SUPPLEMENTAL MATERIAL

Li et al., https://doi.org/10.1084/jem.20161105

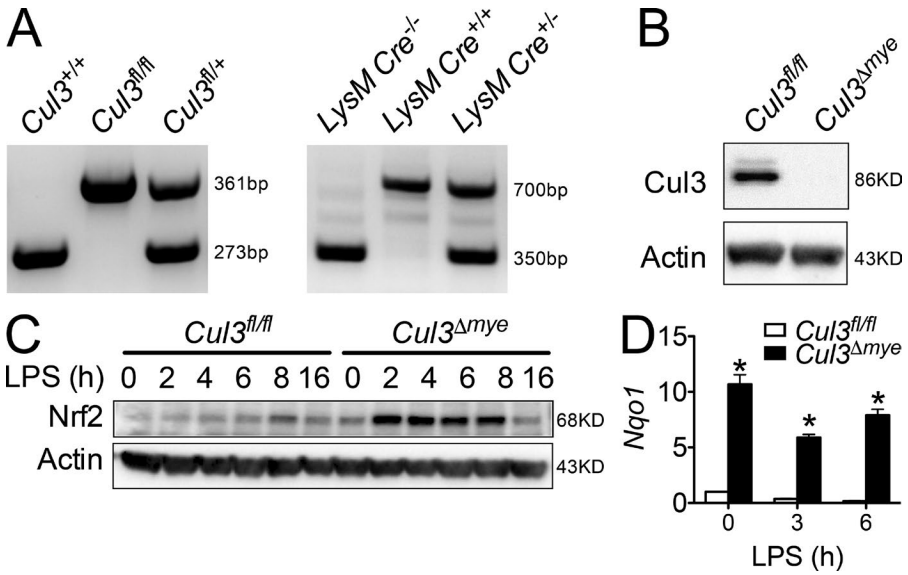

Figure S1. **Generation of myeloid conditional *Cul3* gene deletion mice (*Cul3*$^{\Delta mye}$).** (A) Genotyping was performed to detect floxed *Cul3* allele (left) or lysosome M-Cre transgene (right). Primers used during genotyping PCR were as follows: for floxed *Cul3* allele, forward 5′-TTAAAAACCGGAAAGGCCAG-3′ and reverse 5′-CAGCCAAAACAAACAAACACAC-3′; for lysosome M-Cre transgene, WT forward 5′-TTACAGTCGGCCAGGCTGAC-3′, transgene forward 5′-CCC AGAAATGCCAGATTACG-3′, and common reverse 5′-CTTGGGCTGCCAGAATTTCTC-3′. (B) Immunoblotting was performed to detect CUL3 protein in *Cul3*$^{fl/fl}$ and *Cul3*$^{\Delta mye}$ BMMs. (C and D) *Cul3*$^{fl/fl}$ and *Cul3*$^{\Delta mye}$ BMMs were left untreated or stimulated with LPS for the indicated periods. (C) Immunoblotting was performed to detect Nrf2 protein. (D) RT-PCR was performed to detect *Nqo1* transcript. Data are representative of three independent experiments, and *Nqo1* transcript levels are expressed as mean ± SD. * P, < 0.05 versus controls.

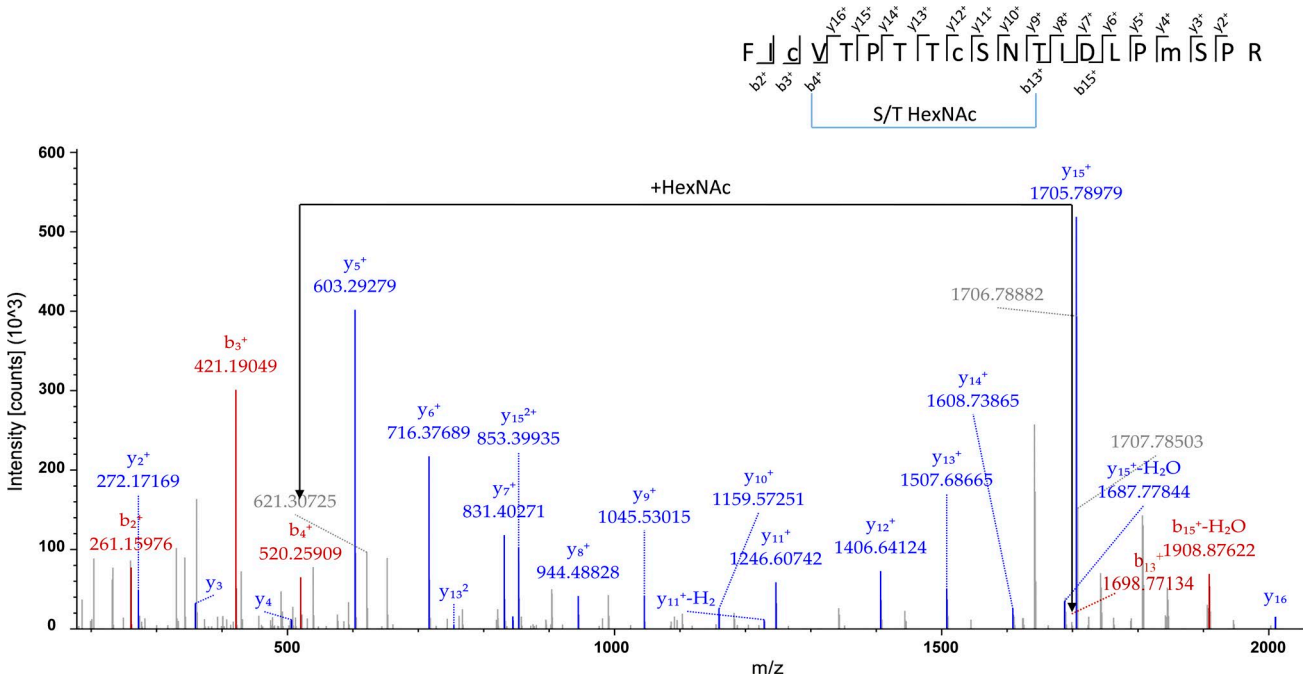

Figure S2. **MS analysis of STAT3 *O*-GlcNAcylation in mouse BMMs.** Total STAT3 was immunoprecipitated from 60 × 10^6 mouse BMMs left untreated or treated with 200 ng/ml LPS for 6 h. LC-MS/MS analysis was performed as described in Fig. 4.

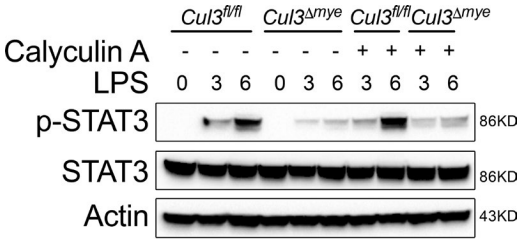

Figure S3. **PP1 is not responsible for the defective STAT3 phosphorylation in *Cul3*$^{\Delta mye}$ macrophages.** *Cul3*$^{fl/fl}$ and *Cul3*$^{\Delta mye}$ BMMs were left untreated or stimulated with LPS for 3 or 6 h with or without the pretreatment with 2 nM PP1 inhibitor calyculin A. STAT3 phosphorylation (Y705) was assayed by immunoblotting.

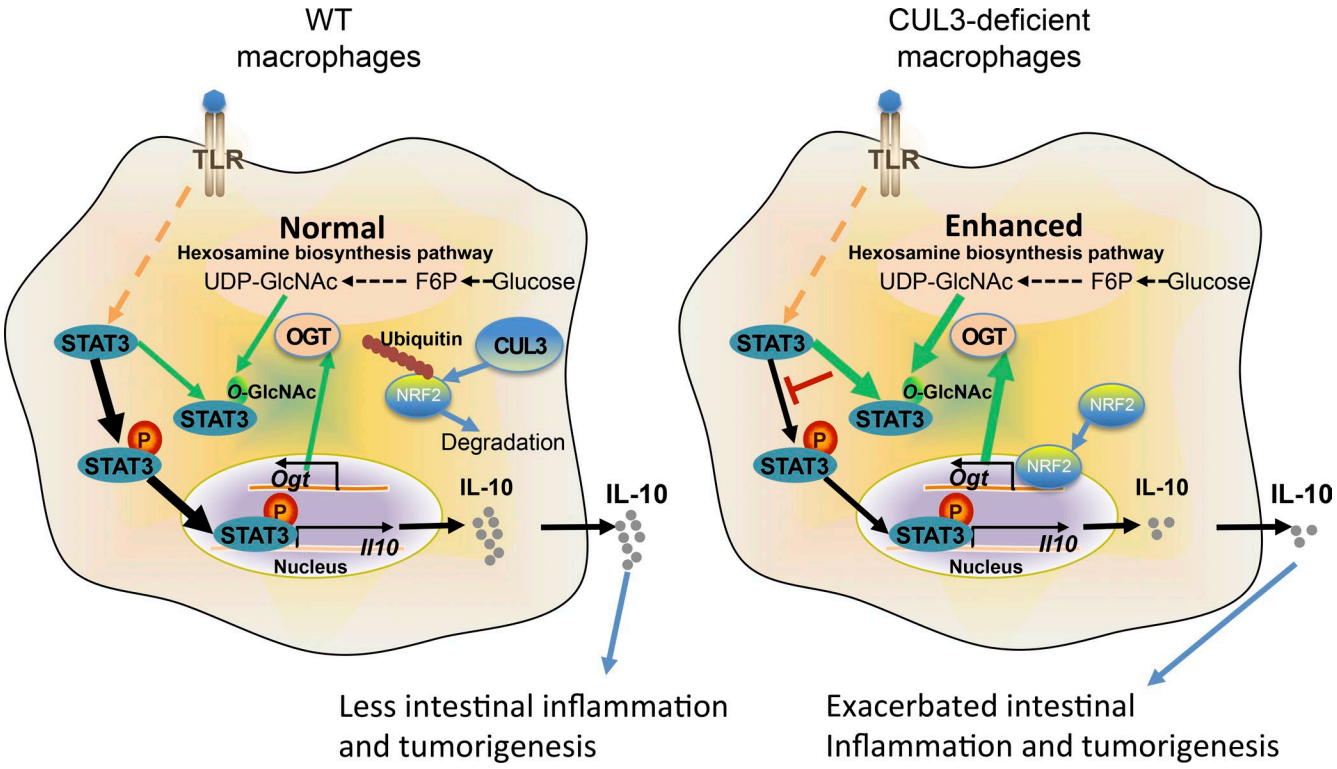

Figure S4. **Schematic of CUL3-Nrf2 signaling-modulated OGT expression and STAT3 *O*-GlcNAcylation on STAT3 phosphorylation and IL-10 production in macrophages.** In WT macrophages, CUL3 serves as a critical E3 ubiquitin ligase for Nrf2 protein ubiquitination and degradation. CUL3 deficiency results in elevated Nrf2 protein, which subsequently causes enhanced Ogt transcription. Therefore, OGT-mediated *O*-GlcNAcylation of STAT3 on T717 is enhanced in CUL3-deficient macrophages, which intrinsically inhibits STAT3 phosphorylation and IL-10 production and exacerbates disease severity in chemically induced colitis and CAC.

Table S1. **Sequences of RT-PCR primers**

Mouse genes	Forward (5′-3′)	Reverse (5′-3′)
Il10	CCCTTTGCTATGGTGTCCTT	TGGTTTCTCTTCCCAAGACC
Il12a	GAGGACTTGAAGATGTACCAG	TCCTATCTGTGTGAGGAGGGC
Cxcl1	CTGGGATTCACCTCAAGAAC	GAAGCCAGCGTTCACCAGAC
Cxcl2	AGTTTGCCTTGACCCTGAAGC	AGGCTCCTCCTTTCCAGG
Ogt	TTCGGGAATCACCCTACTTCA	TACCATCATCCGGGCTCAA
Nqo1	AGGATGGGAGGTACTCGAATC	AGGCGTCCTTCCTTATATGCTA
Actb	AGGGCTATGCTCTCCCTCAC	CTCTCAGCTGTGGTGGTGAA

Table S2. **Primers used for site-directed mutagenesis**

Mutation sites	Forward (5′-3′)	Reverse (5′-3′)
T714A	GTTTATCTGTGTGGCACCAACGACCTG	CAGGTCGTTGGTGCCACACAGATAAAC
T716A	CTGTGTGACACCAGCGACCTGCAGCAATAC	GTATTGCTGCAGGTCGCTGGTGTCACACAG
T717A	CTGTGTGACACCAACGGCCTGCAGCAATAC	GTATTGCTGCAGGCCGTTGGTGTCACACAG
T721A	GCAGCAATGCCATTGACCTGC	GGTCAATGGCATTGCTGCAGG
T714/717A	CATCTGTGTGGCACCAACGGCCTGCAGC	GCTGCAGGCCGTTGGTGCCACACAGATG
T714/716/717A	CATCTGTGTGGCACCAGCGGCCTGCAGC	GCTGCAGGCCGCTGGTGCCACACAGATG

Tables S3 and S4 are included as separate Excel files. Table S3 shows the list of genes with increased and decreased expression levels in LPS-treated *Cul3*$^{\Delta mye}$ macrophages compared with similarly treated WT macrophages. Table S4 shows the list of metabolites in LPS-treated *Cul3*$^{\Delta mye}$ macrophages versus similarly treated WT macrophages.