

Supplemental Material

Supplemental Methods

LC-MS analysis of PGE₂ in colon

Colon epithelial cells were homogenized in 1 × PBS and 2 equivalent volumes of acetone were added and incubated with shaking for 10 minutes. The samples were centrifuged and supernatants were dried under vacuum centrifugation. The samples and purified PGE₂ (Cayman Chemical, Ann Arbor, MI) were suspended in 60% acetonitrile. The samples were injected into an UPLC-QTOFMS system (Waters, Mildford, MA) and separated using an Acquity UPLC™ BEH C₁₈ column (Waters). Standard curve with linear range from 20 to 1000 nM of PGE₂ ($r = 0.99$) was achieved by using dynamic range enhancement (DRE) function of QTOF Premier. PGE₂ concentrations were determined by the integration of peak area and fitting with standard curve.

Nimesulide treatment

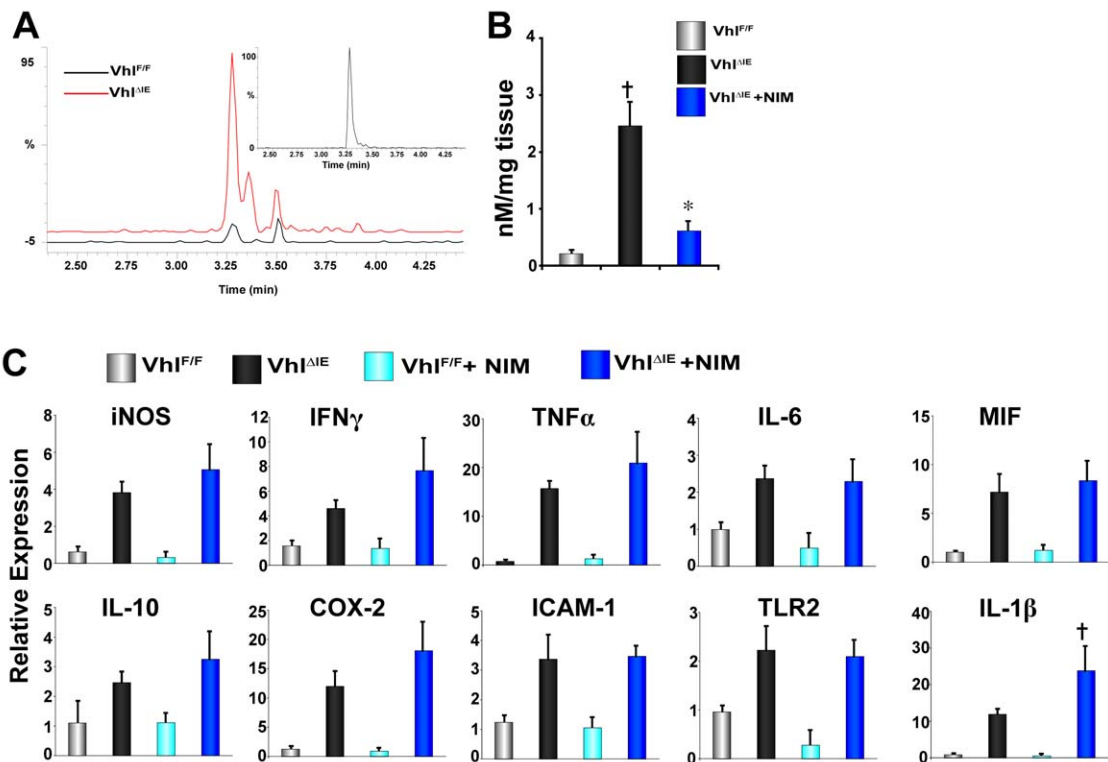
Nimesulide (Sigma, St. Louis, MO) was suspended in corn oil and delivered to the mouse via gavage at 20mg/kg and corn oil only was used as vehicle.

Supplemental Figure Legends

Supplemental Figure 1 Pro-inflammatory gene expression in *Vhl*^{F/F} and *Vhl*^{ΔIE} mice following nimesulide administration. (A) Representative overlaid chromatograms from colon extracts *Vhl*^{F/F} and *Vhl*^{ΔIE} mice; inset consist of a chromatogram from purified PGE₂. (B) Quantitation of PGE₂ levels. (C) qPCR analysis of pro-inflammatory mediators in the colon epithelium from *Vhl*^{F/F} and *Vhl*^{ΔIE} mice treated with vehicle or 20mg/kg of nimesulide.

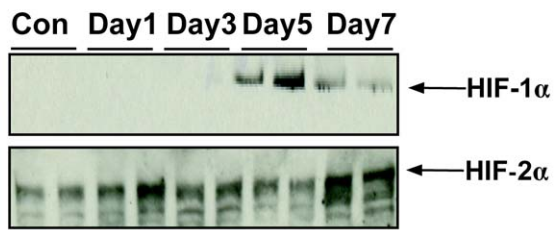
Supplemental Figure 2 Hif-1α and Hif-2α expression following 1, 3, 5, and 7 day DSS treatment. (A) Western blot analysis measuring HIF-1α or HIF-2α expression in colon epithelial cells from wild-type mice treated with DSS for 1, 3, 5, and 7 days. (B) qPCR analysis of HIF target genes in colon epithelial cells from wild-type mice treated with DSS for 1, 3, 5, and 7 days. Expression was normalized to β-actin and each bar represents the mean value ± S.D. (†)= *P*<.01 compared to control treated mice.

Supplemental Figure 1



Supplemental Figure 1

A



B

