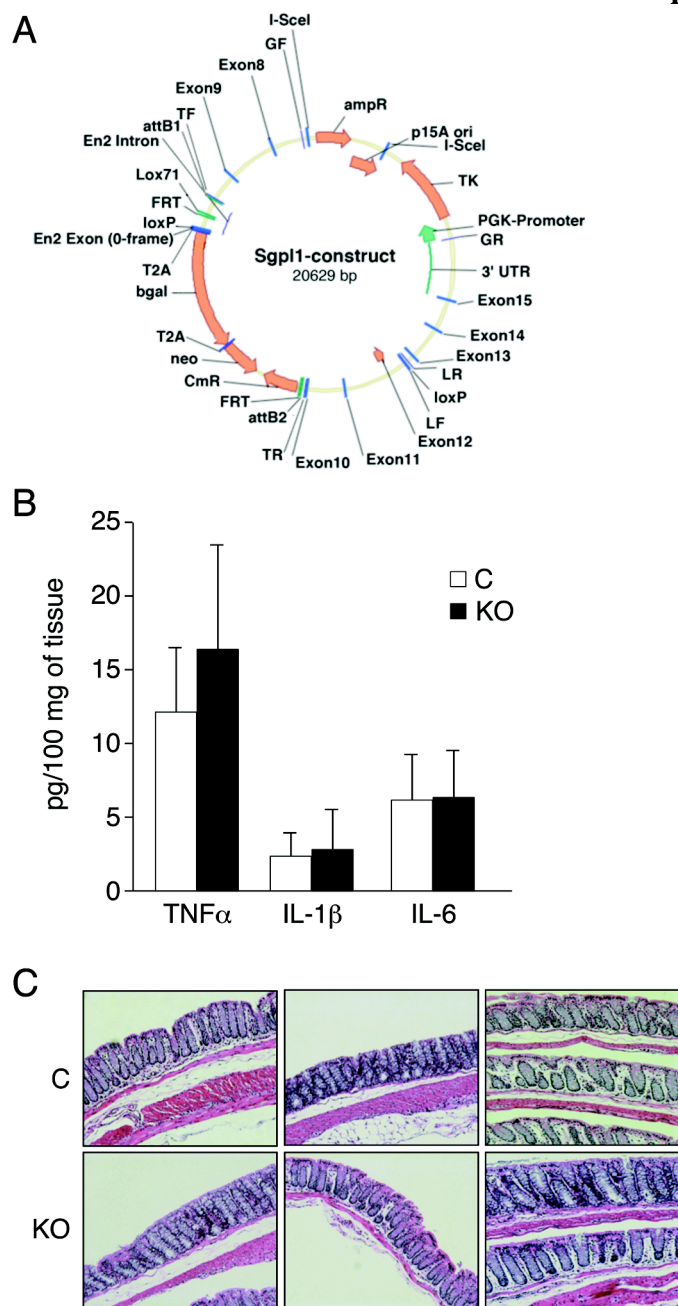
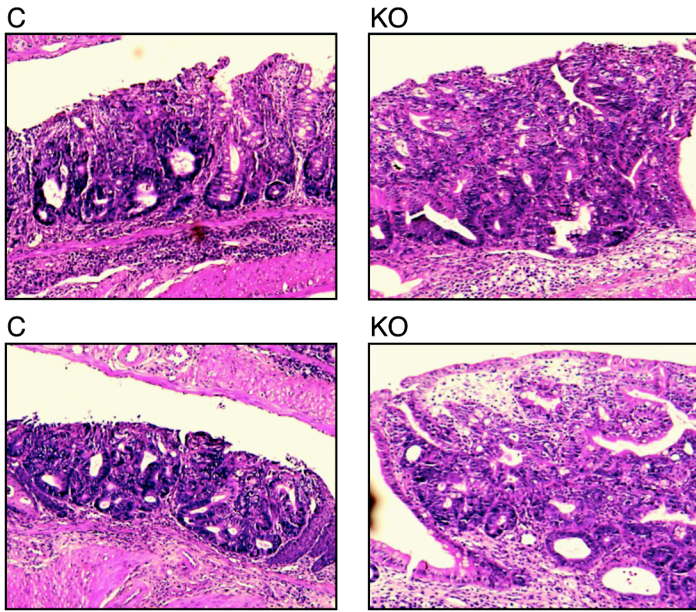


## Online Supplemental Data



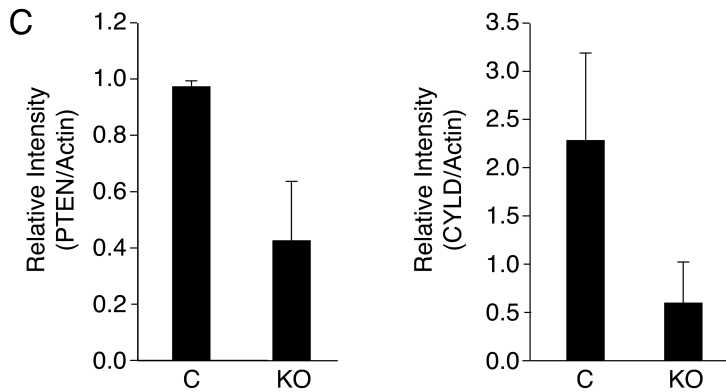
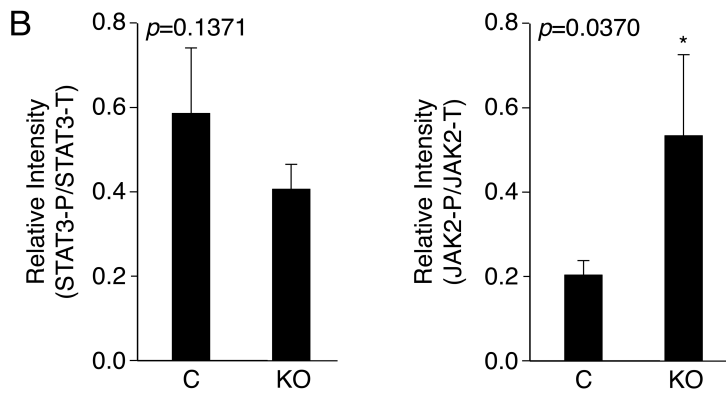
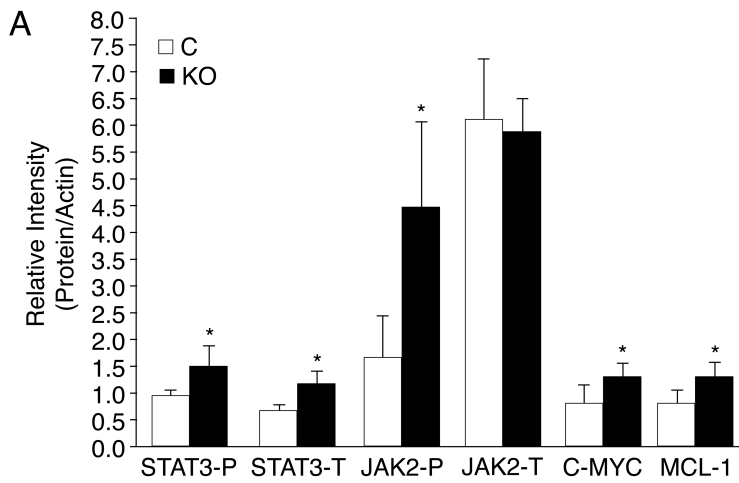
### Supplemental Figure 1.

(A) Trapping vector for generation of conditional SPL knockout mouse models. Targeting vector introduces a *lacZ* reporter 5' to exon 10, resulting in an SPL truncation. The reporter was deleted by crossing to FLPR transgenic mice. The resulting *Sgpl1* allele has "floxed" exons 10-12 that can be excised by *Cre* recombinase. (B) Colon inflammatory cytokines of C vs KO mice at baseline level. (C) Colon H&E staining (10X) of C vs KO mice 42 days after induction of SPL's deletion.



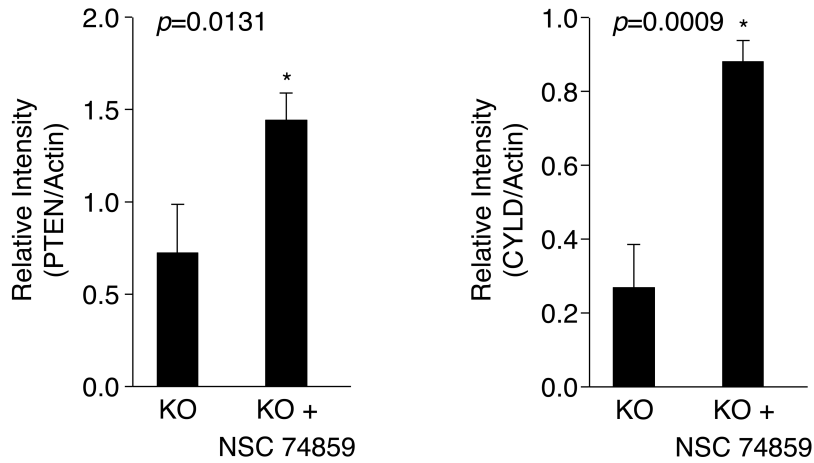
**Supplemental Figure 2.**

Representative pictures (10X) of tumors from C and KO mice treated with AOM/DSS. The analyses were performed by a pathologist blinded to the genotype. No significant differences in tumor grade were found.



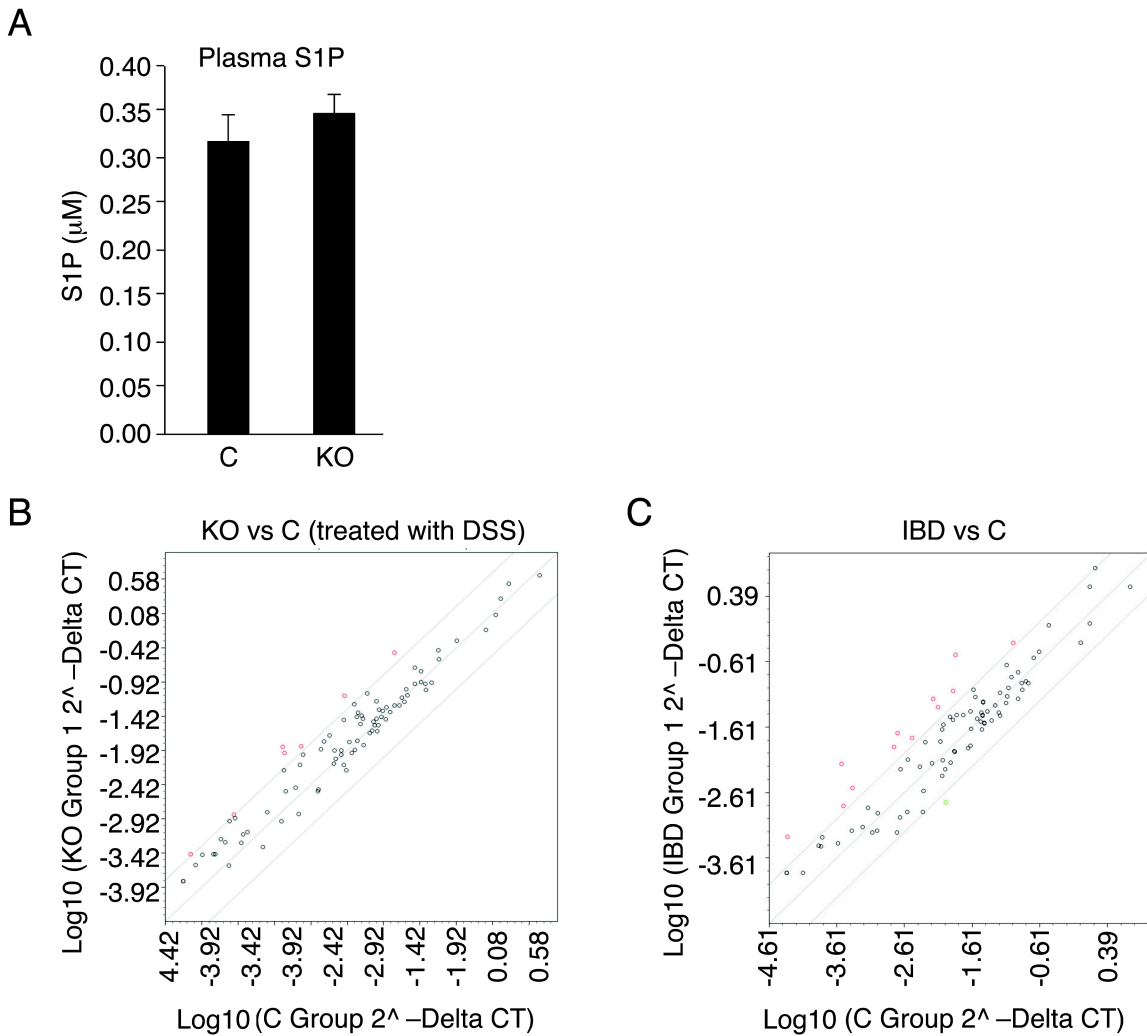
**Supplemental Figure 3.**

Loss of SPL in gut epithelium enhances STAT3 signaling. **(A)** Image quantification of immunoblot shown in Figure 3C demonstrating protein expression levels of activated STAT3 phosphorylated on Tyr 705 (STAT3-P), total STAT3 (STAT3-T), phosphorylated JAK2 (JAK2-P), total Jak-2 (JAK2-T), C-Myc, MCL-1 over ACTIN in C and KO mouse colon tissues following administration of AOM/DSS. White bars = C; black bars = KO. **(B)** Image quantification of immunoblot shown in Figure 3C demonstrating protein expression levels of activated STAT3 phosphorylated (STAT3-P) over total STAT3 (STAT3-T) and phosphorylated JAK2 (JAK2-P) over total Jak-2 (JAK2-T). **(C)** Image quantification of immunoblot shown in Figure 3H demonstrating protein expression levels of PTEN and CYLD in non-neoplastic colon tissues in C and KO mouse colon tissues following administration of AOM/DSS. Results represent quantification of each protein normalized to actin loading control.



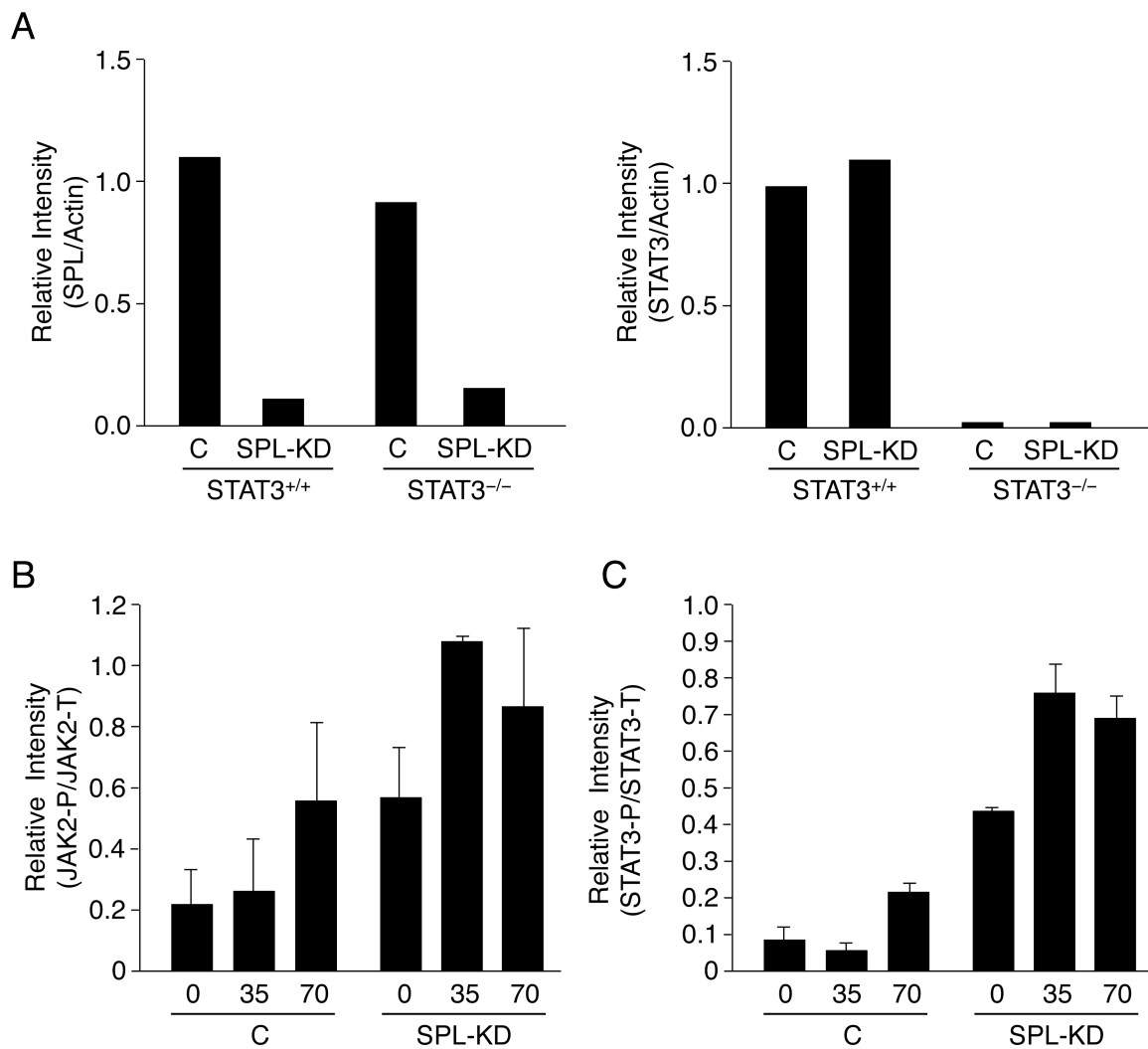
**Supplemental Figure 4.**

Inhibition of STAT3 normalizes PTEN and CYLD expression levels in colon tissues of  $SPL^{GutKO}$  mice. KO mice were treated with AOM/DSS  $\pm$  NSC 74859, 5 mg/kg i.p. or saline every other day on days 30-60. Shown is image quantification of immunoblot shown in Figure 4F, which demonstrates normalization of PTEN and CYLD protein expression levels in KO mice treated with NSC 74859. Results represent quantification of each protein normalized to actin loading control.



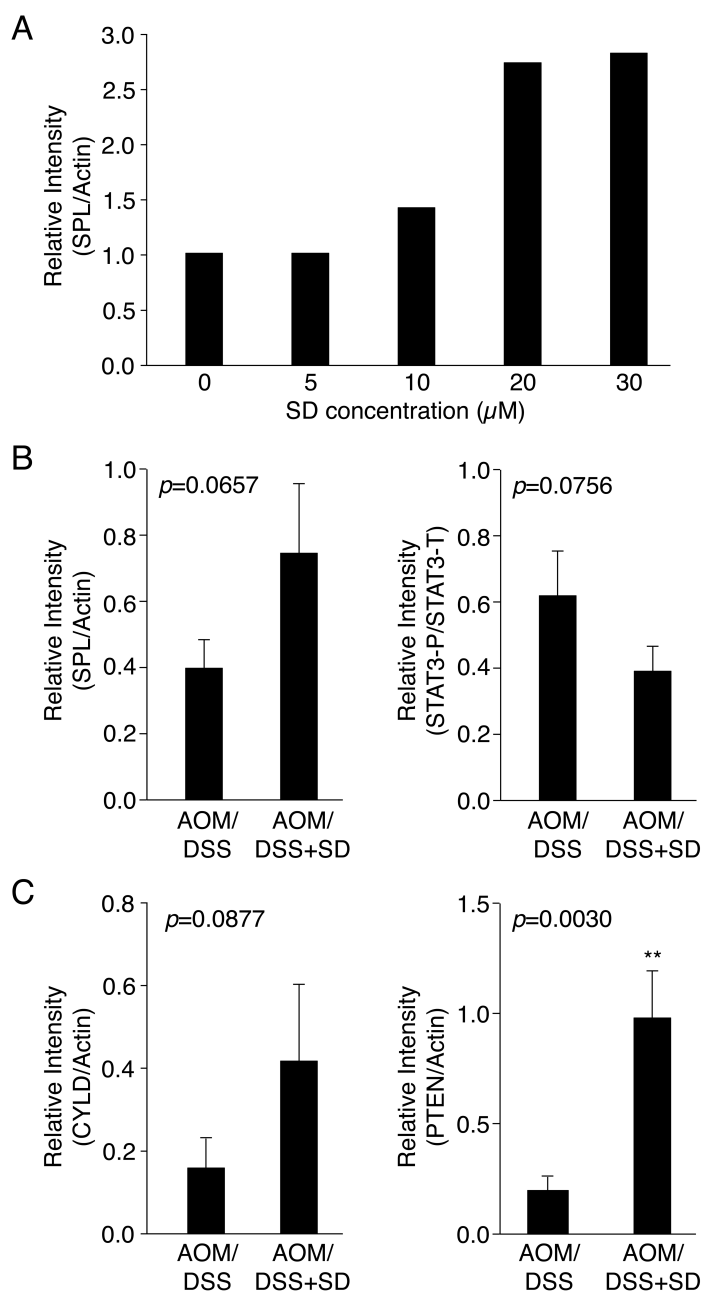
**Supplemental Figure 5.**

The S1P/SPL/STAT3 axis in experimental murine colitis and human IBD. C and KO mice were treated for two cycles of 3% DSS. **(A)** Plasma S1P levels in C mice treated with DSS vs. KO mice treated with DSS. **(B)** Dot plot demonstrating in red the genes upregulated significantly in KO group vs. C group for mouse STAT3 qRT-PCR array results presented in Table S2 and summarized in Table 1. **(C)** Dot plot demonstrating in red the genes upregulated significantly in IBD group vs. C group for human STAT3 qRT-PCR array results presented in Table S3 and summarized in Table 2.



**Supplemental Figure 6.**

STAT3 and SPL silencing in MEFs. (A) Image quantification of immunoblot shown in Figure 6A demonstrating SPL and total STAT3 protein expression levels in STAT3<sup>+/+</sup> and STAT3<sup>-/-</sup> MEFs in which SPL was silenced (SPL-KD) compared to vector-only control cell lines (C). Image quantification of immunoblot shown in Figure 6H demonstrating JAK2-P (B) and STAT3-P (C) levels normalized against total protein levels in S1P-treated MEFs.



### Supplemental Figure 7.

Oral SD induce SPL in vitro and in vivo and prevent STAT3 activation. **(A)** Image quantification of immunoblot shown in Figure 7D. DLD1 cells were treated with SDs at the concentrations shown, harvested 24h later, and SPL protein was measured in whole cell extracts. Results represent quantification of SPL protein normalized to actin loading control. **(B)** Image quantification of immunoblot shown in Figure 7E. WT mice were administered AOM/DSS + 25 mg/kg SD or vehicle only every other day by gavage. Immunoblotting of colon SPL and **(C)** Image quantification of immunoblot shown in Figure 7I, which demonstrates that protein expression levels of PTEN and CYLD are higher in colons of mice treated with AOM/DSS + SD than in mice treated with AOM/DSS alone. Results represent quantification of each protein normalized to actin loading control.

## Supplemental Tables

Gene	Forward 5'→3'	Reverse 5'→3'
<i>Sgpl1</i> Exons 8-9	AAAGCAGCTCATTATTTTGG	GGGTAGAACAGACCAGCATA
<i>Sgpl1</i> Exons 8-12	AAAGCAGCTCATTATTTTGG	ACTGACAATTGAGGATCACC
<i>SGPL1</i>	AGGCCCTAGGGGAAATGGAT	TCAGGAGTCCCTGAGATGGG
<i>S1PR1</i>	CCTGCTTGAGCGAGGCTG	GAGAGCCTTCACTGGCTTCA
<i>SPHK1</i>	CCTTCACGCTGATGCTCA CT	CGTTCACCACCTCGTGCAT
<i>HPRT1</i>	TGACACTGGCAAAACAATGCA	GGTCCTTTTACCAGCAAGCT
<i>Cyld</i>	TTGGCAACTGGGATGGAAGG	CCT GTGTCACGCTAT CTGGG
<i>Spns2</i>	GCACTTTGGGGTCAAGGA	CCCAGGTAGCCAAAGATGG
<i>Il6</i>	TCCGGAGAGGAGACTTCACA	TTGCCATTGCACA ACTCTTTTCT
<i>S1pr1</i>	ACC TAG CCC TCT CGG ACC TAT	CCC AGA CAA CAG CAG GTT AGC
<i>Gapdh</i>	ACCTGCCAAGTATGATGA	GGAGTTGCTGTTGAAGTC
<i>B2M</i>	GTGCTCGCGCTACTCTCTC	GTCAACTTCAATGTCGGAT

### Supplemental Table 1.

Primers used for PCR in this study. Forward and reverse primer pairs “Exon 8–9” and “Exon 8–12” were used to confirm complete recombination of murine *Sgpl1* gene, as described in Online Methods. All other primer pairs were used to quantify expression of SIP-related genes in colon tissues of IBD patients and controls presented in Figure 5 or *Il6* and *Spns2* expression in MEF lines or *Cyld* expression in tumors in Figure 6 or *S1pr1* expression presented in Figure 3.



Symbol	Fold Change (compared to control group)		
	Fold change	95% CI	p-value
<i>Akt1</i>	<b>3.5919</b>	(0.00001, 15.82)	0.840982
<i>Bax</i>	0.8393	(0.62, 1.06)	0.245236
<i>Bcl2</i>	0.5227	(0.23, 0.82)	0.093664
<i>Ccl12</i>	<b>2.2768</b>	(0.00001, 5.44)	0.326097
<i>Ccl3</i>	<b>4.9102</b>	(1.08, 8.74)	<b>0.017191</b>
<i>Ccl4</i>	<b>8.7514</b>	(0.74, 16.76)	<b>0.000037</b>
<i>Ccl5</i>	<b>2.8449</b>	(0.00001, 7.75)	0.174203
<i>Cd4</i>	0.5396	(0.00001, 1.16)	0.625154
<i>Cd40</i>	1.4839	(0.00001, 3.27)	0.439967
<i>Cd40lg</i>	1.2666	(0.21, 2.32)	0.690247
<i>Cd80</i>	<b>2.3788</b>	(0.55, 4.21)	<b>0.033755</b>
<i>Cdc25a</i>	0.7427	(0.41, 1.08)	0.257841
<i>Cdkn1a</i>	0.7964	(0.23, 1.37)	0.367076
<i>Cebpd</i>	0.8454	(0.28, 1.41)	0.682077
<i>Csf1</i>	0.7802	(0.52, 1.04)	0.202664
<i>Csf2</i>	<b>2.8804</b>	(0.00001, 5.95)	0.200016
<i>Csf3</i>	1.4447	(0.00001, 5.11)	0.896709
<i>Csf3r</i>	<b>2.5068</b>	(0.00001, 5.58)	0.168606
<i>Cxcl10</i>	<b>3.7838</b>	(0.00001, 12.11)	0.165209
<i>Cscl12</i>	1.8758	(0.79, 2.96)	0.107166
<i>Cxcr4</i>	1.0353	(0.12, 1.95)	0.677016
<i>Egfr</i>	1.1482	(0.76, 1.53)	0.457087
<i>Fas</i>	1.4291	(0.53, 2.33)	0.261846
<i>Fasl</i>	<b>4.1044</b>	(0.00001, 12.79)	0.274671
<i>Hgf</i>	1.5888	(0.50, 2.68)	0.317858
<i>Ikbkb</i>	0.856	(0.37, 1.34)	0.607861
<i>Il10</i>	<b>2.1434</b>	(0.43, 3.86)	0.124983
<i>Il11</i>	<b>3.8025</b>	(0.00001, 8.94)	0.131262
<i>Il12a</i>	1.5635	(0.00001, 3.29)	0.543198
<i>Il13</i>	1.5916	(0.00001, 4.16)	0.440553
<i>Il15</i>	0.8816	(0.09, 1.67)	0.762276
<i>Il17a</i>	1.9508	(0.00001, 4.83)	0.65151
<i>Il18</i>	1.2411	(0.05, 2.43)	0.462391
<i>Il18r1</i>	<b>3.5161</b>	(0.00001, 8.73)	0.104683
<i>Il1a</i>	<b>6.8166</b>	(0.00001, 20.16)	0.059813
<i>Il1b</i>	<b>5.8974</b>	(0.00001, 16.51)	0.057581
<i>Il1r1</i>	1.1573	(0.54, 1.77)	0.750074
<i>Il2</i>	<b>4.3547</b>	(0.08, 8.63)	<b>0.033866</b>
<i>Il21</i>	0.8764	(0.00001, 1.89)	0.595547
<i>Il22</i>	<b>2.0701</b>	(0.00001, 5.22)	0.62171
<i>Il23a</i>	0.5575	(0.00001, 1.24)	0.2906
<i>Il24</i>	<b>3.8643</b>	(0.00001, 9.34)	0.141145
<i>Il2ra</i>	1.7605	(0.05, 3.47)	0.242651
<i>Il3</i>	<b>2.2199</b>	(0.00001, 4.82)	0.242701
<i>Il4</i>	<b>3.5292</b>	(0.00001, 10.36)	0.26809
<i>Il5</i>	<b>2.7245</b>	(0.00001, 6.72)	0.25941
<i>Il6</i>	<b>6.6635</b>	(0.00001, 17.61)	0.218507
<i>Il6ra</i>	0.9702	(0.60, 1.34)	0.890576
<i>Il6st</i>	1.3829	(1.07, 1.70)	<b>0.038628</b>
<i>Il7</i>	1.2395	(0.22, 2.26)	0.495628

<i>Il9</i>	<b>2.5472</b>	(0.00001, 6.71)	0.228944
<i>Jak2</i>	1.0954	(0.68, 1.51)	0.526612
<i>Jak3</i>	1.0724	(0.15, 1.99)	0.568178
<i>Lif</i>	0.7432	(0.01, 1.48)	0.886325
<i>Lifr</i>	0.8818	(0.69, 1.07)	0.300929
<i>Lta</i>	1.5022	(0.00001, 3.55)	0.881252
<i>Map2k1</i>	0.6381	(0.26, 1.02)	0.210034
<i>Mapk1</i>	1.188	(0.86, 1.51)	0.280621
<i>Mapk14</i>	1.0018	(0.82, 1.19)	0.987244
<i>Mapk3</i>	0.6651	(0.30, 1.03)	0.191967
<i>Mapk8</i>	1.0413	(0.61, 1.47)	0.6789
<i>Met</i>	1.2155	(0.78, 1.65)	0.365847
<i>Mtor</i>	0.7242	(0.21, 1.24)	0.419534
<i>Myc</i>	0.921	(0.36, 1.49)	0.918203
<i>Nfkb1</i>	0.9588	(0.80, 1.12)	0.686409
<i>Nfkbia</i>	1.604	(0.66, 2.54)	0.157212
<i>Osm</i>	<b>2.723</b>	(0.00001, 5.82)	0.104554
<i>Osmr</i>	<b>2.0803</b>	(0.85, 3.31)	<b>0.01639</b>
<i>Pias3</i>	0.6328	(0.16, 1.10)	0.253109
<i>Pim1</i>	1.7646	(0.68, 2.85)	0.099694
<i>Rac1</i>	1.2765	(0.16, 2.39)	0.441131
<i>Rela</i>	0.69	(0.00001, 1.52)	0.720281
<i>Socs1</i>	<b>2.8866</b>	(0.00001, 6.00)	0.135427
<i>Socs3</i>	<b>2.0256</b>	(0.18, 3.87)	0.145293
<i>Src</i>	0.9824	(0.28, 1.69)	0.709482
<i>Stat3</i>	0.9569	(0.76, 1.15)	0.651982
<i>Tlr4</i>	1.2173	(0.80, 1.63)	0.257788
<i>Tnf</i>	0.6605	(0.00001, 1.68)	0.454812
<i>Tnfrsf10b</i>	0.8069	(0.58, 1.03)	0.222959
<i>Tnfrsf1a</i>	0.8642	(0.39, 1.34)	0.681379
<i>Tnfrsf1b</i>	1.0006	(0.13, 1.87)	0.744635
<i>Tnfsf10</i>	1.1983	(0.00001, 2.61)	0.374755
<i>Tnfsf11</i>	<b>3.1063</b>	(0.00001, 7.27)	0.207586
<i>Tyk2</i>	1.0389	(0.12, 1.96)	0.902107
<i>Tnfrsf1A</i>	0.7435	(0.03, 1.46)	

### Supplemental Table 2.

STAT3 target genes upregulated in SPL<sup>GutKO</sup> colons. Comparison of STAT3 target gene expression in colons of C and KO mice treated with DSS. Fold change is presented in red for genes expressed two-fold higher in KO, black for less than two-fold change.  $n = 4$  mice per group.  $P$ -values with  $p < 0.05$  are shown in red.

Symbol	Fold Change (compared to control group)		
	Fold change	95% CI	p-value
<i>AKT1</i>	0.8313	(0.00001, 1.95)	0.815793
<i>BAX</i>	0.7715	(0.25, 1.30)	0.399074
<i>BCL2</i>	<b>0.2492</b>	(0.00001, 1.38)	0.440492
<i>CCL2</i>	1.0524	(0.00001, 2.23)	0.86047
<i>CCL3</i>	<b>2.5953</b>	(0.00001, 6.48)	0.384502
<i>CCL4</i>	<b>2.0644</b>	(0.00001, 4.78)	0.308236
<i>CCL5</i>	0.8802	(0.28, 1.48)	0.566791
<i>CD4</i>	<b>6.4636</b>	(0.00001, 18.87)	0.324758
<i>CD40</i>	<b>3.0797</b>	(0.00001, 7.53)	0.372874
<i>CD40LG</i>	<b>7.502</b>	(0.00001, 15.91)	0.267103
<i>CDKN1A</i>	<b>6.9563</b>	(0.00001, 18.35)	0.182848
<i>CSF1</i>	<b>5.2118</b>	(0.00001, 10.96)	<b>0.038288</b>
<i>CSF2</i>	<b>4.4125</b>	(0.00001, 13.20)	0.368668
<i>CSF3</i>	<b>26.0293</b>	(0.00001, 115.31)	0.273917
<i>CSF3R</i>	<b>21.6679</b>	(0.00001, 50.34)	<b>0.049076</b>
<i>CXCL10</i>	<b>7.6253</b>	(0.00001, 20.96)	0.11377
<i>EGFR</i>	<b>2.9325</b>	(0.00, 5.86)	0.145068
<i>FAS</i>	<b>2.8779</b>	(0.96, 4.80)	0.063704
<i>FASLG</i>	<b>3.1571</b>	(0.17, 6.14)	0.054099
<i>HGF</i>	<b>4.312</b>	(0.00001, 10.67)	0.271933
<i>IL10</i>	<b>4.7638</b>	(0.00001, 10.23)	0.152418
<i>IL11</i>	<b>14.8687</b>	(0.00001, 40.14)	0.112717
<i>IL13</i>	<b>4.2556</b>	(0.00001, 10.42)	0.268468
<i>IL15</i>	<b>2.0229</b>	(0.00001, 5.38)	0.209898
<i>IL17A</i>	<b>11.0818</b>	(0.00001, 28.69)	0.161338
<i>IL18</i>	<b>2.304</b>	(0.00001, 6.47)	0.29052
<i>IL18R1</i>	<b>6.5011</b>	(0.00001, 15.55)	<b>0.042867</b>
<i>IL1A</i>	<b>47.6701</b>	(0.00001, 173.32)	0.092131
<i>IL1B</i>	<b>19.4642</b>	(0.00001, 70.97)	0.155383
<i>IL1R1</i>	<b>9.7779</b>	(1.19, 18.37)	<b>0.00408</b>
<i>IL2</i>	<b>4.4851</b>	(0.00001, 12.64)	0.150278
<i>IL21</i>	<b>8.131</b>	(0.00001, 21.56)	0.124449
<i>IL22</i>	<b>8.9883</b>	(0.00001, 19.32)	0.057783
<i>IL23A</i>	<b>7.7454</b>	(0.00001, 22.16)	0.253731
<i>IL2RA</i>	<b>4.0165</b>	(0.00001, 9.19)	0.132151
<i>IL3</i>	<b>5.7957</b>	(0.00001, 20.59)	0.180493
<i>IL4</i>	<b>12.6404</b>	(0.00001, 34.15)	0.110983
<i>IL5</i>	<b>7.6063</b>	(0.00001, 38.14)	0.295508
<i>IL6</i>	<b>4.31</b>	(0.00001, 11.78)	0.207029
<i>IL6R</i>	<b>2.0054</b>	(0.33, 3.68)	0.189977
<i>IL8</i>	<b>17.2663</b>	(0.00001, 59.01)	0.269463
<i>JAK2</i>	<b>15.6493</b>	(8.00, 23.30)	<b>0.009991</b>
<i>JAK3</i>	<b>10.6673</b>	(0.00001, 25.66)	0.169327
<i>LIF</i>	<b>8.4346</b>	(0.00001, 21.83)	<b>0.043246</b>
<i>LIFR</i>	<b>3.6187</b>	(0.00001, 8.03)	0.222662
<i>LTA</i>	0.8407	(0.00001, 2.27)	0.829857
<i>MAP2K1</i>	<b>2.3767</b>	(0.07, 4.69)	0.137117
<i>MAPK1</i>	<b>2.5953</b>	(2.12, 3.07)	<b>0.000045</b>
<i>MAPK14</i>	1.699	(0.00001, 3.55)	0.436189
<i>MAPK3</i>	0.8371	(0.27, 1.41)	0.958034

<i>MAPK8</i>	1.9433	(0.39, 3.50)	0.251926
<i>MET</i>	<b>3.5202</b>	(1.06, 5.98)	0.096947
<i>MTOR</i>	<b>4.7168</b>	(0.00001, 9.56)	<b>0.028917</b>
<i>MYC</i>	<b>0.2407</b>	(0.00001, 1.54)	0.255186
<i>NFκB1</i>	<b>2.3009</b>	(0.00001, 4.86)	0.215136
<i>NFKBIA</i>	<b>28.1908</b>	(0.00001, 154.57)	0.436417
<i>OSM</i>	1.5272	(0.00001, 8.25)	0.220134
<i>PIAS3</i>	<b>3.3883</b>	(0.00001, 7.08)	0.275247
<i>PIM1</i>	<b>2.2801</b>	(0.73, 3.84)	0.072973
<i>RELA</i>	<b>2.9214</b>	(1.22, 4.62)	0.079613
<i>SOCS1</i>	<b>2.18</b>	(0.00001, 4.77)	0.28915
<i>SRC</i>	1.6646	(0.07, 3.26)	0.329707
<i>TLR4</i>	<b>2.564</b>	(0.32, 4.81)	0.108543
<i>TNF</i>	<b>2.2833</b>	(0.00001, 5.31)	0.405017
<i>TNFRSF10B</i>	<b>3.3659</b>	(1.19, 5.54)	<b>0.048737</b>
<i>TNFRSF1B</i>	<b>5.6288</b>	(0.00001, 13.22)	0.086626
<i>TNFSF10</i>	<b>2.3538</b>	(1.13, 3.58)	0.06796
<i>TYK2</i>	<b>7.2804</b>	(2.22, 12.35)	<b>0.02859</b>
<i>BCL3</i>	<b>5.3375</b>	(0.00001, 11.66)	<b>0.001463</b>
<i>CASP4</i>	<b>4.4462</b>	(0.00001, 10.51)	0.186187
<i>CEBPD</i>	1.5218	(0.00001, 3.30)	0.42364
<i>GLRX</i>	<b>2.204</b>	(0.00001, 6.08)	0.353563
<i>IFITM2</i>	0.6873	(0.00001, 1.92)	0.853617
<i>IL6ST</i>	<b>2.2452</b>	(0.00001, 5.81)	0.603357
<i>JUNB</i>	1.708	(1.19, 2.23)	0.05061
<i>NRP1</i>	<b>2.9386</b>	(1.18, 4.69)	<b>0.012564</b>
<i>PHF21A</i>	0.6266	(0.00001, 2.87)	0.251704
<i>PROS1</i>	<b>4.948</b>	(0.00001, 12.84)	0.063428
<i>PVRL2</i>	<b>2.7068</b>	(0.69, 4.72)	0.17366
<i>SOCS3</i>	<b>13.4096</b>	(0.00001, 36.30)	0.066855
<i>STAT3</i>	<b>3.6223</b>	(0.20, 7.05)	0.235726
<i>TGM2</i>	<b>8.2547</b>	(0.00001, 23.13)	0.342098
<i>TNFRSF1A</i>	0.7435	(0.03, 1.46)	0.780531

### Supplemental Table 3.

STAT3 target genes upregulated in IBD patient colon biopsy samples. Comparison of STAT3 target gene expression in colons of IBD patients and controls matched for anatomical site, age and gender. Fold change is presented in red for genes expressed two-fold or higher in IBD, blue for genes expressed two-fold or lower in IBD, and black for less than two-fold change.  $n = 3$  control patients and 4 IBD patients.  $P$ -values with  $p < 0.05$  are shown in red.