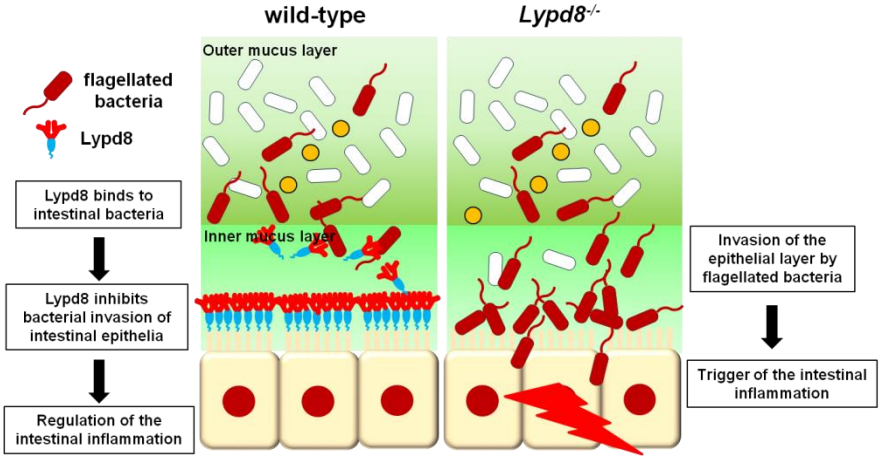
Lypd8 promotes the segregation of flagellated microbiota and colonic epithelia

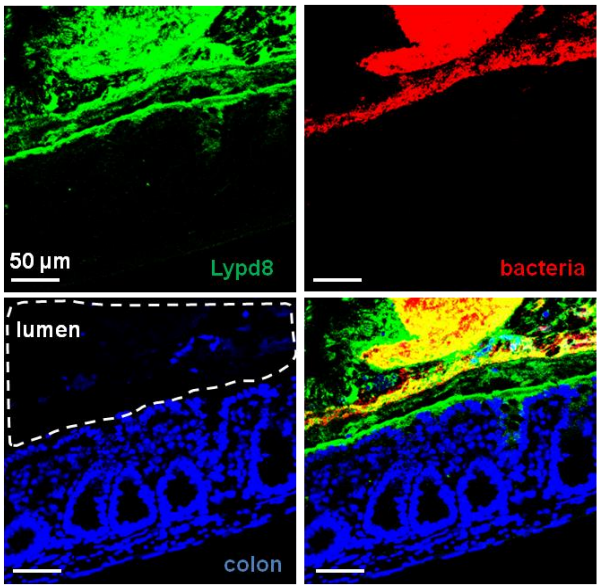
- Invasion of intestinal bacterium induces intestinal inflammation. Mechanisms to inhibit the invasion have remained unclear.
- Takeda's group revealed that the protein, Lypd 8, expressed in epithelial cells on the intestinal gland, regulates intestinal bacterial invasion of the colonic mucosa.
- These findings could shed light into the development of novel therapies for ulcerative colitis, for which there is no definitive treatment at the moment.

Summary Figure



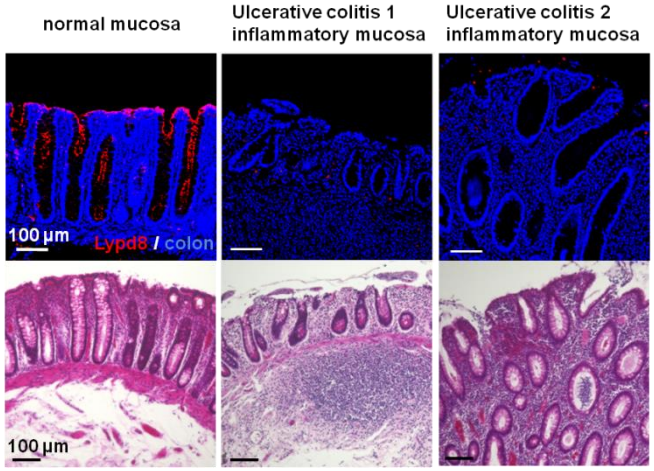
Ly6/PLAUR domain containing 8 (Lypd8), which is a highly glycosylated GPI-anchored protein, is highly and selectively expressed in epithelial cells on the uppermost layer of the intestinal gland and shed into the intestinal lumen. Lypd8 inhibits intestinal bacterial invasion of the colonic mucosa by binding to the intestinal bacteria, and thereby regulates the intestinal inflammation.

Figure 1. Lypd8 expression in the mouse colon.



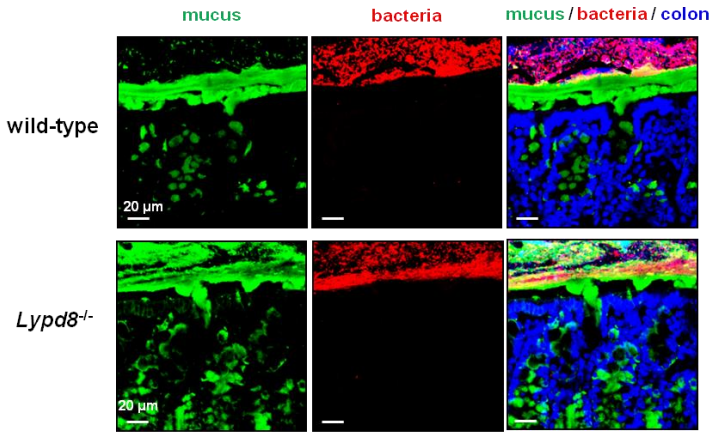
Immunostaining with anti-Lypd8 antibody (Lypd8), FISH using bacteria-specific probe (bacteria), and DAPI (colon) of Carnoy's fixed colon sections. It was found that Lypd8 is highly expressed at the uppermost layer of the intestinal gland and shed into the intestinal lumen.

Figure 2. Decrease of Lypd8 expression in the human colon of ulcerative colitis patients.



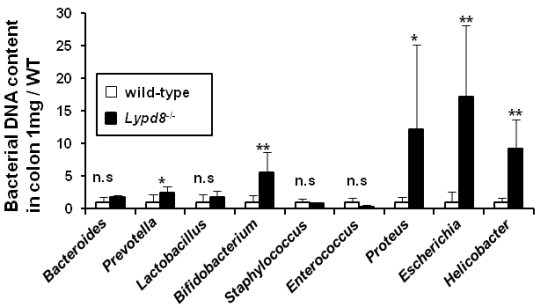
Immunostaining (upper panel) with anti-Lypd8 antibody (Lypd8) and DAPI (colon) and hematoxylin-eosin staining (lower panel) of human colon sections from the normal mucosa of a colon cancer patient and the inflammatory mucosa of two ulcerative colitis (UC) patients. It was found that Lypd8 expression is severely decreased in the inflamed mucosa of UC patients.

Figure 3. Many intestinal bacteria penetrate into the inner mucus layer in *Lypd8*-deficient mice.



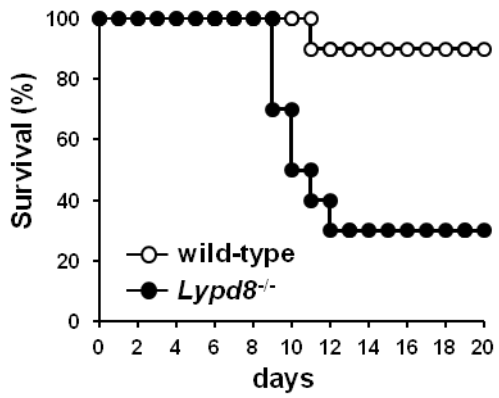
Immunostaining with anti-Muc2 antibody (mucus), FISH using bacteria-specific probe (bacteria) and DAPI (colon) of Carnoy's fixed colon sections. It was observed that many intestinal bacteria penetrate into the inner mucus layer in *Lypd8*-deficient mice, but not in wild-type mice.

Figure 4. The numbers of flagellated bacteria such as *Proteus, Escherichia* and *Helicobacter* are tremendously increased in *Lypd8*$^{-/-}$ colonic tissues.



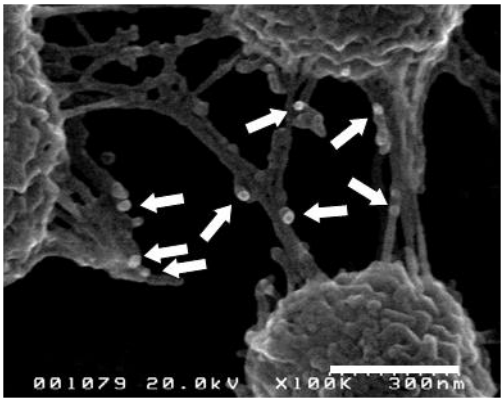
Quantitative PCR of bacterial DNA in colon tissues using bacterial genus–specific primers was performed. Data show each bacterial DNA amount compared to WT mice group. The numbers of *Proteus*, *Escherichia* and *Helicobacter*, all of which are flagellated bacteria, were increased in colonic tissues of *Lypd8*-deficient mice, compared to those of wild-type mice.

Figure 5. *Lypd8*-deficient mice are highly susceptible to dextran sulfate sodium (DSS)-induced colitis.



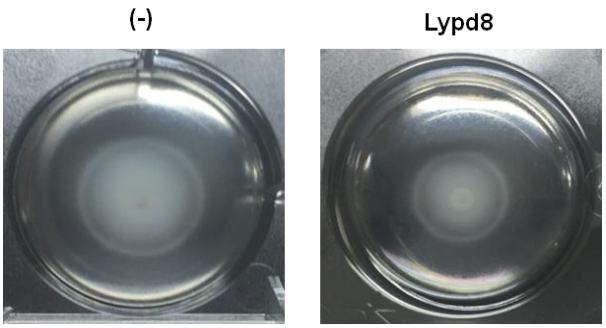
Survival rate after 2% DSS administration of wild type and *Lypd8*-deficient mice is shown. The mortality of *Lypd8*-deficient mice was much higher than that of wild-type mice. Lypd8 deficient mice were highly susceptible to DSS-induced colitis.

Figure 6. Lypd8 binds to flagella of *P. mirabilis*.



Scanning electron microscopic images of *P. mirabilis* labeled for immunogold detection of the bound Lypd8 are shown. White arrows indicate gold-particles.

Figure 7. Lypd8 suppresses the motility of *P. mirabilis*.



Representative photos of *P.mirabilis* motility in semisolid agar with (right) or without (left) Lypd8 protein at 4 h after incubation are shown. It was found that motility of *P. mirabilis* was inhibited in semisolid agar containing Lypd8 protein.