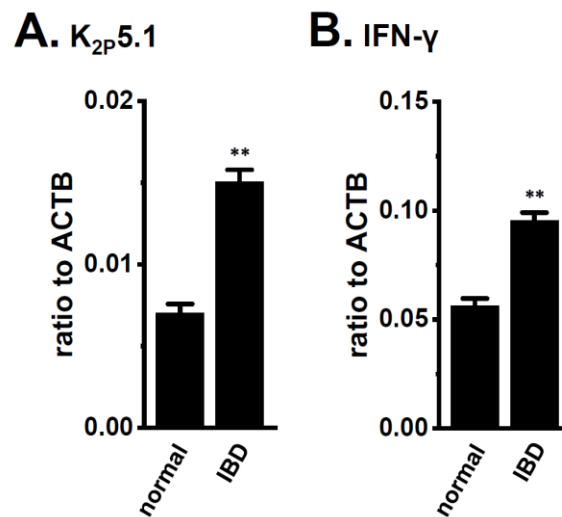
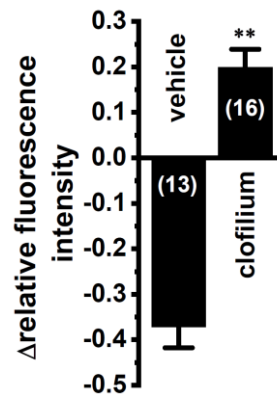


# Possible Contribution of Inflammation-Associated Hypoxia to Increased $K_{2P5.1}$ $K^+$ Channel Expression in $CD4^+$ T cells of the Mouse Model for Inflammatory Bowel Disease

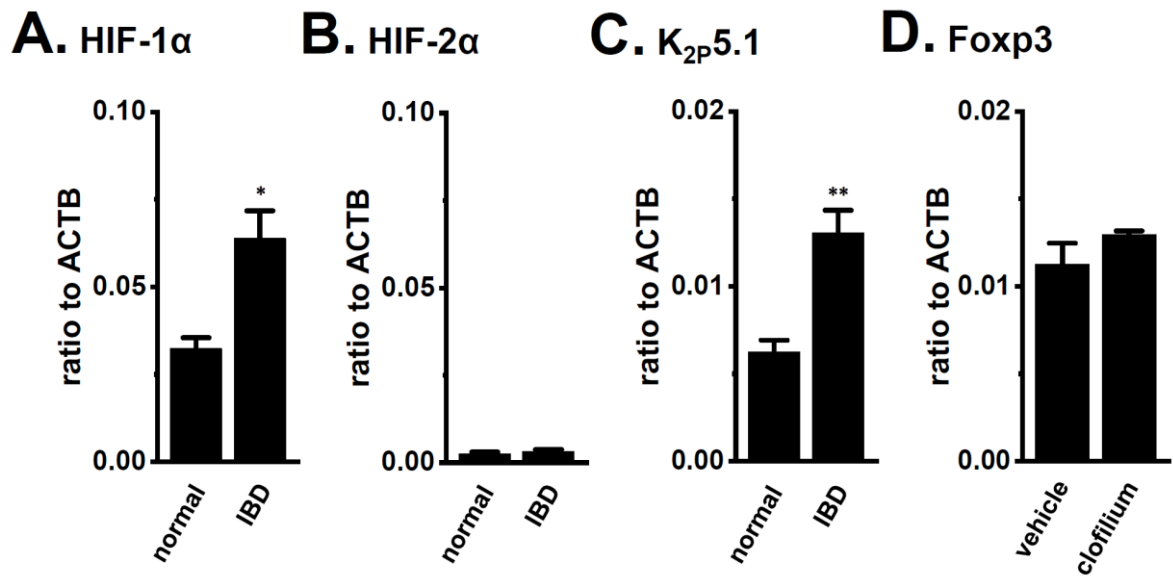
Kyoko Endo, Hiroaki Kito, Ryo Tanaka, Junko Kajikuri, Satoshi Tanaka, Elghareeb E. Elboray, Takayoshi Suzuki and Susumu Ohya



**Figure S1.** Increased expression levels of  $K_{2p5.1}$  and IFN- $\gamma$  transcripts in splenic  $CD4^+$  T cells of IBD model mice. A, B: Real-time PCR assay for  $K_{2p5.1}$  (A) and IFN- $\gamma$  (B) in the splenic  $CD4^+CD25^-$  T cells of 'normal' and 'IBD' model mice (n=4 mice for each). Expression levels were shown as a ratio to ACTB. Results are expressed as means  $\pm$  SEM. \*\*:  $p < 0.01$  vs. normal.



**Figure S2.** Disappearance of  $K_{2p5.1}$  activity by application of clofilium (5  $\mu$ M) in hypoxia-exposed splenic  $CD4^+$  T cells. Summarized results of voltage-sensitive fluorescent dye imaging of alkaline pH (pH 8.5)-induced changes in relative fluorescent intensity of DiBAC $_4$ (3) in the presence ('clofilium') and absence ('vehicle') of 5  $\mu$ M clofilium in hypoxia-exposed splenic  $CD4^+$  T cells. Cells were isolated from two different mice in each group. Cell numbers used in experiments are shown in parentheses. Results are expressed as means  $\pm$  SEM. \*\*:  $p < 0.01$  vs. vehicle control.



**Figure S3.** Expression levels of HIF-1 $\alpha$ , HIF-2 $\alpha$ , and K<sub>2p</sub>5.1 in splenic CD4<sup>+</sup> CD25<sup>+</sup> T<sub>reg</sub> cells of DSS-induced IBD model mice and effects of the treatment with 5  $\mu$ M clofilium for 24 h on expression levels of Fcpx3 in Con-A-stimulated splenic CD4<sup>+</sup> T cells. A-D: Real-time PCR assay for HIF-1 $\alpha$  (A), HIF-2 $\alpha$  (B), and K<sub>2p</sub>5.1 (C) in the splenic CD4<sup>+</sup>CD25<sup>+</sup> T<sub>reg</sub> cells of 'normal' and 'IBD' model mice and Fcpx3 (D) in clofilium-treated CD4<sup>+</sup> T cells (n=4 mice for each). Expression levels were expressed as a ratio to ACTB. Results are expressed as means  $\pm$  SEM. \*, \*\*:  $p < 0.05, 0.01$  vs. normal mice (normal) or vehicle control (vehicle).