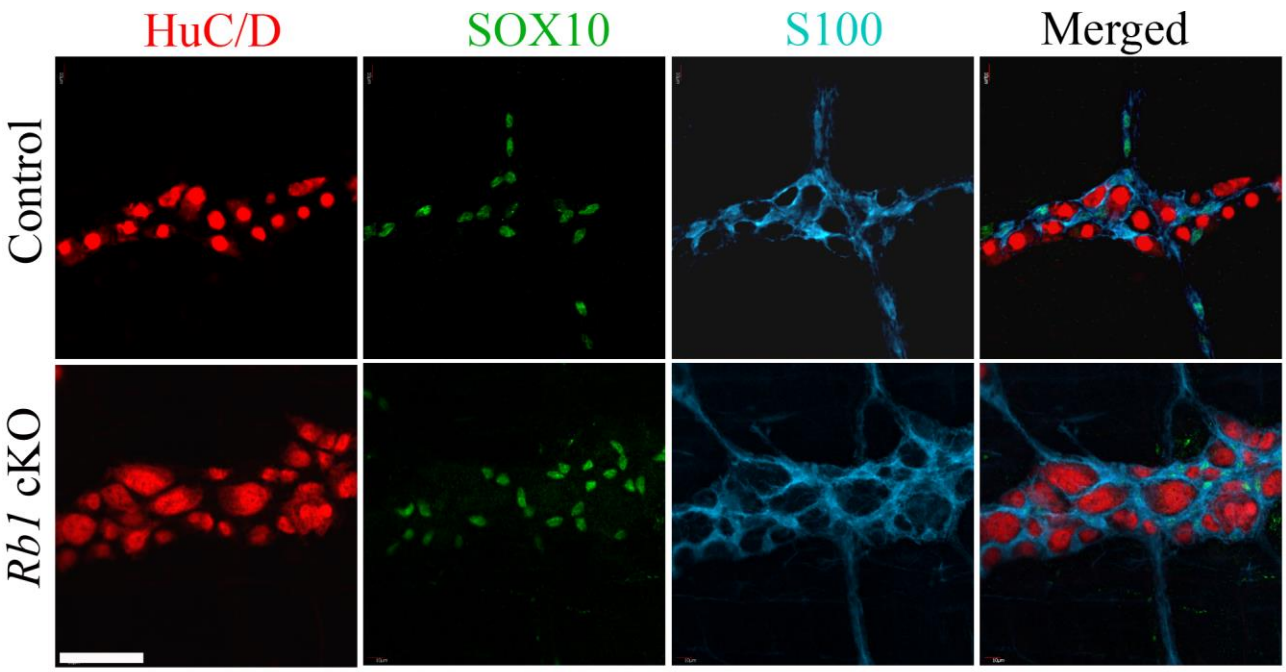
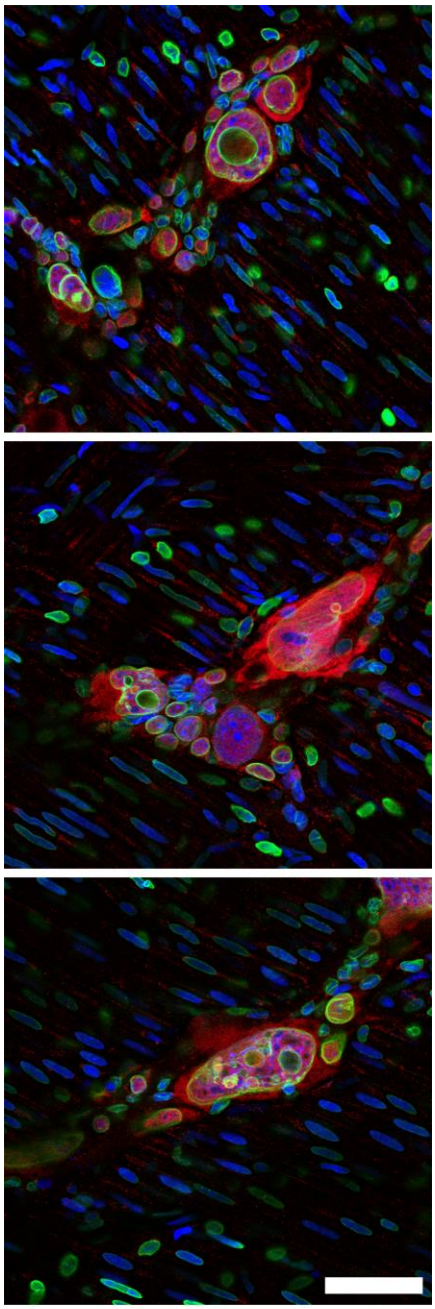
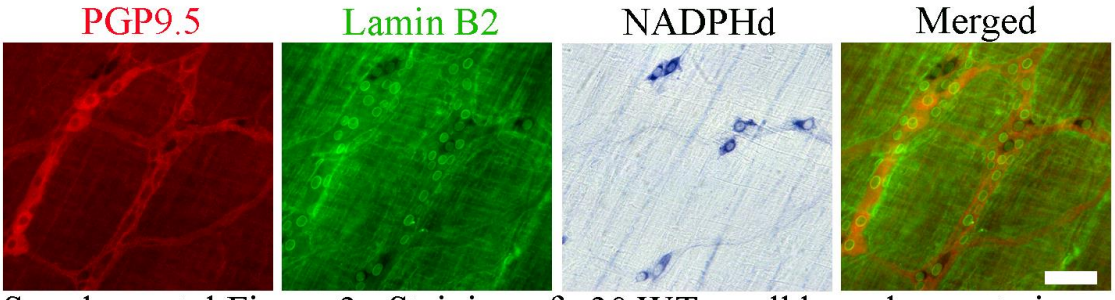


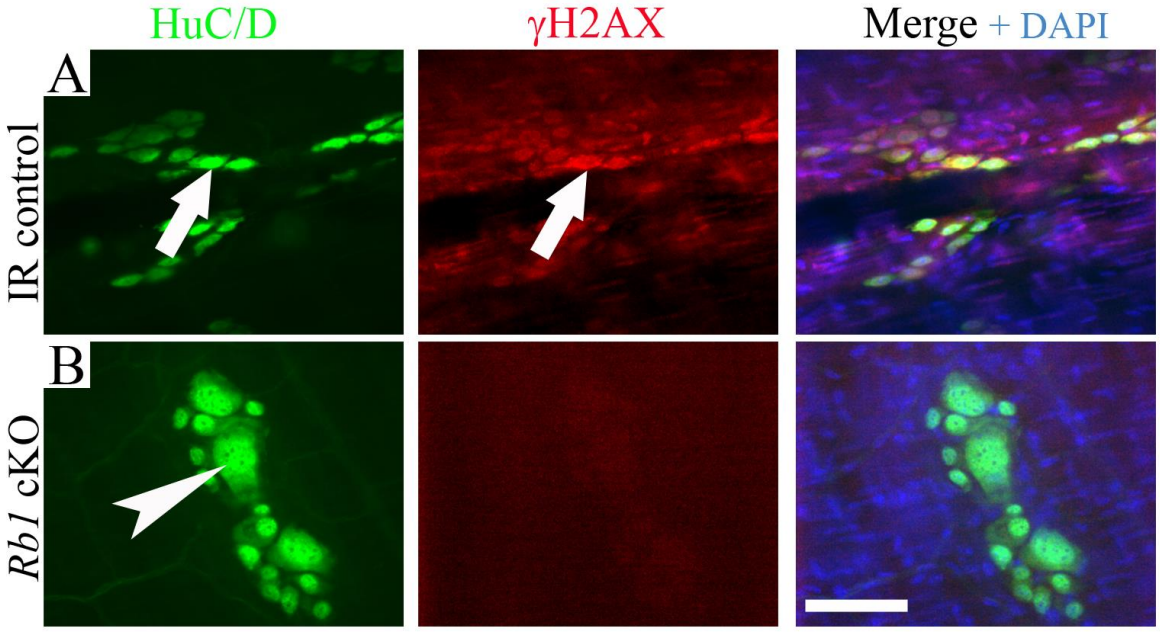
Supplemental Figure 1. Whole mount myenteric plexus from p30 WT and *Rb1* cKO mice was stained with antibodies to HuC/D (neurons), SOX10 (glia), and S100 (glia). Images show that Sox10 and S100 are expressed in the same cells, and that Sox10 is not expressed in neurons. Scale bar = 50 μm.



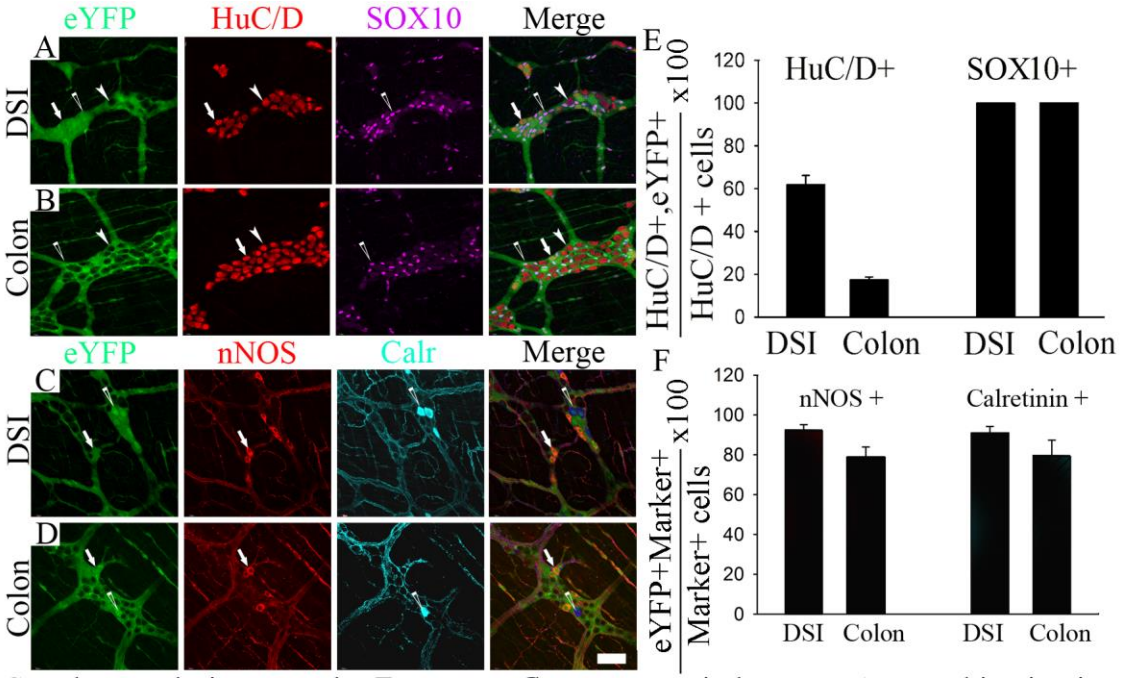
Supplemental Figure 2. Small bowel myenteric plexus from *Rb1* cKO mouse stained with antibodies to LMNA/C (green), HuC/D (red) and with DRAQ5 dye (blue) as in Figure 3 show some of the unusual nuclear morphologies observed in the mutant mouse. Supplemental videos 1-6 show confocal reconstructions of unusual myenteric neurons. Scale bar = 50 μm.



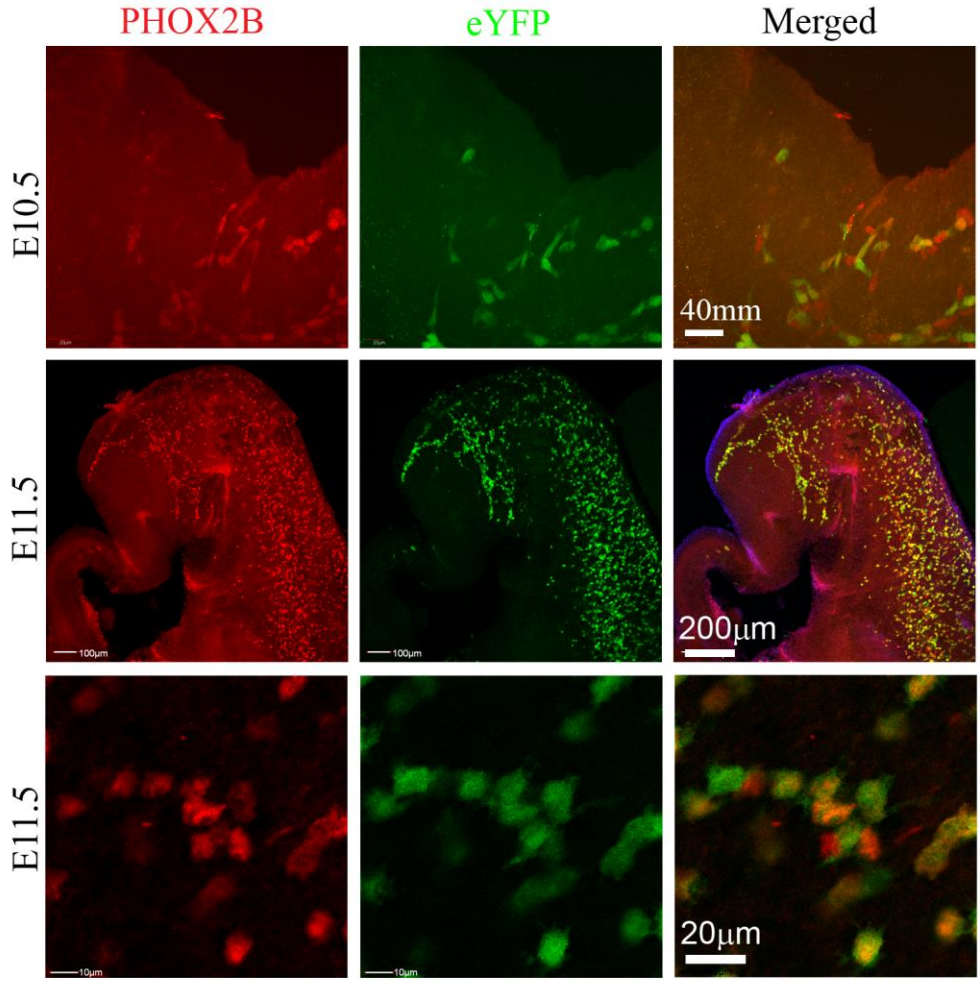
Supplemental Figure 3. Staining of p30 WT small bowel myenteric plexus using a combination of PGP9.5 (red) and Lamin B2 (green) immunohistochemistry, and NADPH diaphorase histochemistry (blue), shows that Lamin B2 is preferentially expressed in all myenteric neurons at this age in this tissue. Scale bar = 50 μm.

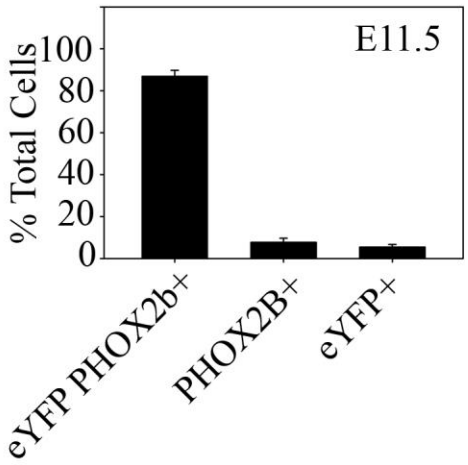


Supplemental Figure 4: *Rb1* cKO enteric neurons did not show DNA damage. We stained mouse distal small bowel muscle layers with an antibody to HuC/D (green) to highlight neurons and with an antibody to γH2AX (red), a protein rapidly recruited to DNA double strand breaks. DAPI was used to stain nuclei. (A) As a positive control we used a mouse irradiated with 1000 rads (IR control). A white arrow indicates a γH2AX positive myenteric neuron. (B) *Rb1* cKO mice without irradiation do not have γH2AX staining in the myenteric plexus. Arrowhead indicates a large HuC/D+ cell. Scale bar = 50 μm.

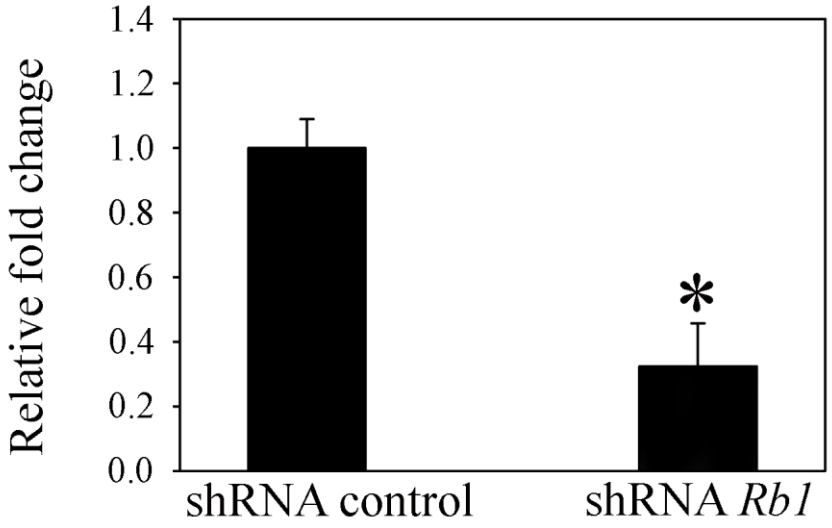


Supplemental Figure 5. The *Tyrosinase Cre* transgene induces DNA recombination in all myenteric glia and a subset of myenteric neurons. (A-D) The myenteric plexus in *TyrCre* transgenic mice bred to a *Cre* dependent *Rosa26 eYFP* reporter line was stained with antibodies to HuC/D, SOX10, nNOS or calretinin (Calr). (A,B) HuC/D+; eYFP+ cells (white arrows), HuC/D+; eYFP negative cell (solid arrowhead), SOX10+; eYFP+ glia (open arrowhead in A, B). (C, D) Calr+; eYFP+ (open arrowhead), nNOS+; eYFP+ (white arrows) are highlighted. (E, F) Quantitative analysis (> 900 cells/bar). (A, B) Scale bar = 50 μm.





Supplemental Figure 6. Antibody staining for PHOX2B was combined with eYFP imaging in *TyrCre;R26REYFP* mice. Images show that eYFP is expressed in many but not all PHOX2B+ cells at E10.5 and in almost all PHOX2B+ cells at E11.5.



Supplemental Figure 7. E12.5 dissociated bowel cells in culture were infected with lentivirus expressing shRNA to *Rb1* or a control shRNA. Quantitative RT-PCR relative to GAPDH showed that the *Rb1* shRNA significantly reduced *Rb1* mRNA levels. * $P = 0.007$.

SUPPLEMENTAL VIDEOS:

We obtained confocal images and generated three dimensional videos using an Olympus FV1000 confocal microscope and a 60x oil immersion objective.

Supplemental Video 1: Confocal image and three dimensional reconstruction of distal small bowel myenteric plexus of a WT mouse stained with antibodies to HuC/D (red), lamin A/C (green) and with the DRAQ5 DNA dye (blue).

Supplemental Videos 2-6: Confocal images and three dimensional reconstructions of distal small bowel myenteric plexus from *Rb1* cKO mice stained with antibodies to HuC/D (red), lamin A/C (green) and with the DRAQ5 DNA dye (blue) show complex shapes to nuclear lamina. Supplemental video 6 shows a magnified region of video 5.

Supplemental Videos 7-12: In vitro organ bath was used to evaluate motility patterns from the small bowel and colon of mice. Videos show a WT (Video 7 and 9) and a severely affected *Rb1* cKO mouse (Video 8 and 10). In each video, the colon is on the bottom and has a green artificial stool pellet inserted before starting video recording. Distal colon is on the left and distal small bowel is on the right. WT small bowel (top) actively contracted, but *Rb1* cKO small bowel (top) was more dilated and contracted less vigorously. Video 9 and 10 (*Rb1* cKO) show the same bowel segments after addition of L-NAME at 12 seconds (WT) or 10 seconds (*Rb1* cKO) after starting the video recording. L-NAME led to a substantial reduction in small bowel wall diameter in *Rb1* cKO mouse consistent with elevated NO synthesis, but the effect was less obvious in WT small bowel. Videos 11 (WT) and 12 (*Rb1* cKO) show colon contractions moving an artificial stool pellet with the images projected at 8x the normal speed. Distal colon is on the right.