Supplemental Material

Supplemental movie 1:

BrdU and RS67506 were infused for 1 week followed by 4 weeks of chase during which RS67506 was given orally. BrdU (red) and HuC/D (green) immunoreactivities were demonstrated. A z-stack of focal planes imaged at intervals of 0.5 µm through a myenteric ganglion. The images were subjected to deconvolution and 3-D construction (Improvision; Volocity 4 software). Cells that are doubly labeled with antibodies to BrdU and punctate HuC/D can be seen on the backside of ganglion cells, in a “germinal niche” between the non BrdU-labeled ganglion cells and the longitudinal muscle (unstained and not visible in the preparation). The distance between BrdU-HuC/D-labeled cells and the nearest myenteric neuron in the ganglion was ~10 µm. Scale: one unit = 8.2 µm.

Supplemental movie 2:

BrdU and RS67506 were infused for 1 week followed by 16 weeks of chase. RS67506 was given orally for 10 weeks. BrdU (red) and HuC/D (green) immunoreactivities were demonstrated. A z-stack of focal planes imaged at intervals of 0.5 µm through a myenteric ganglion. The images were again subjected to deconvolution and 3-D construction. A cell that is doubly labeled with antibodies to BrdU and HuC/D can be seen to have moved very close to a ganglion and is almost within it. The distance between BrdU-HuC/D-co-labeled cell and the nearest myenteric neuron in the ganglion was ~4 µm. Scale: one unit = 8.8 µm.

Supplemental figure 1:
The location of HuC/D-immunoreactive cells that incorporated BrdU, determined by analyzing z-stack of deconvoluted images, was extraganglionic after 4 weeks of chase in WT mice treated with RS67506. A-E, Sequential optical sections separated by intervals of 2.5 μm. (A1-E1, BrdU; A2-E2, HuC/D; A3-E3, Merged images). BrdU-labeled nuclei are limited to focal planes A and B, while HuC/D-immunoreactive neurons are located in focal planes C-E. Mature myenteric neurons do not label with BrdU after 4 weeks of chase. The striations in planes A and B are due to the presence of the periganglionic connective tissue sheath. F, A yz-projection of the z-stack of images illustrated in A-E. The thickness of the projected plane is 15 μm. G, A full thickness xy-projection of the z-stack. Scale bar, 6 μm.

Supplemental figure 2:

The location of HuC/D-immunoreactive cells that incorporated BrdU, determined by analyzing z-stack of deconvoluted images, was closer to myenteric ganglia, but still extraganglionic after 16 weeks of chase in WT mice treated with RS67506. A-E, Sequential optical sections separated by intervals of 1.25 μm. (A1-E1, BrdU; A2-E2, HuC/D; A3-E3, Merged images). BrdU-labeled nuclei are clearly in focus in planes A and B. The punctate HuC/D-immunoreactivity of these cells is evident in A2,3 and B2,3. Mature myenteric neurons, which are still not labeled by BrdU after 16 weeks of chase, do not come into sharp focus until planes D and E are reached. The striations of the periganglionic connective tissue sheath are visible in planes B and C. F, A yz-projection of the z-stack of images illustrated in A-E; the location of the BrdU-labeled cells is shown by the arrow. The thickness of the projected plane is 11.4 μm. G, A full thickness xy-projection of the z-stack. Scale bar, 6 μm.
Supplemental figure 3:

HuC/D-immunoreactive cells that incorporated BrdU, determined by analyzing the z-stack of deconvoluted images, finally enter myenteric ganglia after 24 weeks of chase in WT mice treated with tegaserod. A-C, Sequential optical sections separated by intervals of 1.25 µm. (A1-C1, BrdU; A2-C2, HuC/D; A3-C3, Merged images). BrdU-labeled nuclei are in focus all 3 planes, and in the sharpest focus in B. The HuC/D-immunoreactivity of these cells (no longer punctate) is evident in all 3 focal planes, but like the BrdU-labeled nuclei is sharpest in B. Myenteric neurons thus become labeled by BrdU after 24 weeks of chase, although they are small neurons. F, A yz-projection of the z-stack of images illustrated in A-E; the arrow shows the location of the BrdU-labeled cells. The thickness of the projected plane is 7.2 µm. G, A full thickness xy-projection of the z-stack. Scale bar, 6 µm.