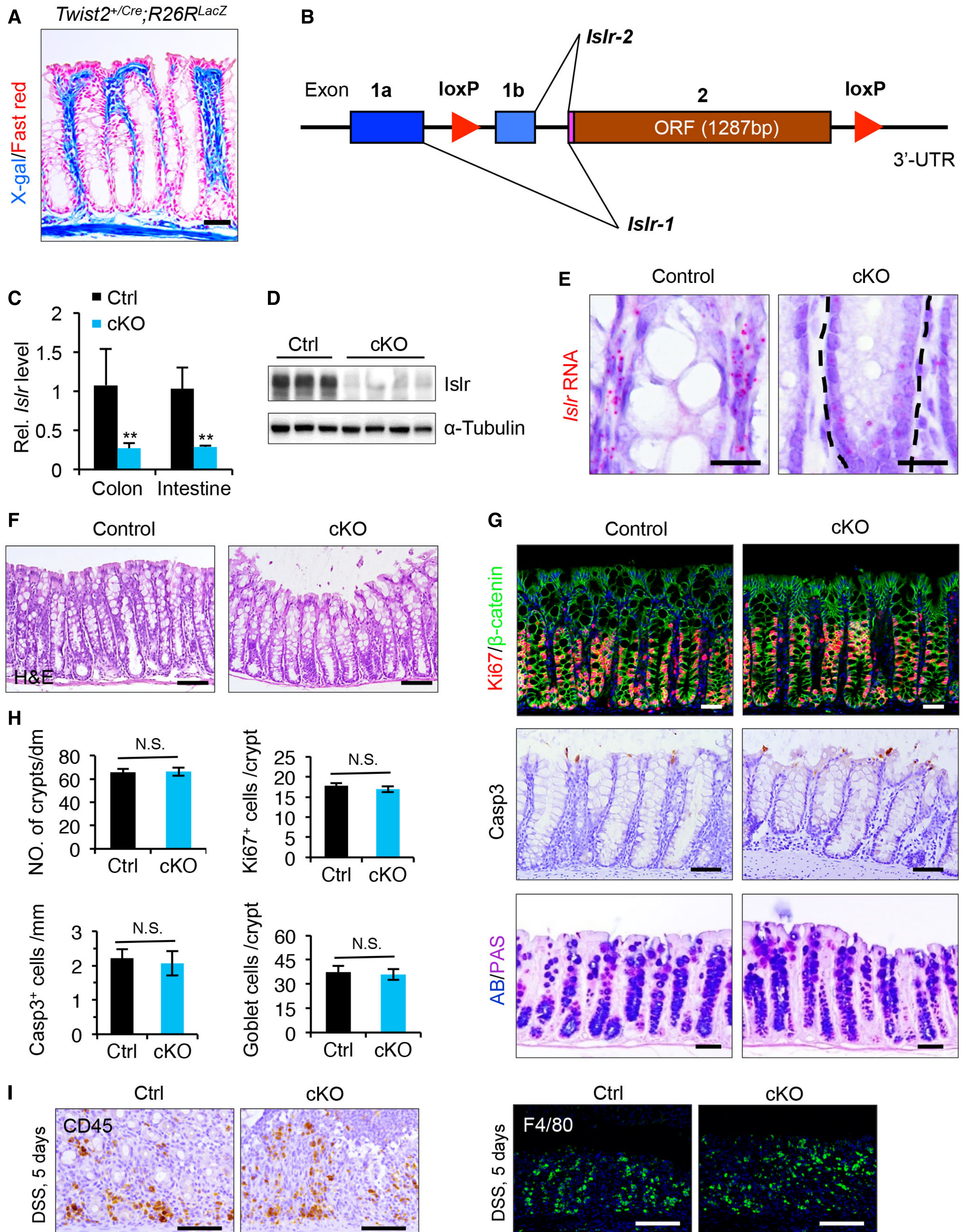

Figure EV1.

Figure EV2. Deletion of *Islr* in stromal cells impaired intestinal epithelial regeneration in TNBS-induced colitis.

A Quantification of body weight change in control (n = 8) and cKO (n = 8) mice after TNBS treatment.
B Gross images of colons from control (n = 5) and cKO (n = 5) mice 4 days after TNBS treatment, and quantification of colon length from control and cKO mice.
C Histological images of colonic inflamed mucosa from control and cKO mice at indicated timepoints after TNBS treatment; n = 4. Scale bar: 100 μm.
D Quantification of the clinical score of inflamed mucosa in control and cKO mice; n = 5.
E Double immunofluorescence for Ki67 and β-catenin in colonic inflamed mucosa from control and cKO mice at indicated timepoints after TNBS treatment; n = 4. Scale bar: 100 μm.
F Quantification of the percentage of $Ki67^+$ cells versus epithelial cells at 3 days after TNBS treatment; n = 4.

Data information: In (A, B, D, and F), data are presented as mean ± SD. **$P < 0.01$; ***$P < 0.001$ (Student's t-test).

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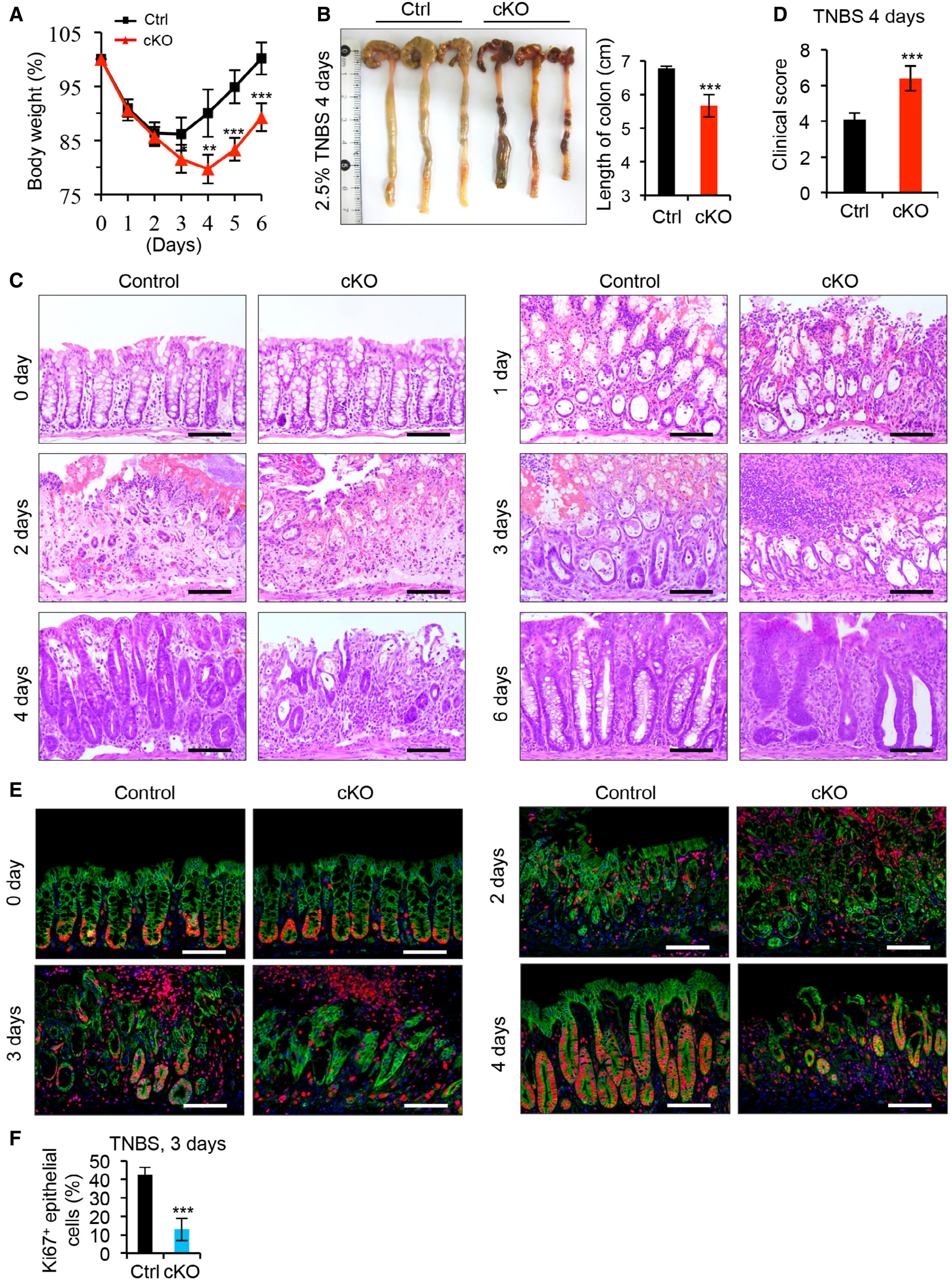

Figure EV2.

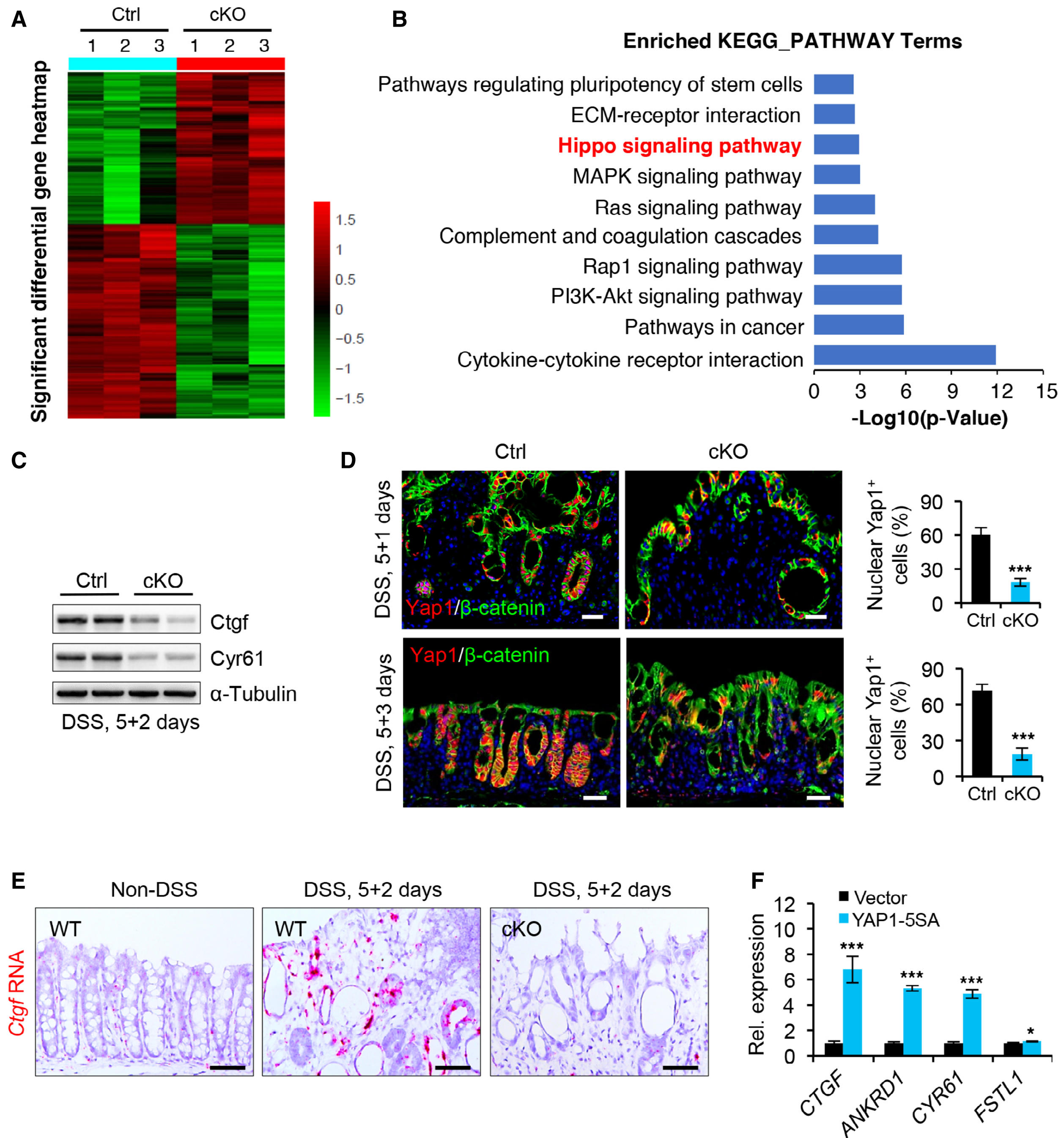

Figure EV3. Deletion of *Islr* in stromal cells suppressed epithelial Hippo signaling.

A Heatmaps of differentially expressed genes (DEGs) in colons from control and cKO mice 1 day after DSS removal. Note: The cutoff is $P < 0.05$ and fold > 2. The parameter of the color key indicated the fold changes converted to log2 of signal value normalized.

B KEGG pathway analysis of DEGs in cKO mice.

C Western blotting for Ctgf and Cyr61 in colon tissues from control and cKO mice 2 days after 5-day DSS treatment. α-Tubulin was used as a loading control.

D Double immunofluorescence for Yap1 and β-catenin in the colons from control and cKO mice 1 or 3 days after 5-day DSS treatment. Control, $n \geq 3$ at each timepoint; cKO, $n \geq 3$ at each timepoint. Scale bar: 50 μm.

E RNAscope *in situ* hybridization assay for Ctgf in colonic epithelium from WT mice or control and cKO mice 2 days after 5-day DSS treatment. Scale bar: 25 μm.

F qRT–PCR analysis for *CTGF*, *ANKRD1*, *CYR61*, and *FSTL1* in NCM460 colon epithelial cells transfected with vector or YAP1-5SA. $^{*}P < 0.05$; $^{***}P < 0.001$.

Data information: In (D and F), data are presented as mean ± SD. $^{*}P < 0.05$; $^{***}P < 0.001$ (Student's *t*-test).

Source data are available online for this figure.

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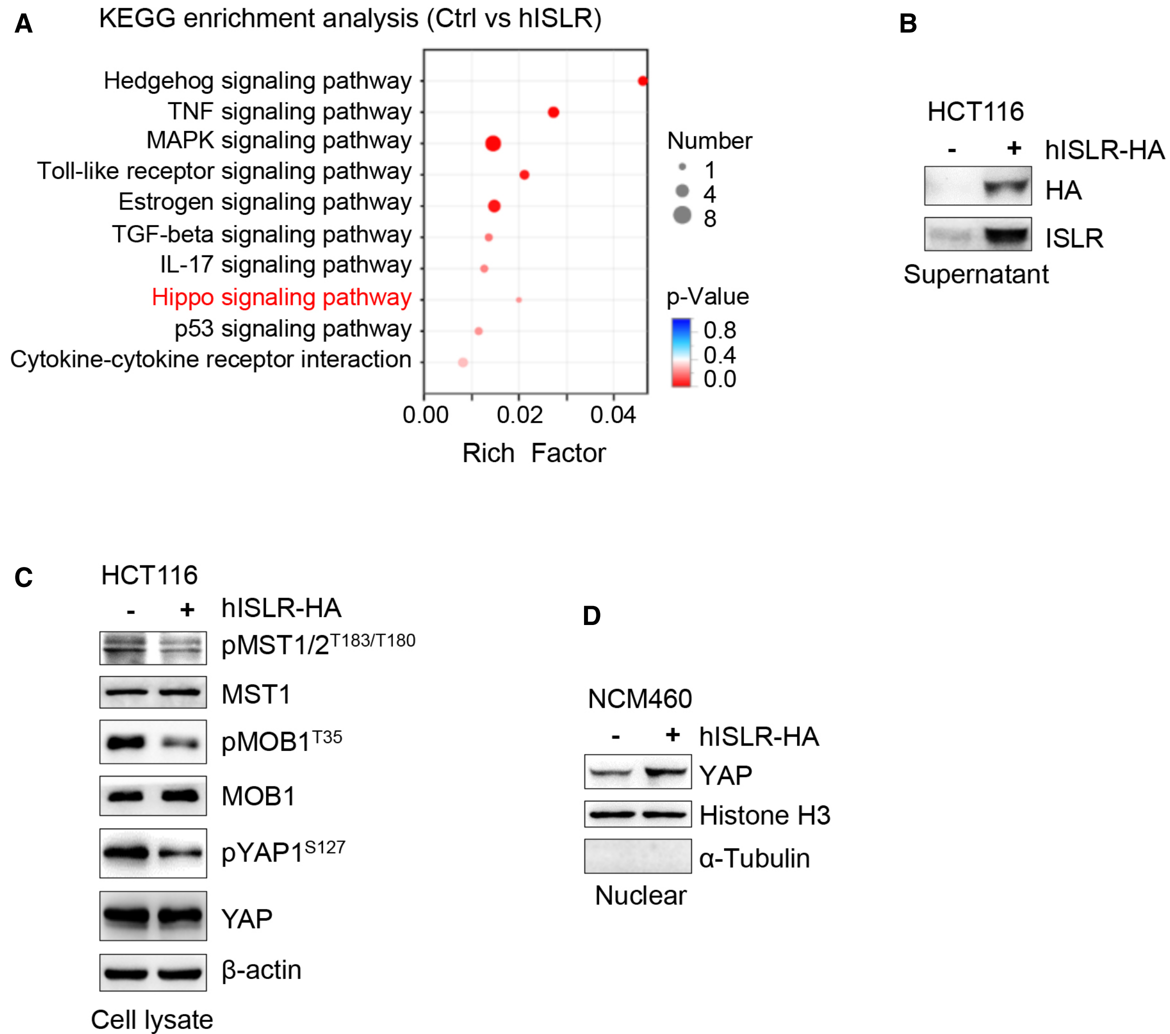

Figure EV4. Stromal cell-secreted Islr suppressed epithelial Hippo signaling and activated YAP.

A KEGG enrichment analysis in DEGs of hISLR overexpressing HEK293FT cells.

B Western blotting for HA and ISLR in the supernatant from HCT116 CRC cells transfected with pcDNA3.1 or pcDNA3.1-hISLR-HA plasmids.

C Western blotting for pMST1/2, MST1, pMOB1 and MOB1, pYAP, and YAP in lysates from HCT116 CRC cells cultured in the supernatants with or without hISLR-HA. β-actin was used as a loading control.

D Western blotting for YAP in nuclear proteins isolated from NCM460 colon epithelial cells cultured in the supernatant with or without hISLR-HA. Histone H3, a positive control for nuclear proteins. α-Tubulin, a positive control for cytoplasmic proteins.

Source data are available online for this figure.

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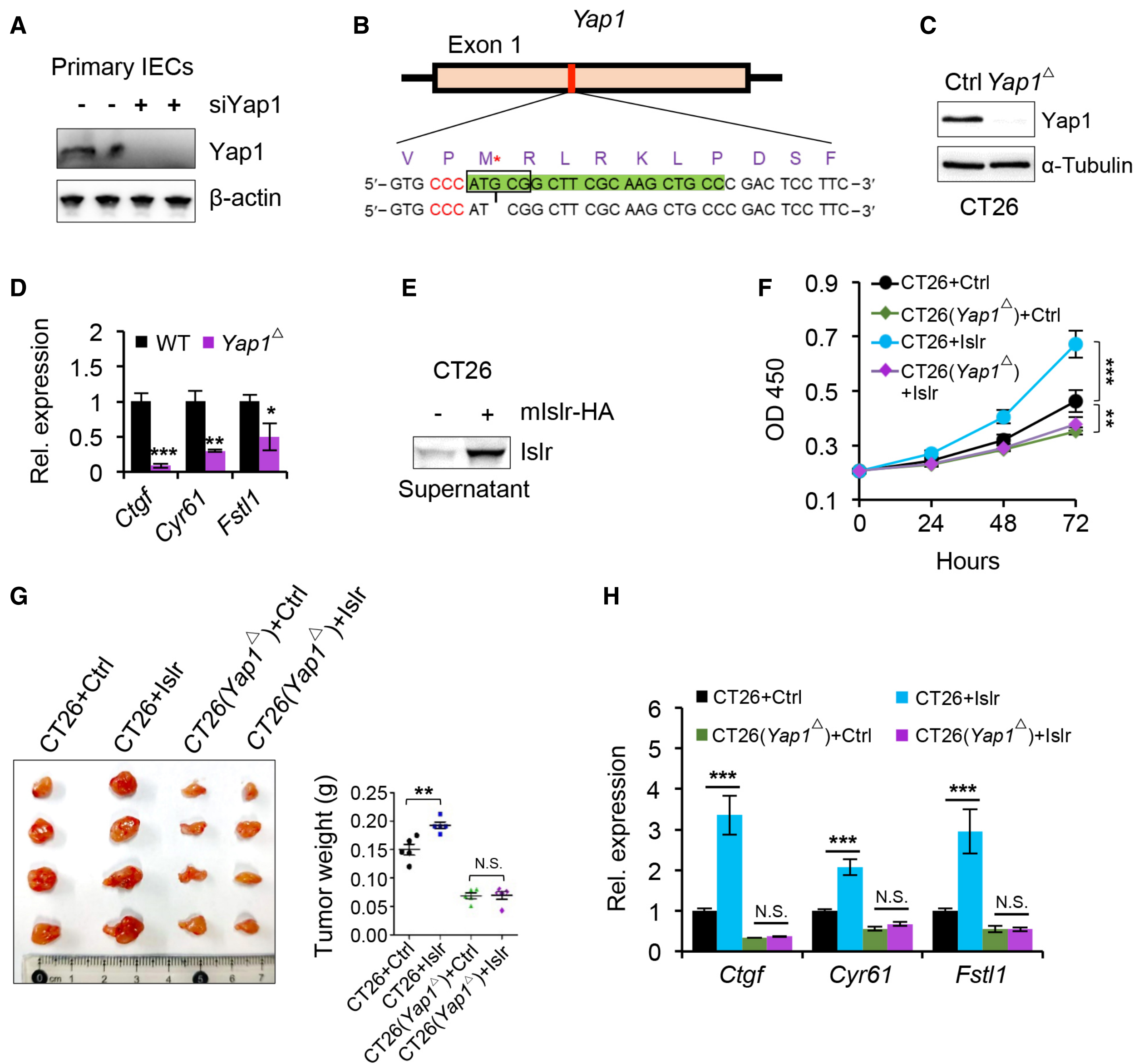

Figure EV5. Deletion of Yap1 abrogated the mIslr-mediated promoting effect of tumor growth.

A Western blotting for Yap1 in primary intestinal epithelial cells 24 h after Yap1 siRNA treatment. β-actin was used as a loading control. The sequences of Yap1 siRNA are in Appendix Table S5.

B Generating Yap1-deficient CT26 CRC cells using Crispr/Cas9 technique. gRNA marked by green color. Protospacer adjacent motif (PAM) sequence marked by red color. G deletion is indicated by an asterisk.

C Western blotting for Yap1 in normal and *Yap1*-deficient (*Yap1*$^{\Delta}$) CT26 CRC cells. α-Tubulin was used as a loading control.

D qRT–PCR for *Ctgf*, *Cry61*, and *Fstl1* in normal and *Yap1*-deficient (*Yap1*$^{\Delta}$) CT26 CRC cells; $n = 3$.

E Western blotting for mIslr in the supernatant from CT26 CRC cells transfected with pcDNA3.1 or pcDNA3.1-mIslr plasmids.

F The growth curve of CT26 CRC cells under indicated conditions; $n = 5$.

G Gross images of xenografted tumors 10 days after transplantation. Quantification of tumor weight; $n = 5$.

H qRT–PCR for *Ctgf*, *Cry61*, and *Fstl1* in CT26 CRC cells at indicated conditions; $n = 3$.

Data information: In (D, F–H), data are presented as mean ± SD. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$ (Student's *t*-test).
Source data are available online for this figure.

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