Supplemental Figure 1. Rate and location of IEC differentiation in intact planarians. Animals were fed BrdU, fixed after 1, 3, or 5 days, cryosectioned, and labeled with anti-BrdU (green) and anti-muscle (magenta). (A, B) At 24h, BrdU-positive intestinal cells are exceptionally rare. (C-F) At subsequent time points, BrdU-positive intestinal cells differentiate. (C, D) At 72h, differentiation of BrdU-positive enterocytes (green, arrows) occurs uniformly in anteroposterior, dorsoventral, and mediolateral axes. (E, F) At 5 days, more BrdU-positive intestinal cells have differentiated, but again, not at specific locations. (A, C, E) anterior (prepharyngeal) cross sections. (B, D, F) posterior (tail) sections. Dorsal is to the top in all images. Scale bars = 100 μm.

Supplemental Figure 2. Visualization of intestinal regeneration using immunofluorescent markers. Regenerates were fixed at three (A, D, G, J), five (B, E, H, K), or seven (C, F, I, L) days after amputation, and labeled with anti-phosphotyrosine (green) to visualize the apical (i.e., luminal) surface of IECs, and anti-muscle (magenta) serum to visualize reestablishment of enteric muscles that surround each intestinal branch. All images are confocal projections of a subset of optical slices from each of the regions shown. (A-C, head fragments.) (A) In head regenerates three days after amputation, anti-phosphotyrosine labeling is diffuse and muscles are disorganized at the severed tips of gut branches (arrows). (B) At five days, new branches projecting around the pharynx are phosphotyrosine-positive and partially surrounded by a nascent layer of enteric muscles (arrows). Some of these muscle fibers extend across the posterior midline (arrowheads), even though phosphotyrosine labeling never does; the role of these fibers is unknown. (C) By seven days, regenerated posterior branches

remain phosphotyrosine-positive, and are almost completely surrounded by enteric muscles. (D-I, anterior and posterior regions of pharyngeal (trunk) regenerates.) (D and G) In early (3 day) trunk fragments, the layer of enteric muscles is thin or absent in the anterior and posterior tips of severed intestinal branches (arrows). (E and H) At five days, both phosphotyrosine labeling and the enteric musculature often become more broadly disorganized in the anterior region (E) where intestinal branches undergo considerable reorganization as the prepharyngeal region increases in length. The muscle layer is still incomplete at the tips of elongating posterior branches (arrows, H). (F and I) At seven days, both anterior and posterior branches have become surrounded by a new layer of enteric muscles even at the distal tips of gut branches, but the layer of muscles is still disorganized. Muscle fibers extend away from gut branches towards the periphery of the animal (arrows); whether these fibers serve a functional role or merely represent an intermediate stage of regeneration is unclear. (J-L, tail fragments.) (J) In early tail regenerates, early anterior projections across the midline (arrow, J) are phosphotyrosine-positive, and are partially surrounded by enteric muscles. However, as in other fragments at this time point (A, D, and G), there are often significant gaps at severed branch tips. (K) At five days, phosphotyrosine labels branches that have recently fused at the anterior midline (arrows). At both five and seven days of regeneration (K and L), muscle fibers surround most intestinal branches. (L) In seven day tail fragments, as in trunk regenerates, muscle fibers often extend anteriorly toward the animal periphery. Anterior is to the top in all images. Scale bars, 100μm.

Supplemental Figure 3. Intestinal remodeling varies from animal to animal. Animals were fed Alexa 546-conjugated dextrans (red) to label differentiated phagocytes, followed by amputation one day later. (A-D) In head fragments at eight days, the contribution of labeled IECs to regenerating tail branches varies both between left and right sides of individuals (compare insets a' to b', yellow arrowheads) as well as in the posterior extent of remodeled tissue (insets c' and d'). (E, e') In tail fragments, projections across the midline can be observed as early as 3 days after amputation. (F-I) At five days after amputation, the degree to which primary anterior branch regeneration has progressed is variable. In over 20 fragments examined, zero (F), one (G), two (H), or even three (I) branches cross the midline (yellow arrowheads, G-I). Animals in all panels were imaged live. All scale bars, 500 μm.

Supplemental Figure 4. Intestinal remodeling requires neoblasts. Animals were fed Alexa 546-conjugated dextrans (red) to label differentiated phagocytes, irradiated one day later, and amputated one day after irradiation. Two representative specimens for both head and tail fragments are shown. (A-b') In five day head regenerates, labeled branches normally project posteriorly around the regenerated pharynx. (C-c') In about one quarter of the irradiated head fragments, posterior branches begin to remodel, angling towards the posterior, but it appears that regeneration halts prematurely (C-c', yellow arrows). (D-d') In most irradiated fragments, however, the intestine does not remodel (D-d'). (E-f') In tail fragments, fusion of anterior branches has begun by 5 days after amputation in almost all fragments (E-f', yellow arrows). (G-h') In irradiated tail fragments, we only observed midline projections in 1/12 specimens (G-g', arrows).

However, in all others, regeneration of the primary anterior branch does not occur. All specimens were imaged live five days after amputation. All scale bars, 500 μm.

Supplemental Figure 5. Amputation does not stimulate enterocyte proliferation. (A, B) Sagittal sections from two-day head and tail regenerates. Mitotic cells (arrows, green) are found near the tips of severed branches (A) or dorsal and ventral to the intestine, but not within the enteric muscle boundary. (C, D) Sagittal sections from five day head and tail regenerates. Again, mitotic cells (green, arrows) are never located on the lumenal side of the enteric muscle boundary. Dashed yellow lines in C and D represent the approximate plane of amputation; tissue generated during regeneration is posterior (to the right) in C, and anterior (to the left) in D. Solid yellow lines highlight the enteric muscle boundary. Green, anti-phosphohistone-H3-Ser10-positive cells. Weak green signal within the intestine in B is non-speicifc labeling of secretory vesicles in goblet cells. Magenta, enteric and body wall muscles. Anterior is to the left in all images. Scale bars, 100 μm.

Supplemental Figure 6. New intestinal cells differentiate at the anterior midline and peripharyngeal branches in five day tail regenerates. Intact animals were fed BrdU, amputated 24h later, allowed to regenerate for 5 days, then fixed and processed for BrdU immunofluorescence. Representative anterior (A), pharyngeal (B), and posterior (C) transverse sections are shown. (A, B) IECs differentiate preferentially in midline regions of the regenerating anterior branch (A, yellow arrowheads), and the medial wall of peripharyngeal branches projecting around the regenerating pharynx (B),

yellow arrowheads). (C) In posterior branches that do not remodel, relatively few new intestinal cells differentiate. Green, BrdU-positive cells; magenta, muscles. Dorsal is to the top in all images. Scale bars, 100 μm.

Type of branching	# of animals (n=11)	% of animals
Increase in anterior primary branch length	11/11	100%
Increase in posterior primary branch length	11/11	100%
Increase in secondary branch length	9/11	82%
New secondary branches	11/11	100%
New tertiary branches	8/11	73%
“Unzipping” of tertiary branches	8/11	73%
Anteriorization of peripharyngeal branches	8/11	73%

Supplemental Table 1. Types of intestinal branch development in growing planarians. Animals were fed fluorescent dextrans and imaged at one day, 14 days, and 32 days, and scored for the elaboration of branches in different categories.