Supplemental Data

Supplementary Figure 1



Representative Images for the Quantification of Mu\betaD3 staining intensity. Five images were selected out of the complete dataset summarized in Figure 5 (90 individual stained fields representing a total of 30 mice) representing the complete range of Mu β D3 staining intensity. Selection of images for creation of the staining intensity scale was completed without knowledge of mouse treatment or genotype. This scale was provided to the panel of four blinded researchers to aid in the standardization of Mu β D3 staining intensity scoring.



Supplementary Figure 2

HA-28 promotes expression of intracellular H\betaD2 protein in HT-29 cells. Western blot showing H β D2 protein relative to GAPDH protein expression in whole cell lysates of HT-29 cells treated with medium alone or HA-35 or HA-28, HA-35 at equal-molar concentrations (10 μ M) for 9 h. A. Representative Western blot of H β D2 protein expression relative to GAPDH protein expression in whole cell lysates of HT-29 cells treated with media alone, media supplemented with HA-35 or HA-28 (10 μ M C. Average densitometric quantification of Western blot results of three experiments in which HT-29 cultures were treated with medium alone, HA-35, or HA-28. H β D2 protein expression is normalized to GAPDH protein in whole cell lysates. Significance of differences in normalized H β D2 expression were evaluated by comparison of each treatment to medium treatment using Student's *t*-test, with '**' indicating *P* < 0.01.



Supplementary Figure 3

TLR4 protein expression is minimal in the colonic epithelium of TLR4^{-/-} **mice relative to wild-type or CD44**^{-/-} **mice.** Fluorescent micrographs of TLR4-immunostained (green) murine proximal colon tissue. 'NS' indicates an immunostaining control in which no TLR4 antibody was utilized. Images are representative of 3 animals of each genotype, housed under standard conditions and not treated with exogenous HA. No apparent difference in TLR4 protein expression was observed between wild-type and CD44^{-/-} animals. Residual TLR4 staining in the TLR4^{-/-} colonic epithelium relative to non-stained control may be accounted for by low affinity interactions between the polyclonal TLR4 primary antibody and proteins with structural homology to TLR4, such as other members of the Toll-like receptor family.