Supporting Information

Deficiency in the anti-apoptotic protein DJ-1 promotes intestinal epithelial cell apoptosis and aggravates inflammatory bowel disease via p53

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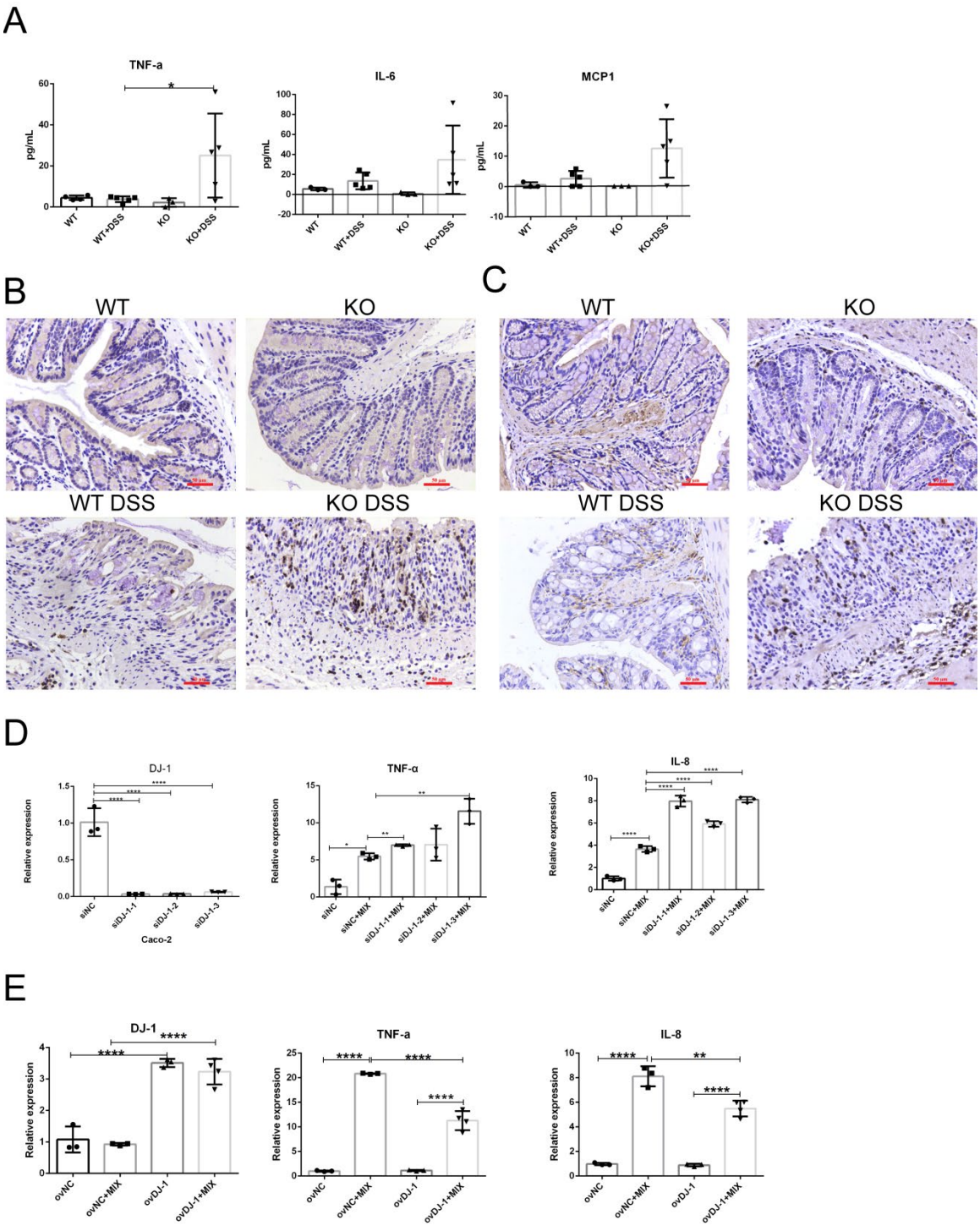
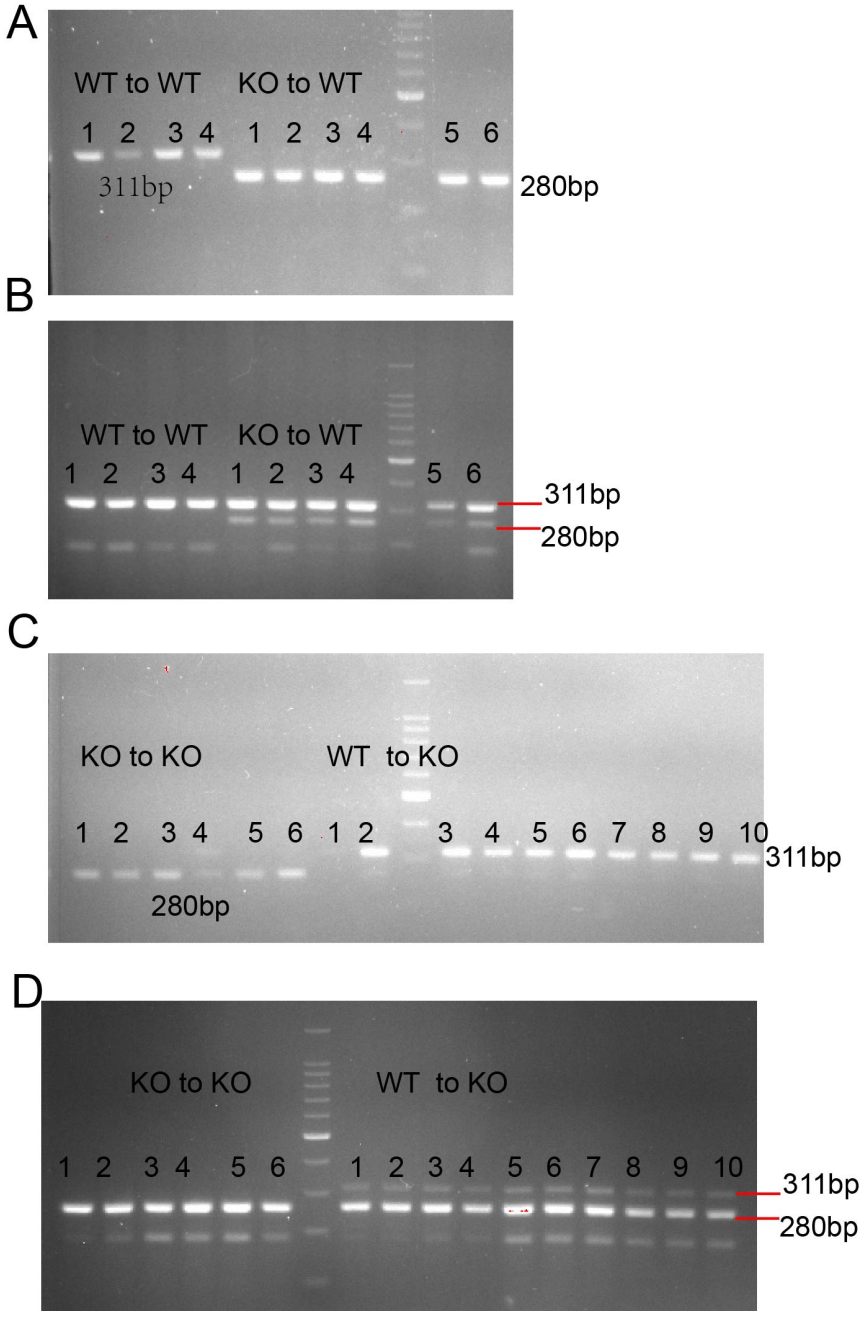


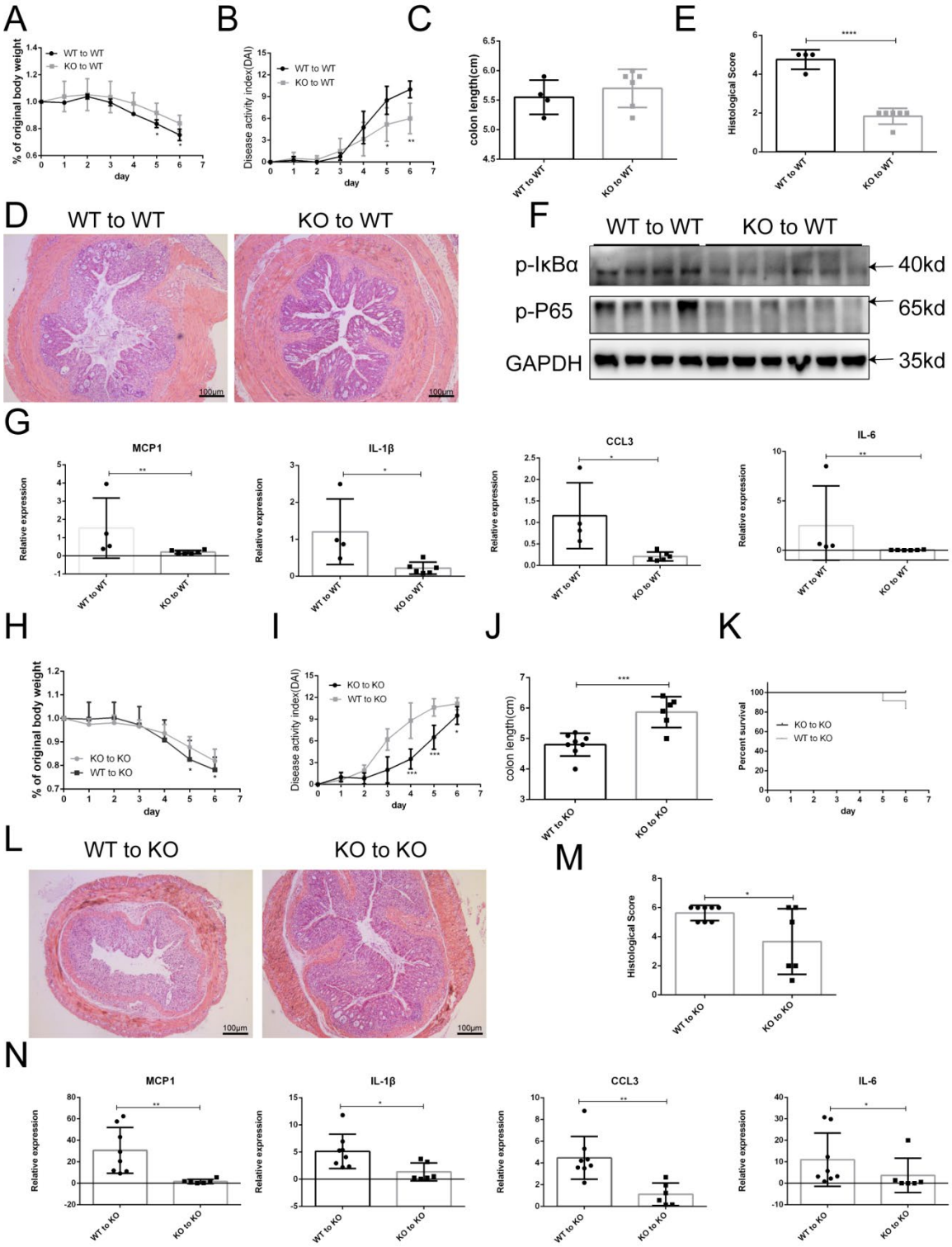
Supporting Figure 1: DJ-1 deficiency contribute to more inflammatory responses in colonic tissues. (A) Relative cytokine levels in mice serum were measured by flow cytometry. Values are expressed as the mean ±SD,**P* <0.05. (B) Representative IHC of the neutrophil infiltration marker myeloperoxidase (MPO) staining in colonic sections from the mice without or after DSS administration (original magnification, 200×). (C) Representative IHC of F4/80 in colonic

sections from the mice treated as in A (original magnification, 200×). (D and E) mRNA expression of cytokine and chemokine in Caco-2 cell after knock down (D) or overexpression (E) of DJ-1 for 24 h, followed by MIX treatment for another 24 h. Values are expressed as the mean ± SD. $^{**}P < 0.01$, $^{***}P<0.001$.



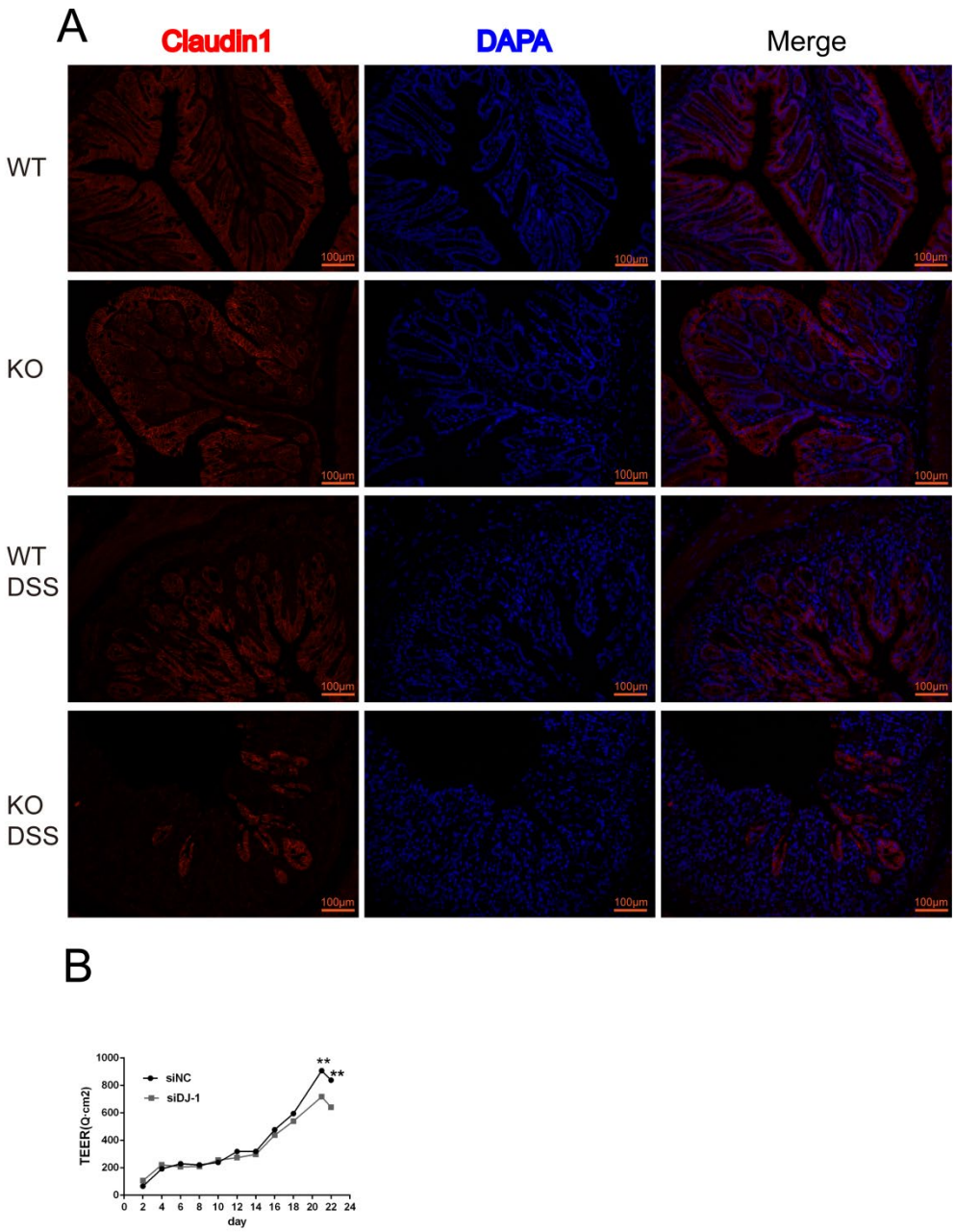
Supporting Figure 2： A: PCR analysis of whole blood cells DNA from WT recipient mice 6 week after radiation demonstrated the bone marrow were successful rebuild in WT recipient. B: PCR analysis of liver tissue from WT recipient mice 6 week after radiation. C: PCR analysis of whole blood cells DNA from KO recipient mice 6 week after radiation demonstrated the bone marrow were successful rebuild in KO recipient. D: PCR analysis of liver tissue from KO

recipient mice 6 week after radiation.

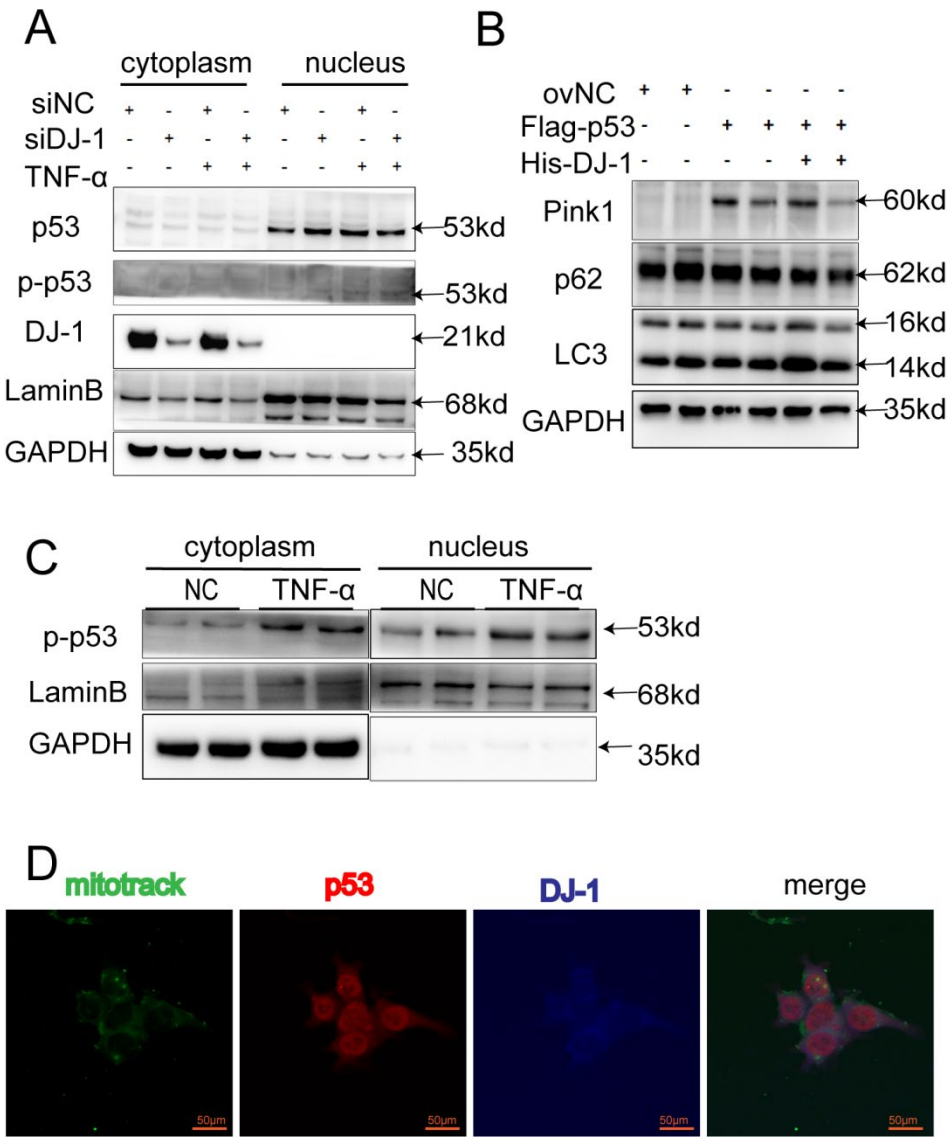


Supporting Figure 3: Absence of DJ-1 in hematopoietic cells is essential for colitis in the

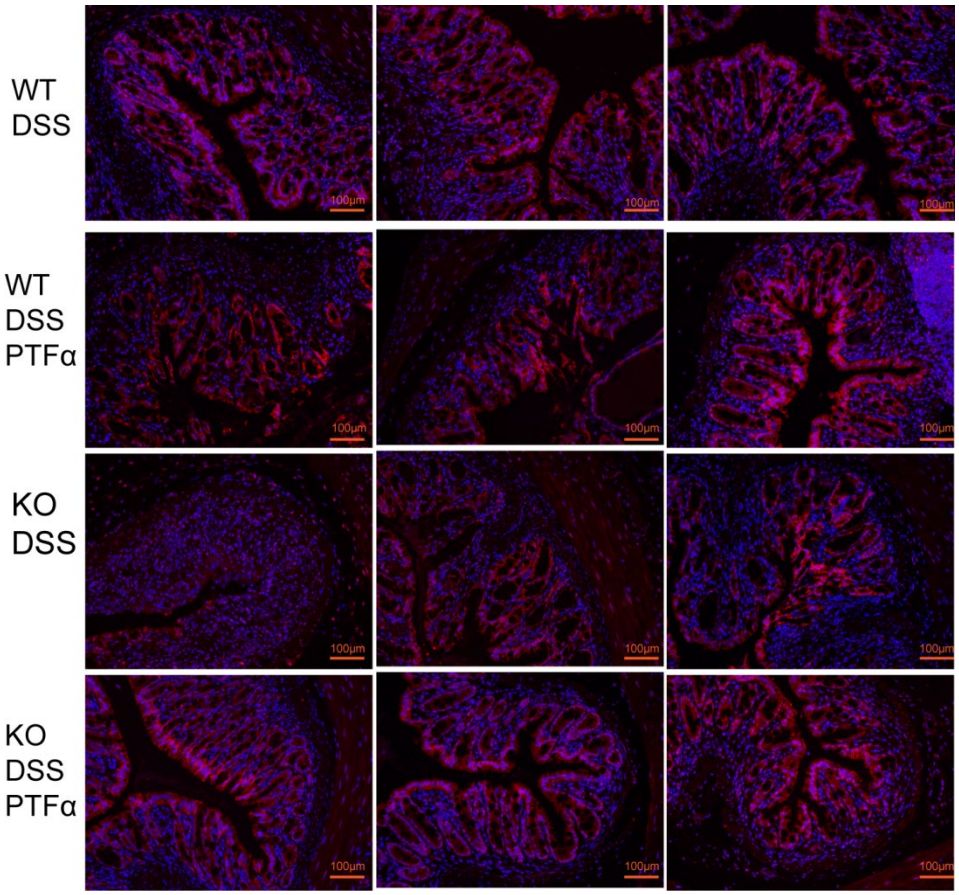
DSS-induced colitis. (A-G) The WT recipient mice received Wild-type bone marrow (WT to WT, *n*=4) and DJ-1 KO bone marrow (KO to WT, *n*=6) mice were treated with 3.5% DSS for 6 days. Body weight (A) and DAI (B) were scored daily. Mice were killed on day 6 and colon lengths (C) were measured. Values are expressed as the mean ± SD. **P < 0.01, ***P<0.001. Histological changes (D) in colon tissues from those mice were examined by hematoxylin and eosin staining. Semiquantitative scoring of histopathology (E) (original magnification, 50×) was then performed as described in the Experimental Procedures. (G) Relative mRNA levels of cytokine and chemokine in colon. (F) Western blotting of Iκbα-NF-κb protein expression in colon from mice after DSS administration. (H-N) The KO recipient mice received Wild-type bone marrow (WT to KO, *n*=10) and DJ-1 KO bone marrow (KO to KO, *n*=6) mice were treated with 3.5% DSS for 6 days. Body weight (H), DAI scores (I), colon lengths (J) and survival rate (K) were measured. Histological changes (L) (original magnification, 50×) by HE and Semiquantitative scoring of histopathology (M) were then performed. (N) Relative mRNA levels of cytokine and chemokine in colon. Values are expressed as the mean ± SD. ***P* < 0.01, ***P<0.001.



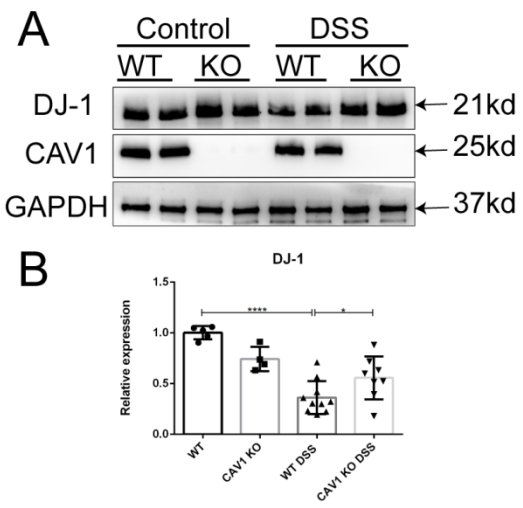
Supporting Figure 4: (A) The immunostaining of cell tight junction protein Claudin1 (Red) staining in colonic sections from the mice treated as in Figure 2 (original magnification, ×200). (B) Caco-2 cells were cultured on the transwell inserts and DJ-1 were knock down by siRNA every week, when the TEER reach 800Q*cm^2, transwell inserts were inserted into multiple plate wells containing RAW264.7 cells. To imitate the colon inflammation, LPS (1ug/ml) was added to the basolateral compartment of this co-culture system for 24 h.



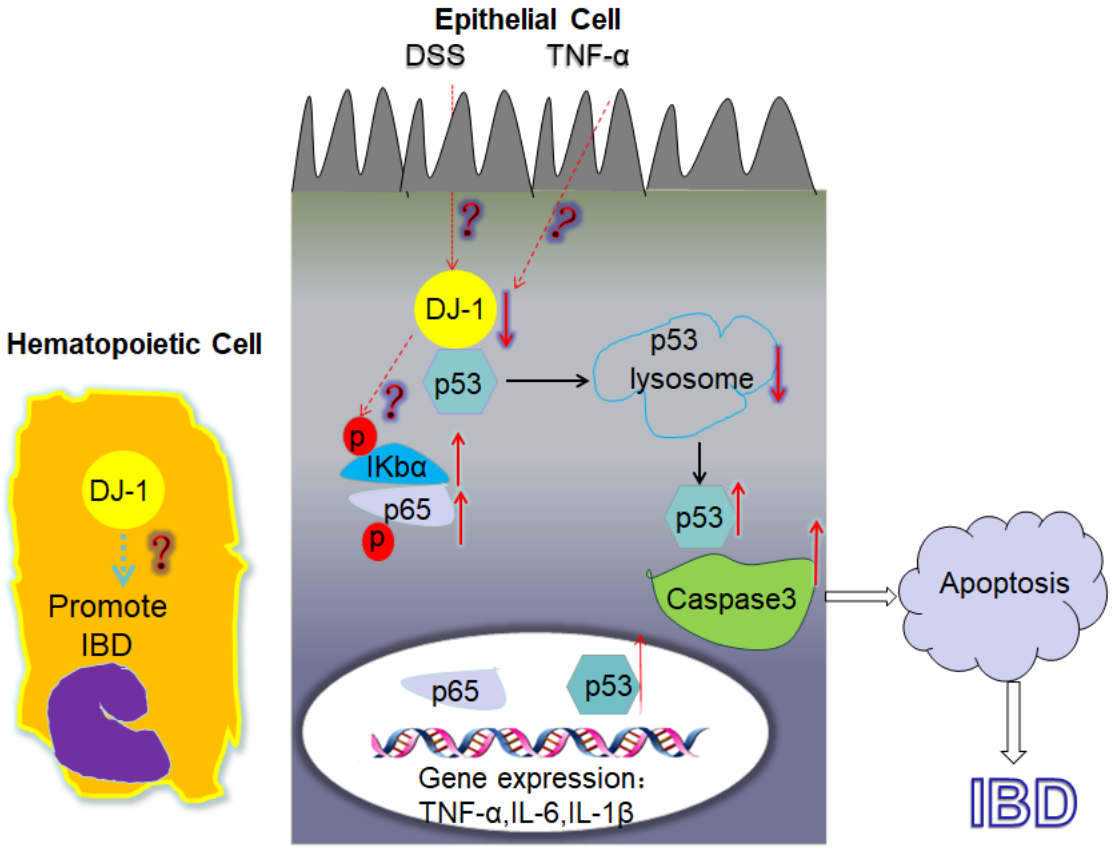
Supporting Figure 5: (A) DJ-1 was knocked down in HCT116 cells by siRNA for 24 h and then the cells were stimulated with 100 ng/mL TNF-α for 24 h. Protein were isolated from cytoplasm and nucleus. (B) 293T cell infected with Flag-tagged p53 and his-tagged DJ-1, and stimulated with 100 ng/mL TNF-α for 24 h.(C) HCT116 cell were stimulated with 100 ng/mL TNF-α for 24 h，Protein were isolated from cytoplasm and nucleus. (D) Representative immunofluorescent staining of mitotrack (green), DJ-1-specific antibody (anti-DJ-1, blue) and p53 antibody (anti-p53, red) (original magnification, 400×) in HCT116 cell treated with TNF-α for 24 h.



Supporting Figure 6: PFT-α was administered by intraperitoneal injection into WT mice (*n*=8) and DJ-1$^{-/-}$ mice (*n*=6), and the control group (WT *n*=5，KO *n*=7) was intraperitoneally injected with DMSO. Claudin1 (red) staining of colon sections of mice treated as before. Nuclei were stained with DAPI (blue) (original magnification, 200×).



Supporting Figure 7: (A-B) Wild-type (*n*=10) and CAV1 KO (*n*=8) mice were treated with 3.5% DSS for 6 days. The DJ-1 protein level (A) and mRNA (B) level were measured in the colon tissue.



Supporting Figure 8: Mechanism schematic diagram of DJ-1 regulating IBD.

Table S1: Antibodies for Western blot

Primary antibody	Company	Cat#
DJ-1	abcam	#18257（WB） # 76008 (IP)
p53	abcam	#26 (WB for mouse) #3133(for human)
p-P65	CST	#3039
p-JNK	CST	#4668
Actin	CST	#3077
GAPDH	CST	#5174
p-Iκbα	CST	#9246
p-IKKα/β	CST	#2697
Cleaved Caspase-3	CST	#9661
Tubulin	CST	#2148
Cleaved Caspase-7	CST	#8438
BAX	CST	#2772
PUMA	CST	#4976

Table S2: Primers used for qPCR

Gene	Primer sequence
M DJ-1 F	AAAACGCAGGGACCATACGAT
M DJ-1 R	AGGCGACTCAGATAAATTCTGTG
M MCP1 F	TTAAAAACCTGGATCGGAACCAA
M MCP1 R	GCATTAGCTTCAGATTTACGGGT
M CCL3 F	TTCTCTGTACCATGACACTCTGC
M CCL3 R	CGTGGAATCTTCCGGCTGTAG
M IL-8 F	TGTTGAGCATGAAAAGCCTCTAT
M IL-8 R	AGGTCTCCCGAATTGGAAAGG
M TNF-α F	CAGGCGGTGCCTATGTCTC
M TNF-α R	CGATCACCCCGAAGTTCAGTAG
M GAPDH F	AGGTCGGTGTGAACGGATTTG
M GAPDH R	GGGGTCGTTGATGGCAACA
M IL-6 F	TAGTCCTTCCTACCCCAATTTCC
M IL-6 R	TTGGTCCTTAGCCACTCCTTC
M IL-1β F	CTCACAAGCAGAGCACAAGC
M IL-1β R	TCCAGCCCATACTTTAGGAAGA
H GAPDH F	TCAACGACCACTTTGTCAAGCTCA
H GAPDH R	GCTGGTGGTCCAGGGGTCTTACT
H TNF-α F	GAGGCCAAGCCCTGGTATG
H TNF-α R	CGGGCCGATTGATCTCAGC
H DJ-1 F	AACCGGAAGGGCCTGATAG
H DJ-1 R	GCAAGAGGGTGTGTTGTAACT
H IL-8 F	ACTGAGAGTGATTGAGAGTGGAC
H IL-8 R	AACCCTCTGCACCCAGTTTTC