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Supplemental Information

RHOA GTPase Controls YAP-Mediated EREG Signaling in Small Intestinal Stem Cell Maintenance

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Supplemental Figure Legends

Supplemental Figure 1. (Related to Figure 1) Expression of RhoA protein in small intestine and ISCs and genotyping of rhoA in villin-CreERT2+; RhoA flox/flox and control WT mice. (A-B) Representative immunofluorescent imaging of anti-RhoA, DAPI and Lgr5-eGFP in WTmouse small intestine at a magnification of 20X (A) or 63x (B). (C) Purified genomic DNAs isolated from small intestines of villin-CreERT2+; RhoA flox/flox mice and controls were subject to PCR genotyping as described in Methods to identify RhoA flox/flox and CreERT2+ alleles. (D) Genomic DNA samples of small intestine duodelum, jejunum and ileum were processed for PCR genotyping as described in Methods to identify RhoA deletion.

Supplemental Figure 2. (Related to Figure 1) Morphologic changes of RhoA knockout small intestines. (A) TEM analysis of the epithelium in control WT and RhoA KO small intestine sections. The EM image shows a disorganization of the epithelium structure and nuclear organization in RhoA KO small intestine epithelium. (B,C) The histology of different regions of small intestine in control WT and RhoA KO mice. Representative H&E staining shows the disrupted jejunum and ileum, particularly the crypts, in RhoA KO mice.

Supplemental Figure 3. (Related to Figure 2) Analyses of Goblet cells, enteroendocrine cells, Paneth cells multinucleated cells and proliferating cells in RhoA KO duodenum. Representative immunofluorescence images of Mucin-2 (A), chromogranin A (B), laminin B/E-Cad (C), cleaved caspase-3 (D), phospho-histone H3 (E) and lysosome (F, G) distributions in Control WT and RhoA KO small intestines.

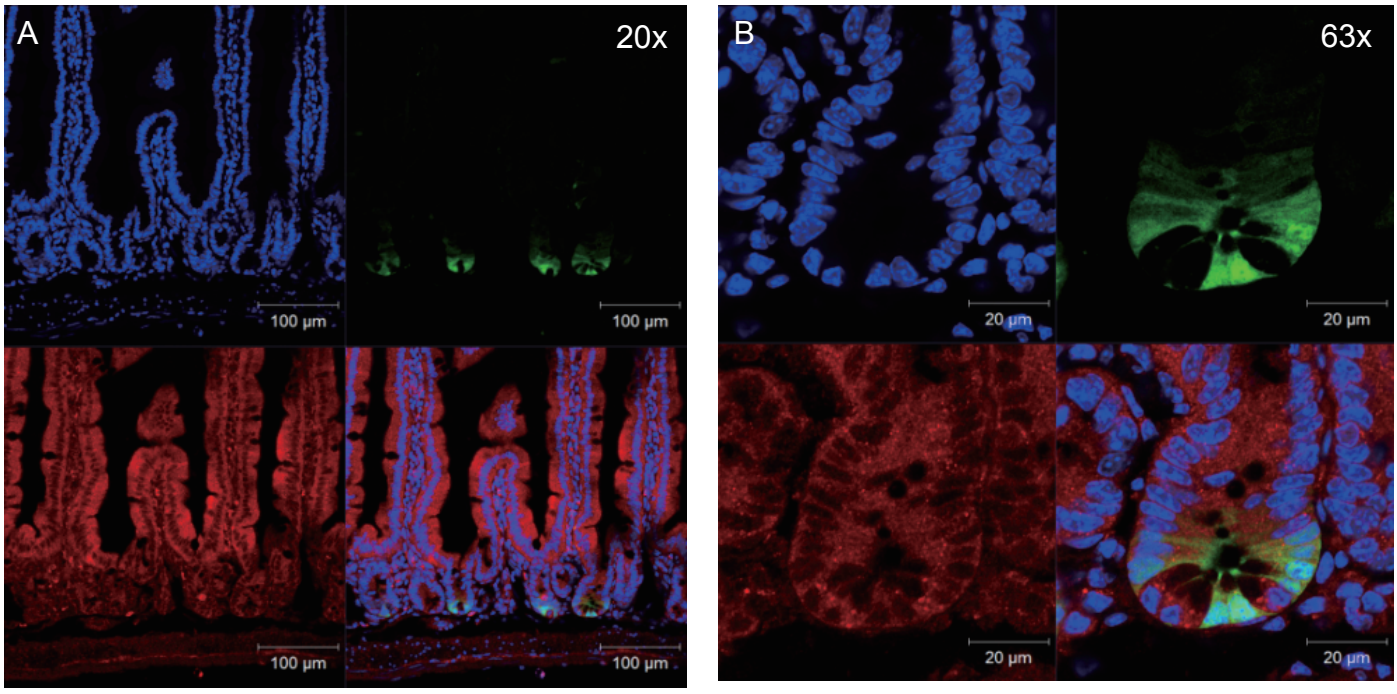
Supplemental Figure 4. (Related to Figure 3) Analysis of ISCs in vivo and in vitro. (A) Direct fluorescence detection of Lgr5-EGFP signal in Control WT and RhoA KO duodenum sections. (B) Induced RhoA deletion in vitro causes a defective E-cadherin distribution in enteroid culture. Enteroids at day five post-induction by the addition of 4-OHT. Enteroids were stained with anti-RhoA (red) and anti-E-cadherin (green). (C) Direct fluorescence imaging of the Lgr5-EGFP signal (arrow head) from cultured enteroids

derived from Lgr5-EGFP-IRES-Cre-ERT2;Villin-CreERT2;rhoAfl/+ (WT) or Lgr5-EGFP-IRES-Cre-ERT2;Villin-CreERT2;rhoAfl/fl mice (KO), and the ROCK inhibitor Y27632 (10 μM) treated control.

Supplemental Figure 5. (Related to Figures 4 & 5) Rescue of RhoA KO phenotypes by an active YAP transgenic mutant. (A) The representative H&E staining images of Control WT, RhoA KO, KO/YAP (S112A) mutant and YAP (S112A) mutant small intestine duodenum. n=3 mice for each genotype. (B) Representative immunofluorescence staining of total YAP showing constitutively active YAP increase YAP protein level. (C) Representative immunofluorescence staining of beta-catenin showing constitutively active YAP does not impact membrane beta-catenin expression.

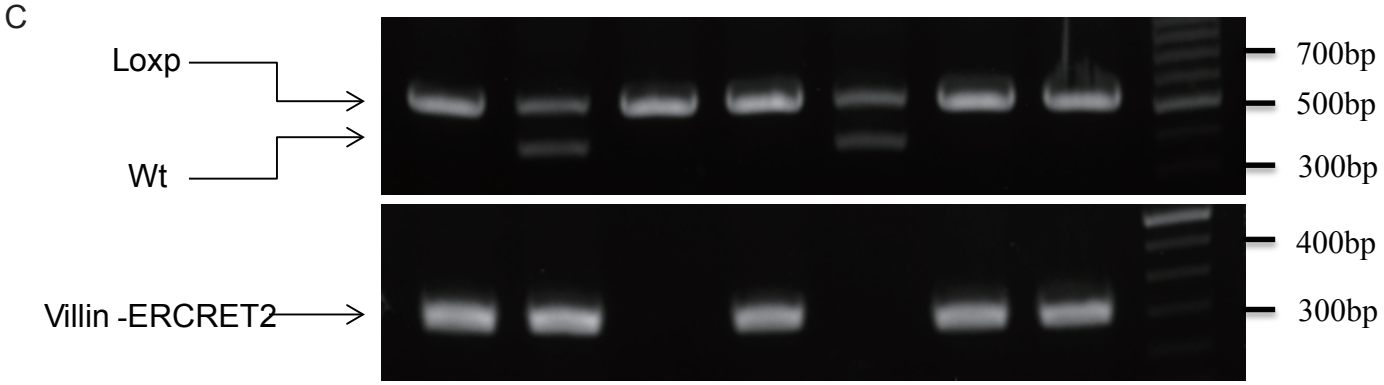
Supplemental Figure 6. (Related to Figures 6 & 7) Rescue of RhoA KO phenotypes by an active beta-catenin mutant. (A) H&E staining of Control WT, RhoA KO, KO/beta-catenin Catnblox(ex3) rescue and beta-catenin Catnblox(ex3) expressing duedenum. (B) Representative immunofluorescence staining of YAP showing beta-catenin Catnblox(ex3) does not impact YAP expression.

Supplemental Figure 7. (Related to Discussion) RhoA KO defects in small intestine in part mimic that of X-ray irradiation injuries. (A) H&E staining of Control WT and irradiated Control WT (IR) small intestine sections. (B-D) Representative immunofluorescence images of anti-E-cadherin, Ki-67, cleaved caspase 3 and lysozyme in Control, IR small intestine duodenum sections. (E) qRT-PCR analysis of the expression of ISC markers in WT and WT+ IR small intestinal crypts. Open bar, WT; black bar, WT + IR. Results are normalized to GAPDH expression and expressed as fold changes. n=3 mice for each genotype.



DAPI/Lgr5-eGFP/RhoA

C



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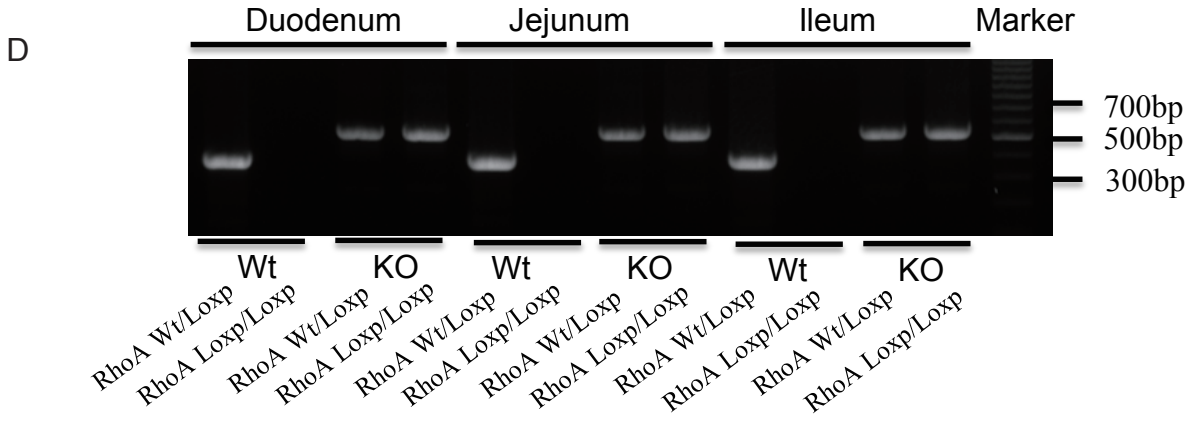
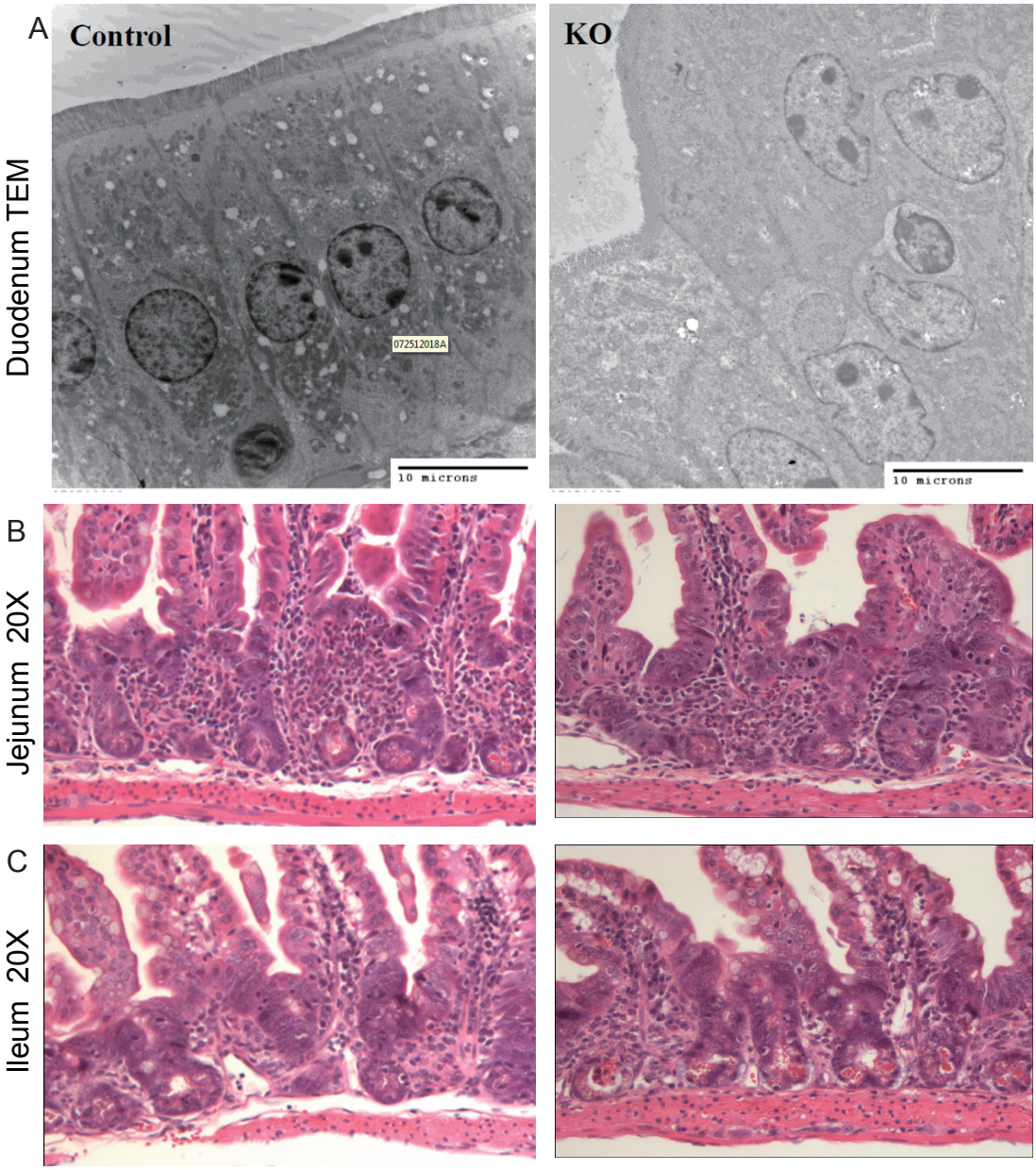
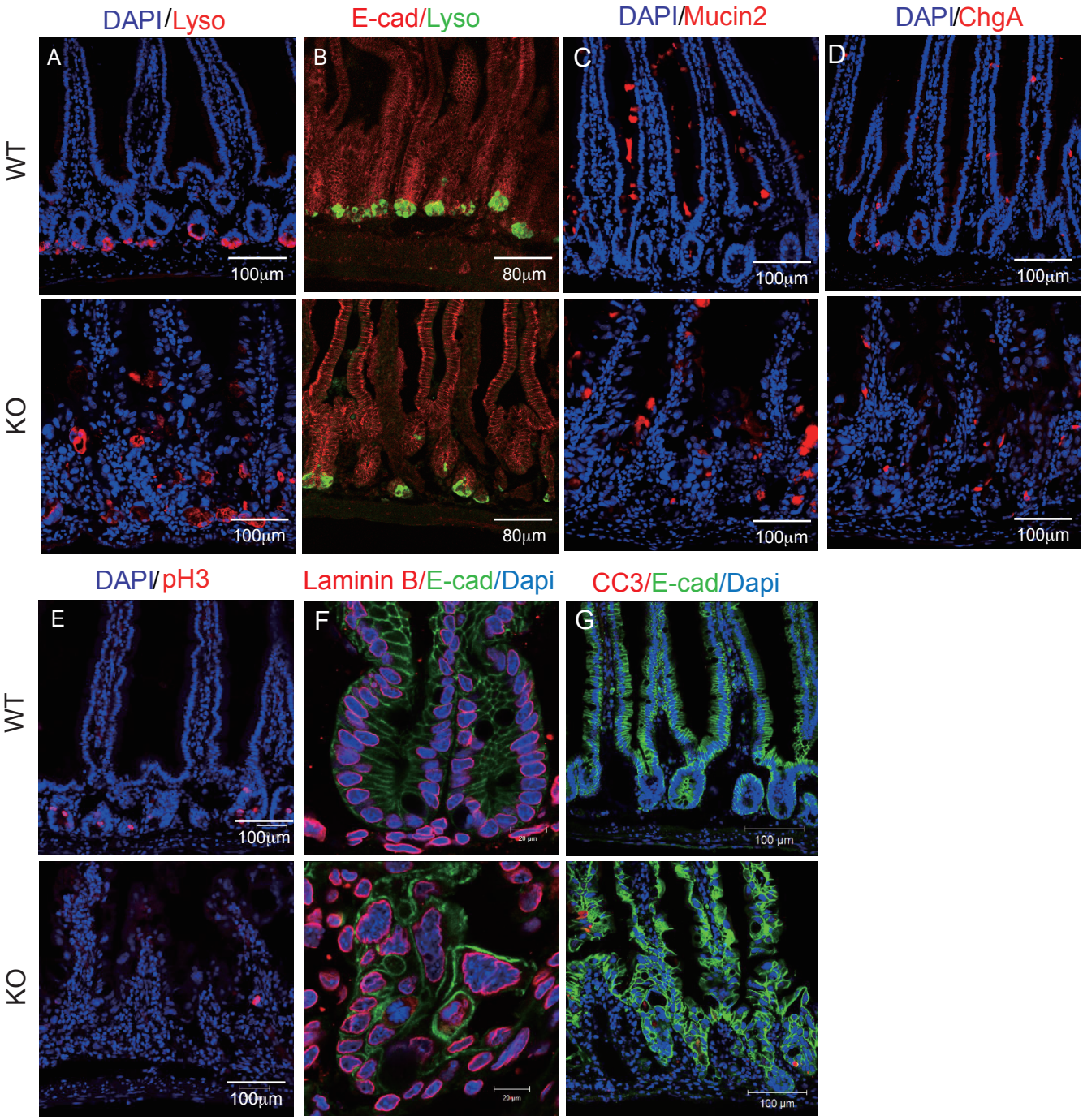
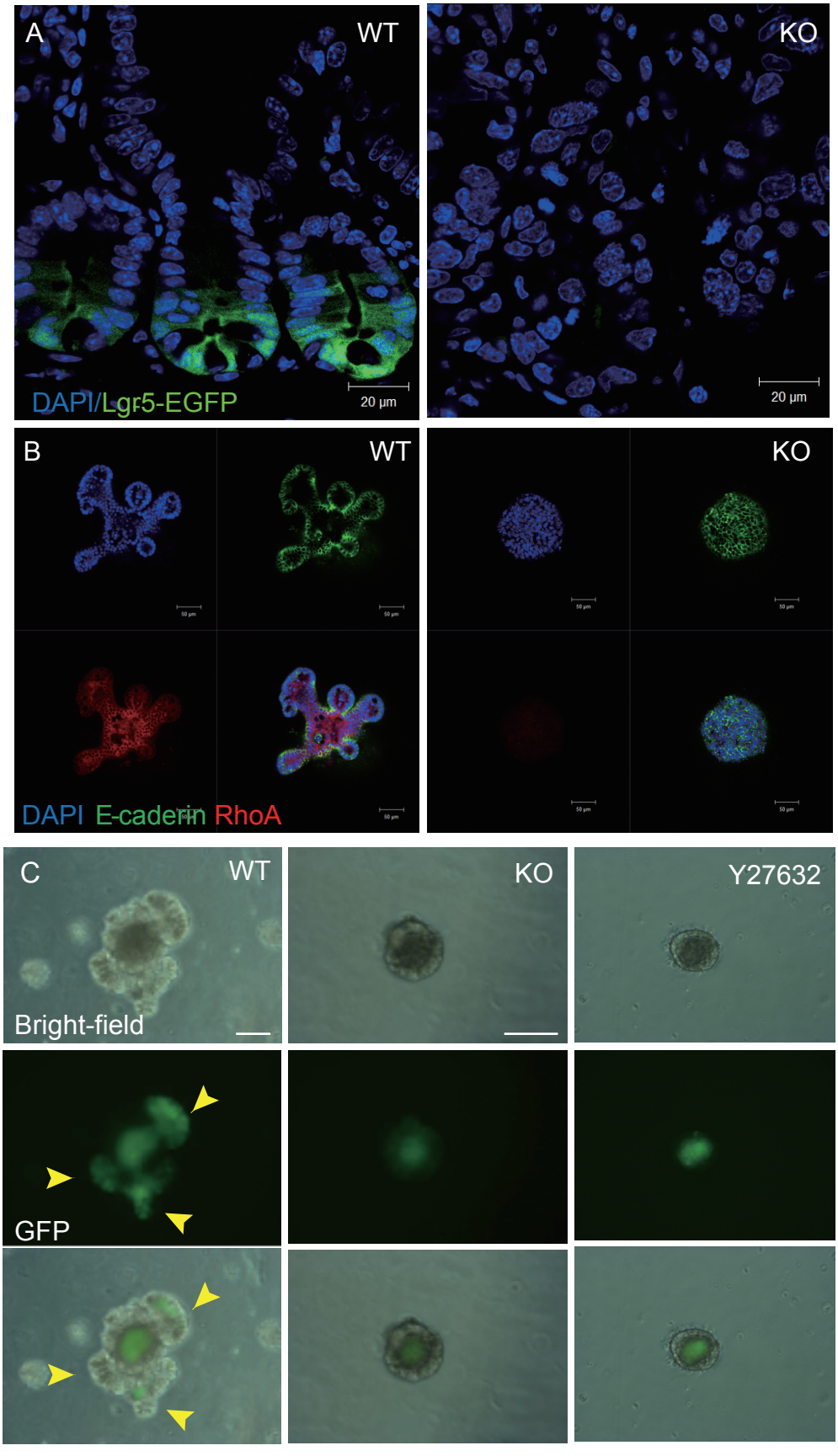
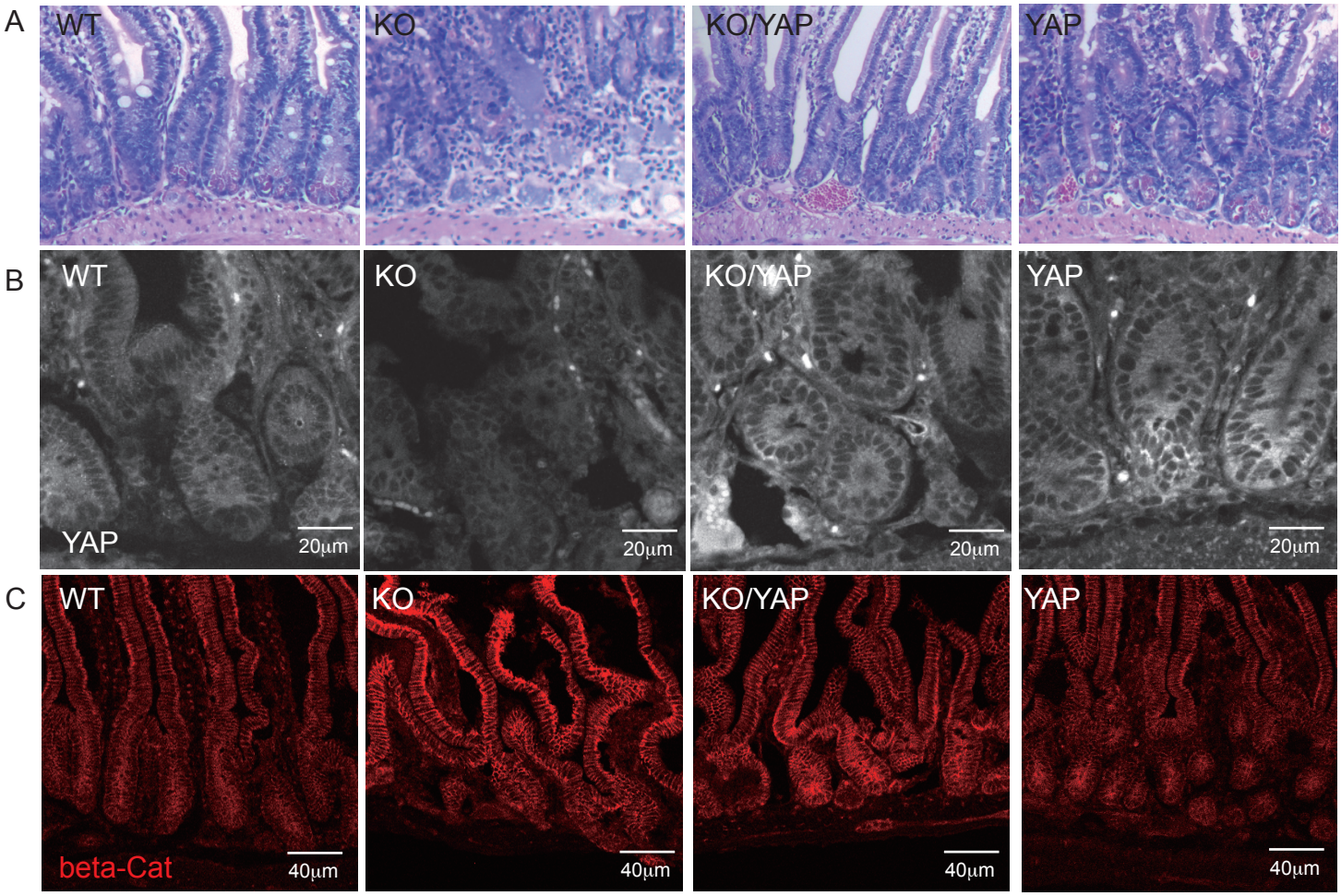
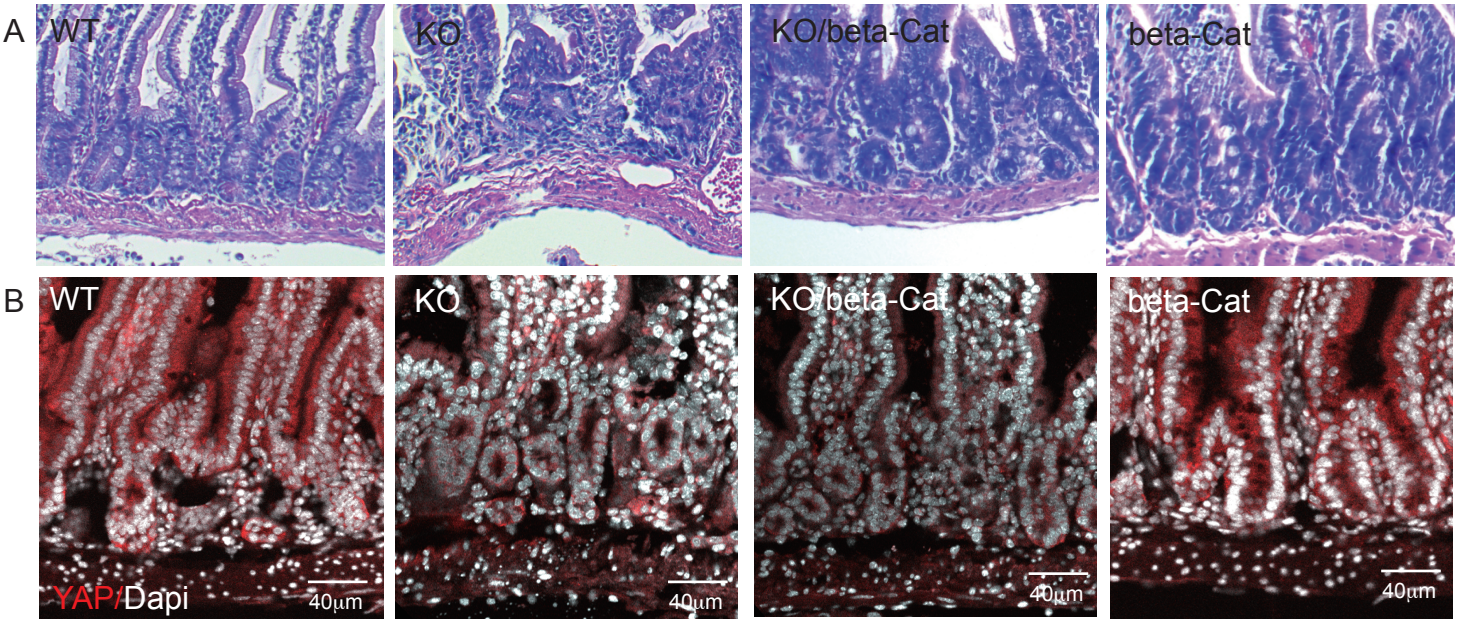


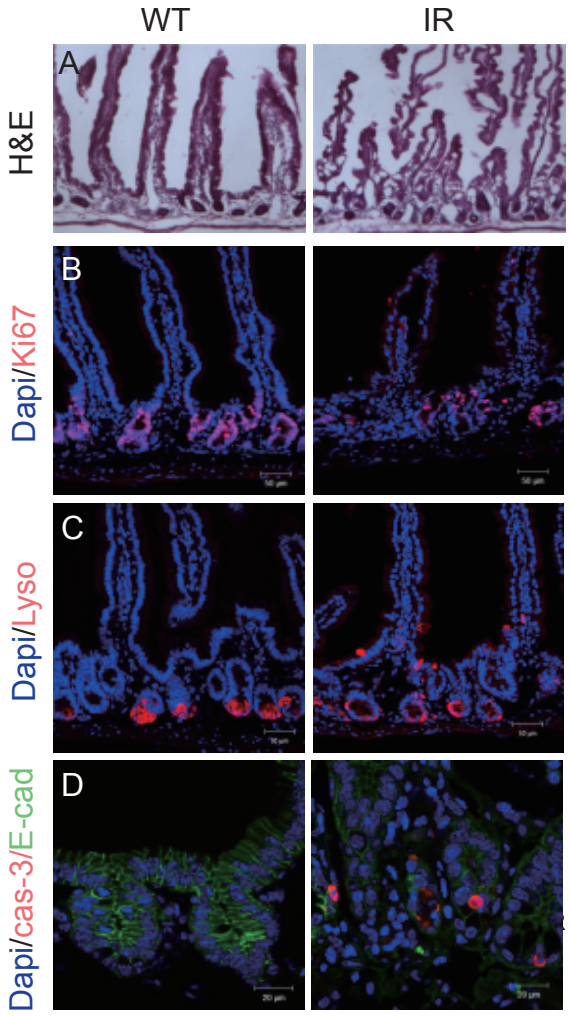

A
Control
KO
Duodenum TEM
072512018A
10 microns
10 microns
B
Jejunum 20X
C
Ileum 20X

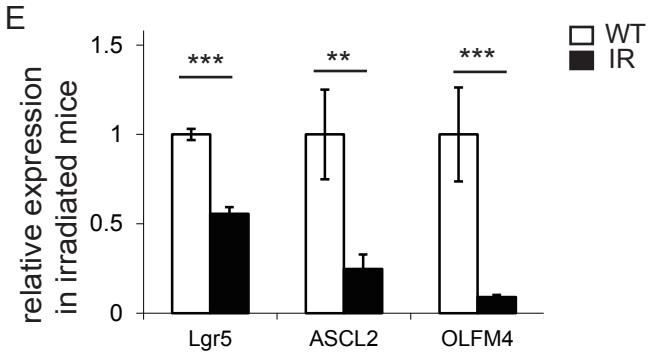

DAPI/Lyso
E-cad/Lyso
DAPI/Mucin2
DAPI/ChgA
A
B
C
D
WT
KO
100μm
80μm
100μm
100μm
100μm
80μm
100μm
100μm
DAPI/pH3
Laminin B/E-cad/Dapi
CC3/E-cad/Dapi
E
F
G
WT
KO
100μm
100μm
100 μm
100 μm


A
WT
KO
DAPI/Lgr5-EGFP
20 μm
20 μm
B
WT
KO
DAPI E-caderin RhoA
C
WT
KO
Y27632
Bright-field
GFP


A
WT
KO
KO/YAP
YAP
B
WT
KO
KO/YAP
YAP
YAP
20μm
20μm
20μm
20μm
C
WT
KO
KO/YAP
YAP
beta-Cat
40μm
40μm
40μm
40μm


A
WT
KO
KO/beta-Cat
beta-Cat
B
WT
KO
KO/beta-Cat
beta-Cat
YAP/Dapi
40μm
40μm
40μm
40μm


WT
IR
A
H&E
B
Dapi/Ki67
C
Dapi/Lyso
D
Dapi/cas-3/E-cad


E
relative expression
in irradiated mice
1.5
1
0.5
0
***
**
***
WT
IR
Lgr5
ASCL2
OLFM4