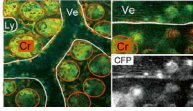
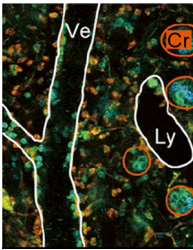


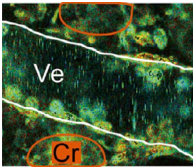
## SUPPLEMENTAL MATERIAL

Mizuno et al., <http://www.jem.org/cgi/content/full/jem.20132112/DC1>

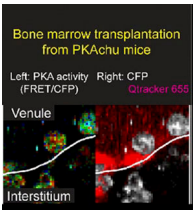
**Video 1. Activation of ERK in neutrophils during adhesion to endothelial cells of the inflamed small intestine.** This video shows time-lapse FRET/CFP and CFP images of the lamina propria of the intestinal mucosa in an Eisuke mouse, which was subjected to 1  $\mu\text{g}/\text{ml}$  LPS and 100 nM fMLP 2 h before imaging. Cr, crypt; Ly, lymphatic vessel; Ve, venule. Gamma, 1.3. Video is representative of five mice analyzed independently. Time is shown in minutes:seconds.



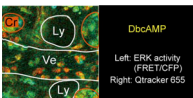
**Video 2. Requirement of ERK activity during the neutrophil recruitment cascade.** This video shows time-lapse FRET/CFP images of the lamina propria of the intestinal mucosa in an Eisuke mouse, which was subjected to 1  $\mu\text{g}/\text{ml}$  LPS and 100 nM fMLP 2 h before imaging. At time 0, 5 mg/kg PD0325901 was injected intravenously. Cr, crypt; Ly, lymphatic vessel; Ve, venule. Gamma, 1.7. Video is representative of five mice analyzed independently. Time is shown in minutes:seconds.



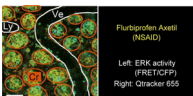
**Video 3. Activation of PKA during the neutrophil recruitment cascade.** This video shows time-lapse FRET/CFP images of the lamina propria of the intestinal mucosa in a PKAchu mouse, which was subjected to 1  $\mu\text{g}/\text{ml}$  LPS and 100 nM fMLP 2 h before imaging. Cr, crypt; Ve, venule. Gamma, 1.14. Video is representative of five mice analyzed independently. Time is shown in minutes:seconds.



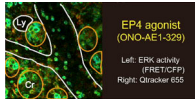
**Video 4. PKA activity in neutrophils derived from a PKAchu mouse and transplanted into a wild-type mouse.** This video shows time-lapse FRET/CFP and CFP/Qtracker 655 images of the lamina propria of the inflamed intestinal mucosa in a C57BL/6 mouse, which received bone marrow from a PKAchu mouse. The mouse was pretreated with 1  $\mu\text{g}/\text{ml}$  LPS and 100 nM fMLP 2 h before imaging. Qtracker 655 was injected intravenously to visualize the endothelial cells before the observation. Circled neutrophils in the bottom video show the extravasation. Gamma, 1.44. Video is representative of three mice analyzed independently. Time is shown in minutes:seconds.



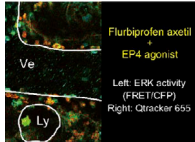
**Video 5. PKA inhibition of ERK activity, recruitment to endothelial cells, and migration of neutrophils.** This video shows time-lapse FRET/CFP and Qtracker 655 images of the lamina propria of the intestinal mucosa in an Eisuke mouse, which was subjected to 1  $\mu\text{g}/\text{ml}$  LPS and 100 nM fMLP 2 h before imaging. At time 0, a cAMP analogue, 0.3 g/kg dbcAMP, was injected intravenously. Cr, crypt; Ly, lymphatic vessel; Ve, venule. Gamma, 1.2. Video is representative of three mice analyzed independently. Time is shown in minutes:seconds.



**Video 6. Accelerated migration and activation of ERK of neutrophils after NSAID injection.** This video shows time-lapse FRET/CFP and Qtracker 655 images of the lamina propria of the intestinal mucosa in an Eisuke mouse, which was subjected to 1  $\mu\text{g}/\text{ml}$  LPS and 100 nM fMLP 2 h before imaging. At time 0, 7.5 mg/kg flurbiprofen axetil was injected intravenously. Cr, crypt; Ly, lymphatic vessel; Ve, venule. Gamma, 1.3. Video is representative of three mice analyzed independently. Time is shown in minutes:seconds. Bar, 50  $\mu\text{m}$ .



**Video 7. An EP4 agonist inhibits the rolling step and the migration of neutrophils.** This video shows time-lapse FRET/CFP and Qtracker 655 images of the lamina propria of the intestinal mucosa in an Eisuke mouse, which was subjected to 1  $\mu$ g/ml LPS and 100 nM fMLP 2 h before imaging. At time 0, an EP4 agonist, 0.25 mg/kg ONO-AE1-329, was injected intravenously. Cr, crypt; Ly, lymphatic vessel. Gamma, 1.3. Video is representative of three mice analyzed independently. Time is shown in minutes:seconds.



**Video 8. The promoting effect of NSAID on neutrophil recruitment was lost in the presence of an EP4 agonist.** This video shows time-lapse FRET/CFP and Qtracker 655 images of the lamina propria of the intestinal mucosa in an Eisuke mouse, which was subjected to 1  $\mu$ g/ml LPS and 100 nM fMLP 2 h before imaging. 7.5 mg/kg flurbiprofen axetil and 0.25 mg/kg ONO-AE1-329 were injected intravenously at time 20 and 60, respectively. Ly, lymphatic vessel; Ve, venule. Gamma, 1.14. Video is representative of three mice analyzed independently. Time is shown in minutes:seconds.