Supplemental Materials

Selenoprotein S maintains intestinal homeostasis in ulcerative colitis by inhibiting necroptosis of colonic epithelial cells through modulation of macrophage polarization

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Table S1. Sequences of oligonucleotide primers for qRT-PCR

Gene Names	Forward Primer (5'→3')	Reverse Primer (5'→3')
GPX1	AGTCCACCGTGTATGCCTTC	GTGTCCGAACTGATTGCACG
GPX2	CCTACCGGCCATTTCCTTTA	GACATCTCCCAGAAGGGTTTAG
GPX3	CCCTTAGTGCATTCAGGCTTAG	GATACCAGTGGACAGAGTGAGA
GPX4	GCCGTCTGAGCCGCTTACTTA	CGTCGATGTCCTTGGCTGAG
Txnrd1	ACAGCGAGGAGACCATAGA	CCACGGTCTCTAAGCCAATAG
Txnrd2	CATCTTCTGGCTGAAGGAGTC	ACAGTGGTATCCAGTCCAATTC
Txnrd3	GAAGGACCGGAAACAGTAGAA	CGATCTTCTCCAGCCCTATTT
Dio1	TGGTTTGTCCTGAAGGTCCG	GTCAGGTGCAACTGCCAAAG
Dio2	TCCTACAGTCACAATGCTACAC	GGTTAGTGTACCTGCCTCTTAC
Dio3	CTCGAAACAGCGCCTAAAGTA	GAAGTCCATCCCTTACCATGTC
SelN	ATGATCTGTCTGCCCAACGG	GTCCTTCCCTCAGGAACTGC
SelK	GTGCAGGAGAAGATGGTGTATG	TGCGTATCAGTCAGGCTAGA
SelW	GCCGTTCGAGTCGTGTATTG	GCCACAAATGTCCAGGCATC
SelT	GAAGTTATGAGCTGAGGGAAGG	CAGTGTGTGTCCAGTAGTCAAG
SelH	GAGCATTGTACGAGCTGACG	AGTCCAGAGTTCAACACGGC
SelM	CCACCACCAACTACCGACC	GGCCTTCACCTCCTTTAGGC
SelF	GAAGTAGCACCACAGTCCATAA	GGTGAGAGCAACAAGTGTAGAA
SelI	GGAGTGTTCTGCCGTTACTT	GAGGAACCGCCACAATTAGA
SelV	GCTATGGCCTTCGGTACATTAT	CTGTAACCTGGGTAGCTCTTTC
SelP	GCCAATAAAACTGCGGAGCC	GCAAGCCTTCACTTGCTGTG
SelO	CACTATCGACTATGGACCCTTTG	GGGCTGCTTACTGTATGTGTAG
SelR	AGTTCGTCCCTAAAGGCAAAG	AGGTTCTGACACTAACGTGGG
SelS	CTTTGCGAGGAGGTGGTTAT	GTCAGAGCGACACTAACAAGAG
Sephs2	CCCACCAATGGCTGGATAAT	GCAGCAGTCCTGTTTAGAGTAG
MIG	AACGTTGTCCACCTCCCTTC	CACAGGCTTTGGCTAGTCGT
TNF-α	CCTGTAGCCCACGTCGTAGC	AGCAATGACTCCAAAGTAGACC
iNOS	CAACAGGGAGAAAGCGCAA	CTCACATACTGTGGACGGGT
IL-6	ATCCAGTTGCCTTCTTGGGACTGA	TTGGATGGTCTTGGTCCTTAGCCA
IL-12	GGAAGCACGGCAGCAGAATA	AACTTGAGGGAGAAGTAGGAATGG
MCP1	CTCACCTGCTGCTACTCATTC	TTACGGCTCAACTTCACATTCA
Arg1	TGTGAAGAACCCACGGTCTG	ACGTCTCGCAAGCCAATGTA
Fizz-1	AACTGCCTGTGCTTACTCGT	CAAGAAGCAGGGTAAATGGGC
IL-4	ACTTGAGAGAGATCATCGGCATTT	AGCACCTTGGAAGCCCTACAG
IL-10	ACTTGGGTTGCCAAGCCTTA	GACACCTTGGTCTTGGAGCTTA
MRC1	GTCAGAACAGACTGCGTGGA	AGGGATCGCCTGTTTTCCAG
CCL24	CCCTGAACTTGGACATAGGGG	AAAATGCTGCTGTTGAAATCCT

Gene Names	Forward Primer (5'→3')	Reverse Primer (5'→3')
Uba52	TTTCTCTTCAACGAGGCGGC	GGTATGGCCGCACTTCTTCT
YAP	CCCTCGTTTTGCCATGAACC	TCCGTATTGCCTGCCGAAAT
MST1	AGACCCGAATGTTGAGCGAG	TTTCAAACACCACCTGGTCTGG
LATS1	CGTCCCCTGCAGGTTAGTTA	AGGTCTTCCTACATCCGCCT
NF-κB-p105	GGTCAAAATTTGCAACTATGTGGG	GCGTGCAGGTGGATGTTTTT
NLRP3	CAAGGCTGCTATCTGGAGGAAC	TCGCAGCAAAGATCCACACA
ASC	CCATCCTGGACGCTCTTGAA	GTGAGCTCCAAGCCATACGA
Caspase1	ACTGCTATGGACAAGGCACG	GCAAGACGTGTACGAGTGGT
GSDMD	ATCAAGGAGGTAAGCGGCAG	CCTTCTCCCATGCCTGACAA
IL-1β	TGGACCTTCCAGGATGAGGACA	GTTCATCTCGGAGCCTGTAGTG
IL-18	GACAGCCTGTGTTCGAGGATATG	TGTTCTTACAGGAGAGGGTAGAC
CAT	TCACTGACGAGATGGCACAC	ATCGAACGGCAATAGGGGTC
GST	GAACTATACAGGTCGGCGATG	AGCTTGGGATGAAAGACTCCG
SOD1	GCTTCTCGTCTTGCTCTCTCTG	TCTGCTCGAAGTGGATGGTT
SOD2	TAACGCGCAGATCATGCAGCTG	AGGCTGAAGAGCGACCTGAGTT
FADD	CCCCTCAGCACACTTGAACT	GTGACTTGCCACAGGACAGT
RIPK1	GGCCATAGAAGGCATGTGCT	GCCACACCAAGATCGGCTAT
RIPK3	GGCTGGCACTCCTCAGATTC	GTGCCGTGTCTTCCATCTCC
MLKL	CCGGACAGCAAAGAGCACTA	CTGCCAGAAAGACTCCTACCG
Claudin1	TTTGGCCAGGCCCTCTTTAC	GGAGCACCTTATCCCCGTTT
Occludin	GAGTTTCAGGTGAATGGGTCAC	AGGCAAATATGGCGATGCAC
ZO-1	TTCCGGGGAAGTTACGTGC	CCATTGCTGTGCTCTTAGCG
ACTB	CCTCTATGCCAACACAGTGC	CCTGCTTGCTGATCCACATC

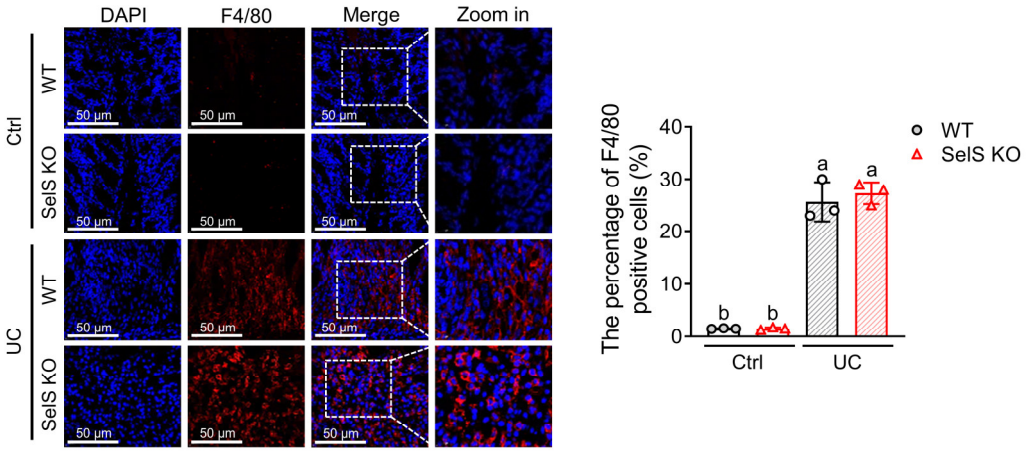

Figure S1. SelS deletion enhances inflammatory response of the colon tissue in UC mice independent of macrophage infiltration. Immunofluorescence staining and quantification analysis of F4/80 in the colon tissue of WT and SelS KO mice in Ctrl and UC groups, n = 3. Scale bar, 50 μm.

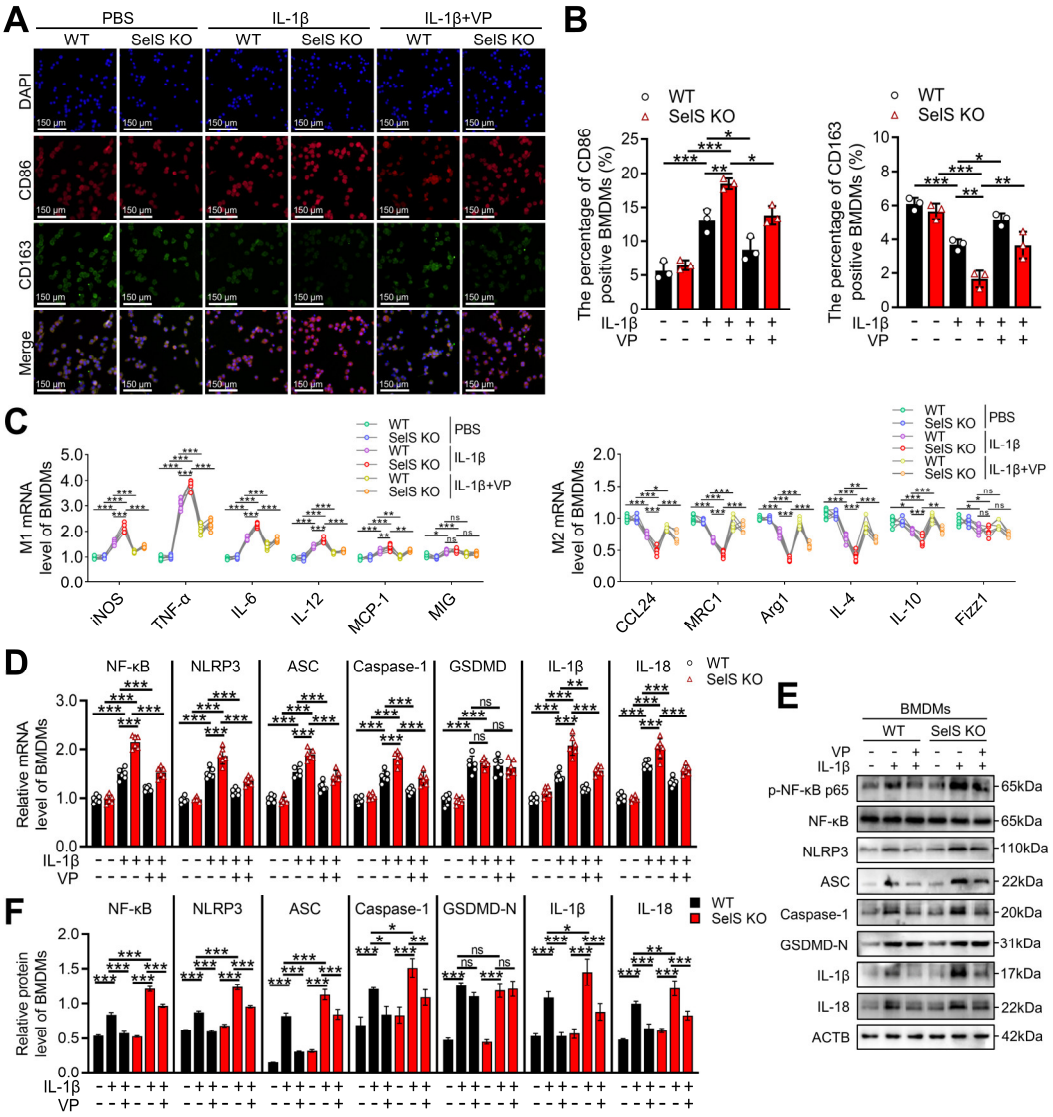

Figure S2. SelS deficiency in BMDMs promotes YAP-mediated M1 polarization and NF-κB/NLRP3 pathway activation. (A-E) BMDMs transfected with siNC or siSelS were pretreated with or without VP (0.32 μM) for 30 min before PBS or IL-1β (100 ng/mL) stimulation for 6 h. **(A)** Immunofluorescence staining and quantitative analysis of CD86 and CD163 in BMDMs, n = 3. Scale bar, 150 μm. **(B)** M1 and M2 related mRNA expressions in BMDMs, n = 6. **(C)** mRNA expressions related to the NF-κB/NLRP3 pathway and GSDMD in BMDMs, n = 6. **(D-E)** Protein levels related to the NF-κB/NLRP3 pathway and GSDMD in BMDMs, n = 3.

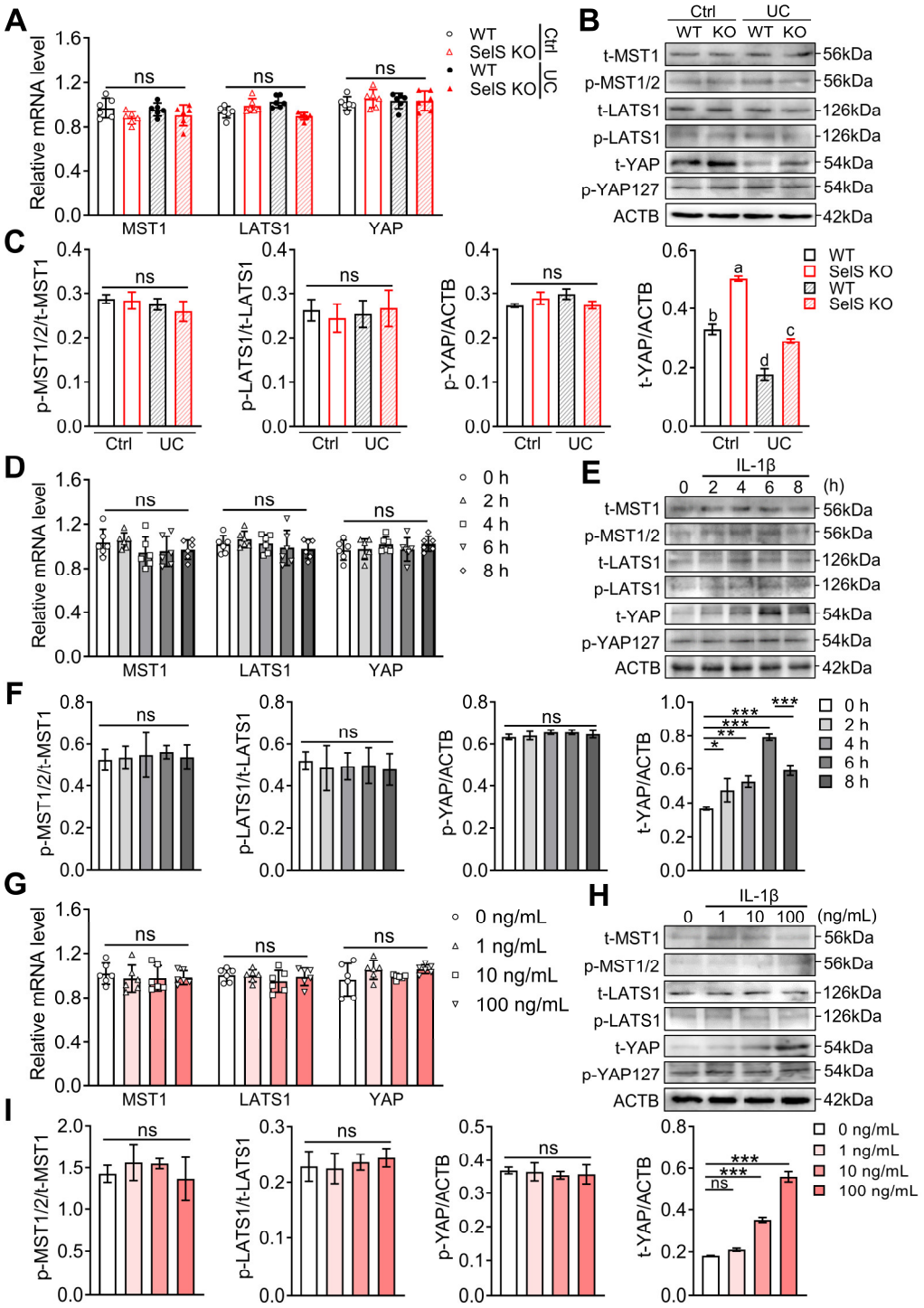

Figure S3. IL-1β increases YAP protein level independent of the traditional Hippo pathway. **(A)** mRNA expressions of MST1, LATS1, and YAP in the colon tissue of WT and SelS KO mice in Ctrl and UC groups, n = 6. **(B-C)** Protein levels of MST1/2, LATS1, and YAP in the colon tissue of WT and SelS KO mice in Ctrl and UC groups. t, total; p, phosphorylated, n = 3. **(D-F)** J774.1 were treated with IL-1β (100 ng/mL) for the indicated times. **(D)** mRNA expressions of MST1, LATS1, and YAP in J774.1, n = 6. **(E-F)** Protein levels of MST1, LATS1, and YAP in J774.1, n = 3. **(G-I)** J774.1 were stimulated with indicated concentrations of IL-1β for 6 h. **(G)** mRNA expressions of MST1, LATS1, and YAP in J774.1, n = 6. **(H-I)** Protein levels of MST1, LATS1, and YAP in J774.1, n = 3.

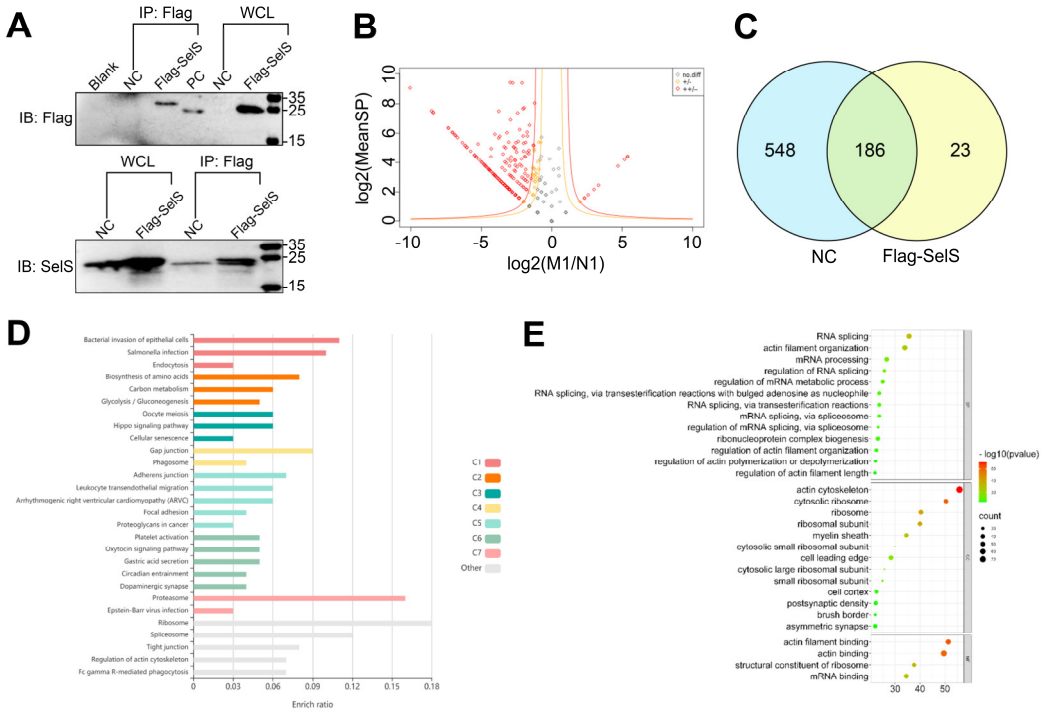

Figure S4. CoIP-MS screens the interacting proteins of SelS in Hepa1-6. (A) The IB of Flag or SelS was followed by IP with anti-Flag antibody in WCL, n = 3. Blank, blank control; NC, transfection of empty plasmids; Flag-SelS, transfection of Flag-SelS overexpression plasmid; PC, positive control; WLC, whole-cell lysates. **(B)** Analysis of differentially expressed proteins in IP by mass spectrometry. **(C)** Venn diagram showing the protein composition in IP of NC group and Flag-SelS group. **(D)** KEGG pathway enrichment of differential proteins in IP of NC group and Flag-SelS group. **(E)** GO function annotation of differential proteins in IP of NC group and Flag-SelS group.

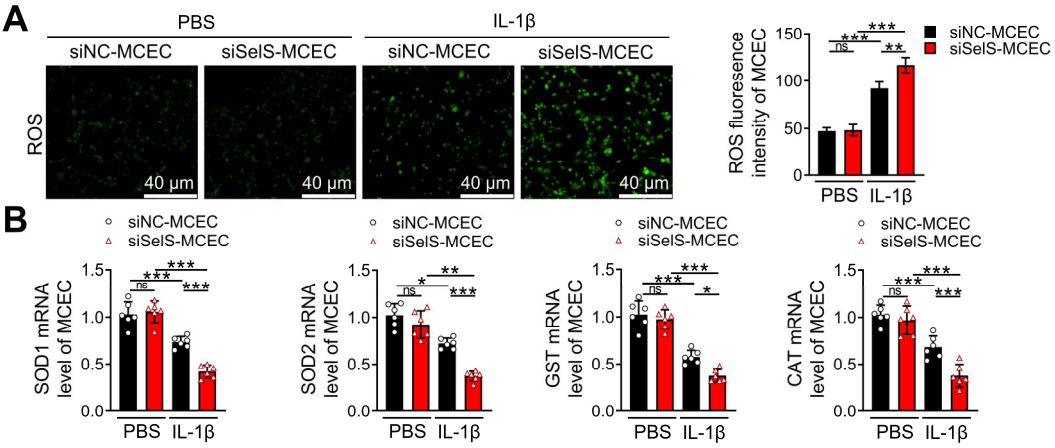

Figure S5. SelS deficiency in MCECs promotes oxidative stress. (A) Representative images of ROS levels in siNC and siSelS MCECs under IL-1β (100 ng/mL) stimulation, n = 3. Scale bar, 40 μm. **(B)** mRNA expressions of antioxidase in siNC and siSelS MCECs under IL-1β (100 ng/mL) stimulation, n = 6.

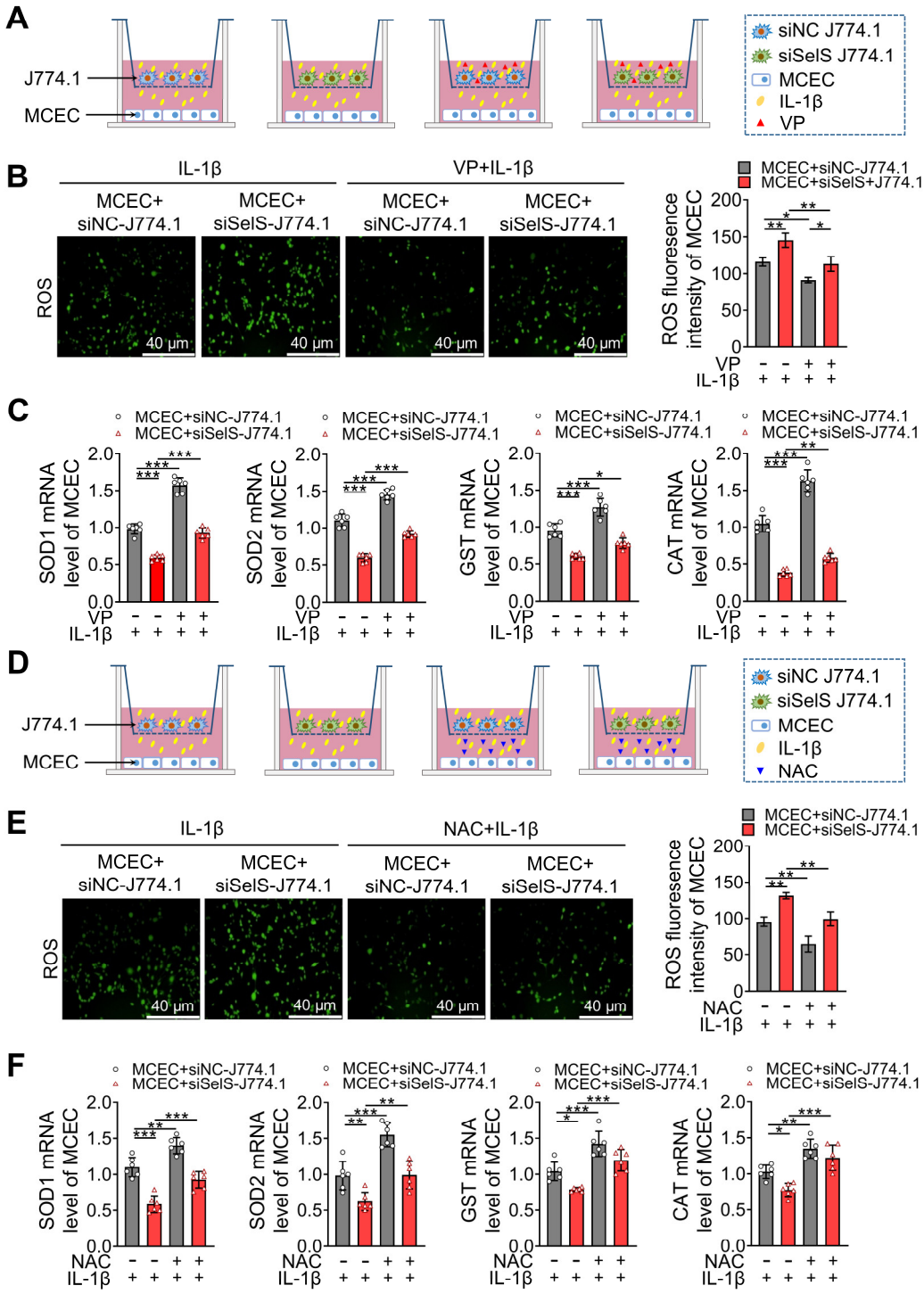

Figure S6. SelS deficiency in J774.1 increases oxidative stress in MCECs by promoting YAP expression. (A-C) MCECs were co-cultured with 0.32 μM VP-pretreated siNC or siSelS J774.1 under IL-1β (100 ng/mL) stimulation. **(A)** Co-culture pattern diagram of MCECs and J774.1. **(B)** Representative images of ROS levels in MCECs, n = 3. Scale bar, 40 μm. **(C)** mRNA expressions of antioxidase in MCECs, n = 6. **(D-F)** 1 mM NAC-pretreated MCECs were co-cultured with siNC or siSelS J774.1 under IL-1β (100 ng/mL) stimulation. **(D)** Co-culture pattern diagram of MCECs and J774.1. **(E)** Representative images of ROS levels in MCECs, n = 3. Scale bar, 40 μm. **(F)** mRNA expressions of antioxidase in MCECs, n = 6.

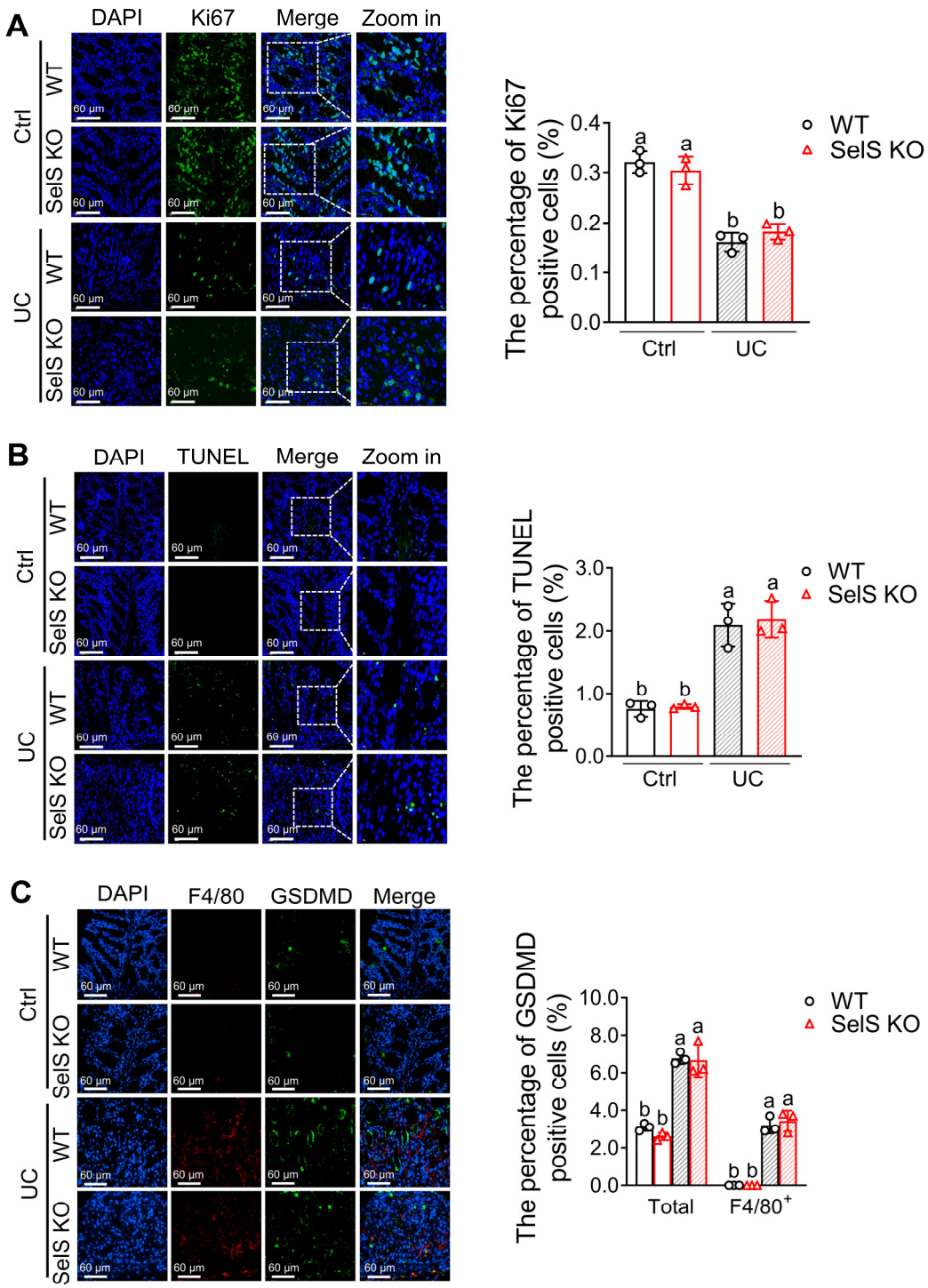

Figure S7. SelS deletion promotes colon injury in UC mice independent of cell proliferation, apoptosis, and pyroptosis. (A) Immunofluorescence staining and quantitative analysis of Ki67 in the colon tissue of WT and SelS KO mice in Ctrl and UC groups, n = 3. Scale bar, 60 μm. **(B)** TUNEL staining and quantitative analysis in the colon tissue of WT and SelS KO mice in Ctrl and UC groups, n = 3. Scale bar, 60 μm. **(C)** Immunofluorescence staining and quantitative analysis of F4/80 and GSDMD in the colon tissue of WT and SelS KO mice in Ctrl and UC groups, n = 3. Scale bar, 60 μm.

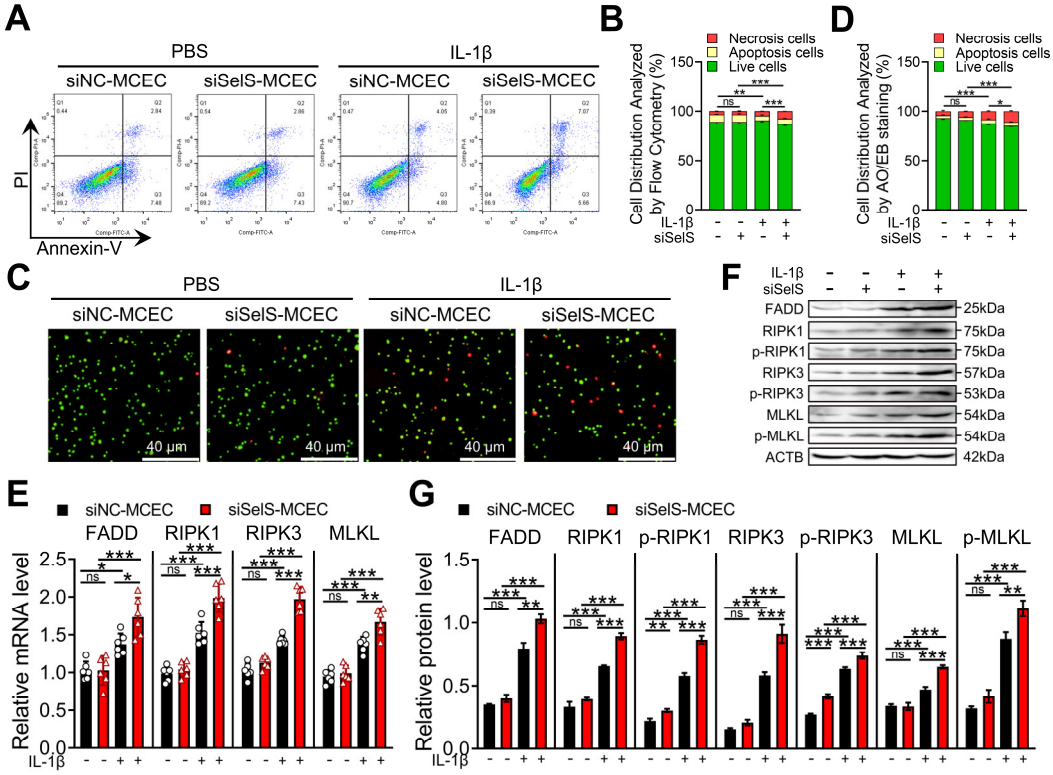

Figure S8. SelS deficiency in MCECs promotes necroptosis. (A-G) siNC and siSelS MCECs were treated with IL-1β (100 ng/mL). **(A-B)** Flow cytometry detection and quantification analysis for FITC/PI staining in MCECs, n = 3. **(C-D)** AO/EB staining and quantification analysis in MCECs, n = 3. Scale bar, 40 μm. **(E)** mRNA expressions related to necroptosis in MCEC, n = 6. **(F-G)** Protein levels related to necroptosis in MCECs, n = 3.

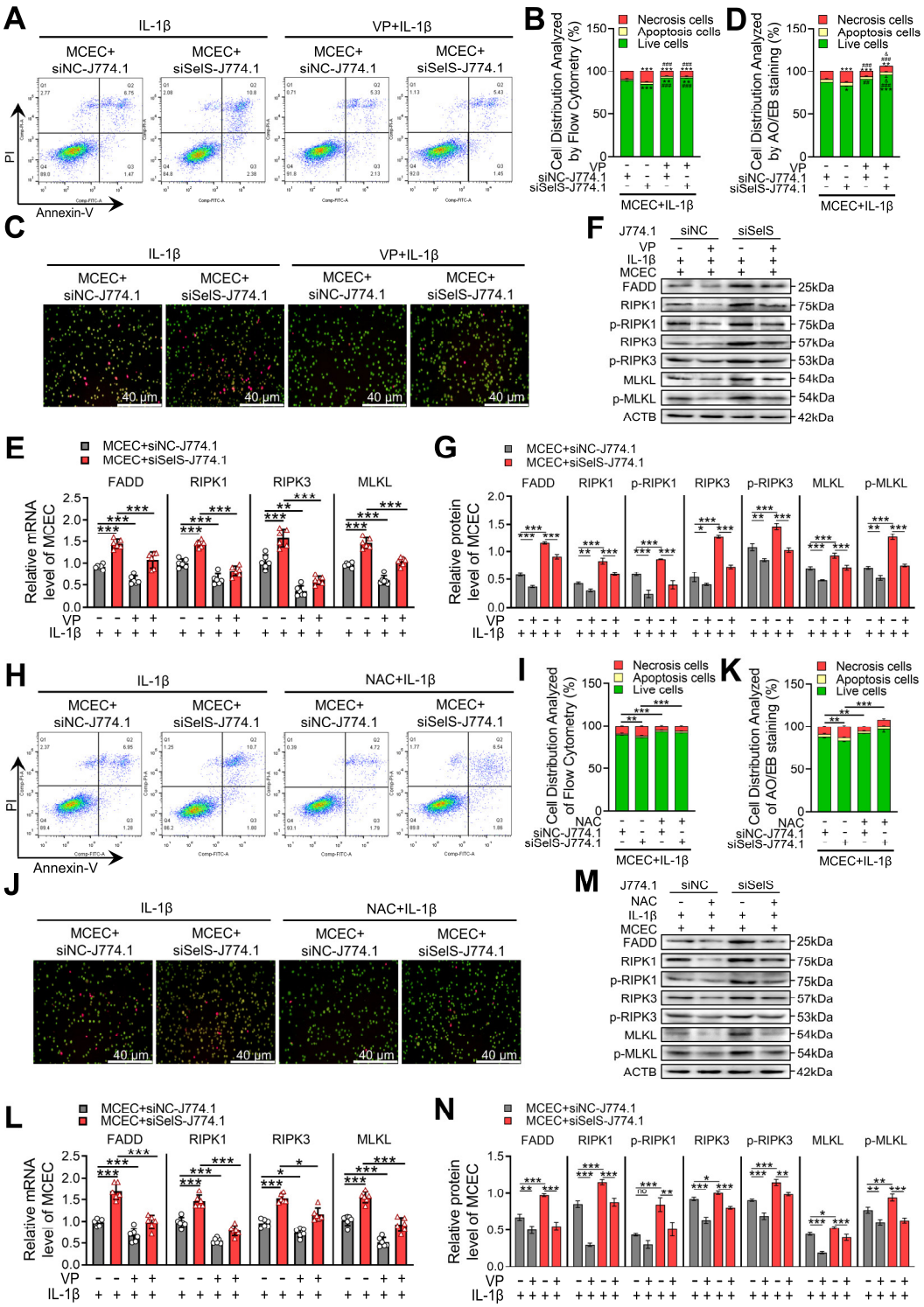

Figure S9. SelS deficiency in J774.1 promotes YAP expression to induce ROS-dependent necroptosis in MCECs. (A-G) MCECs were co-cultured with 0.32 μM VP-pretreated siNC or siSelS J774.1 under IL-1β (100 ng/mL) stimulation. **(A-B)** Flow cytometry detection and quantification analysis for FITC/PI staining in MCECs, n = 3. **(C-D)** AO/EB staining and quantification analysis in MCECs, n = 3. Scale bar, 40 μm. **(E)** mRNA expressions related to necroptosis in MCECs, n = 6. **(F-G)** Protein levels related to necroptosis in MCECs, n = 3. **(H-N)** 1 mM NAC-pretreated MCECs were co-cultured with siNC or siSelS J774.1 under IL-1β (100 ng/mL) stimulation. **(H-I)** Flow cytometry detection and quantification analysis for FITC/PI

staining in MCECs, n = 3. **(J-K)** AO/EB staining and quantification analysis in MCECs, n = 3. Scale bar, 40 μm. **(L)** mRNA expressions related to necroptosis in MCECs, n = 6. **(M-N)** Protein levels related to necroptosis in MCECs, n = 3.

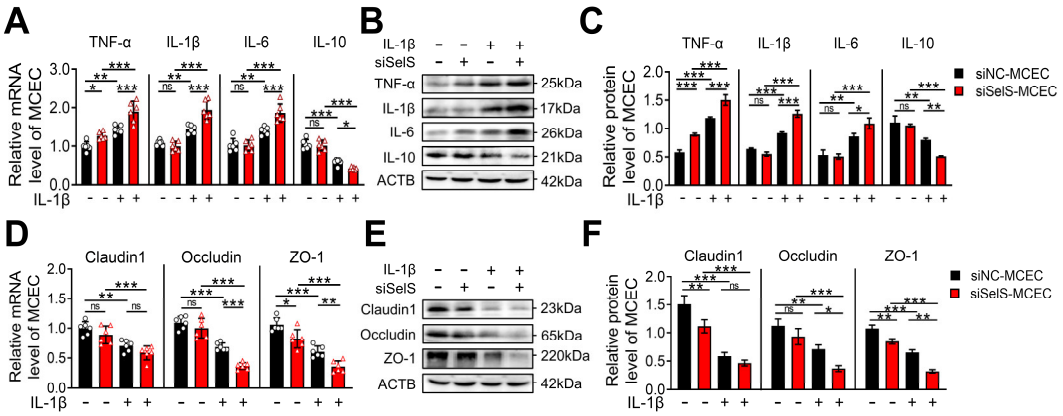

Figure S10. SelS deficiency in MCECs promotes inflammatory response and tight junction impairment. (A-G) siNC and siSelS MCECs were treated with IL-1β (100 ng/mL). **(A)** mRNA expressions related to inflammatory cytokines in MCECs, n = 6. **(B-C)** Protein levels related to inflammatory cytokines in MCECs, n = 3. **(D)** mRNA expressions related to tight junction in MCECs, n = 6. **(E-F)** Protein levels related to tight junction in MCECs, n = 3.

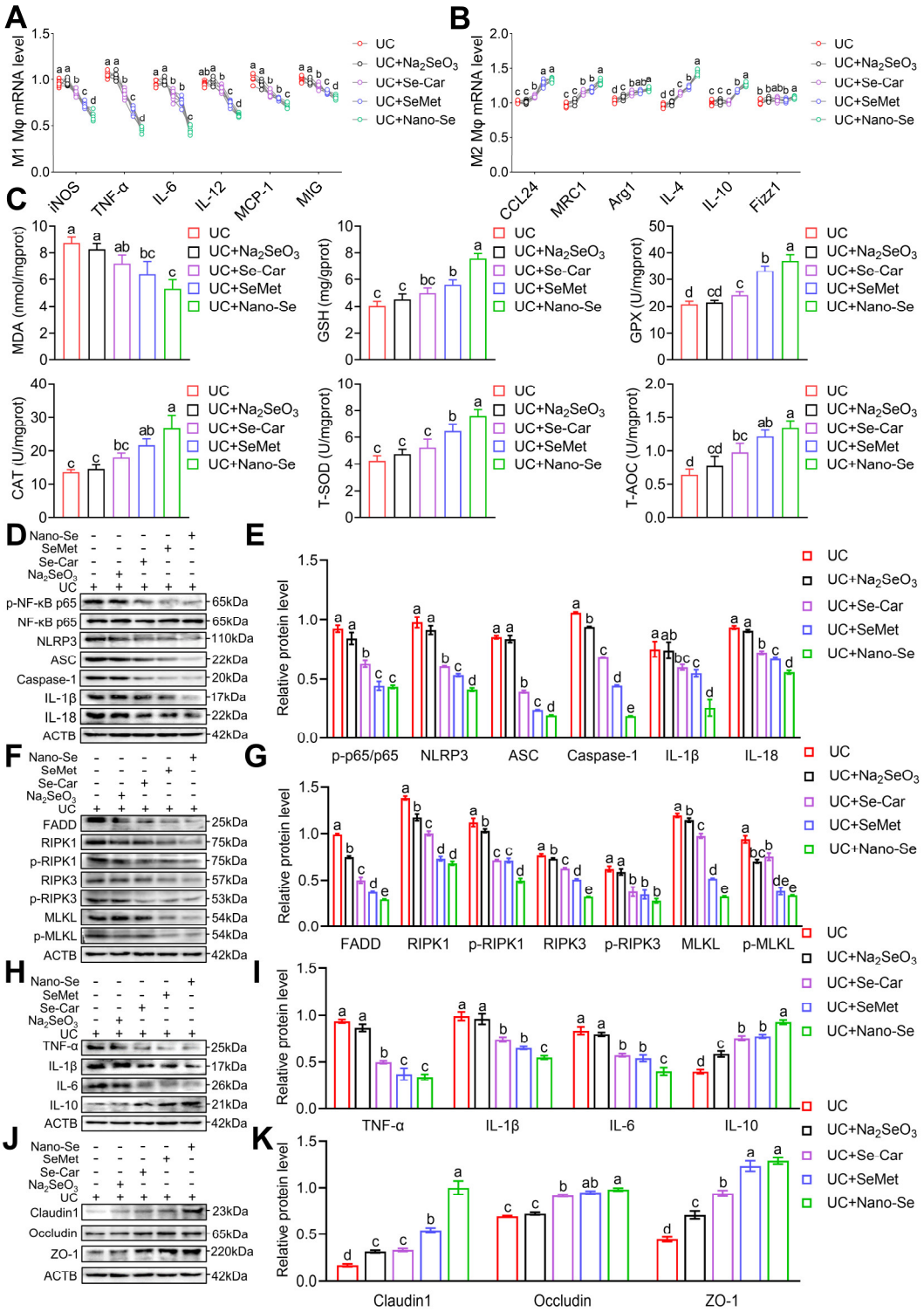

Figure S11. Selenium supplementation reverses macrophage polarization, oxidative stress, necroptosis, inflammatory response, and tight junctions in the colon tissue of UC mice. (A-G) WT mice with UC were given selenium supplements containing 2 mg/kg selenium. **(A-B)** mRNA expressions related to M1 and M2 in the colon tissue, n = 6. **(C)** Levels of pro-oxidant indicators and antioxidant markers in the colon tissue, n = 6. **(D-E)** Protein levels related to the NF-κB/NLRP3 pathway in the colon tissue, n = 3. **(F-G)** Protein levels related to necroptosis in the colon tissue, n = 3. **(H-I)** Protein levels related to inflammatory cytokine in the colon tissue, n = 3. **(J-K)** Protein levels related to tight junction in the colon tissue, n = 3.