Expanded View Figures

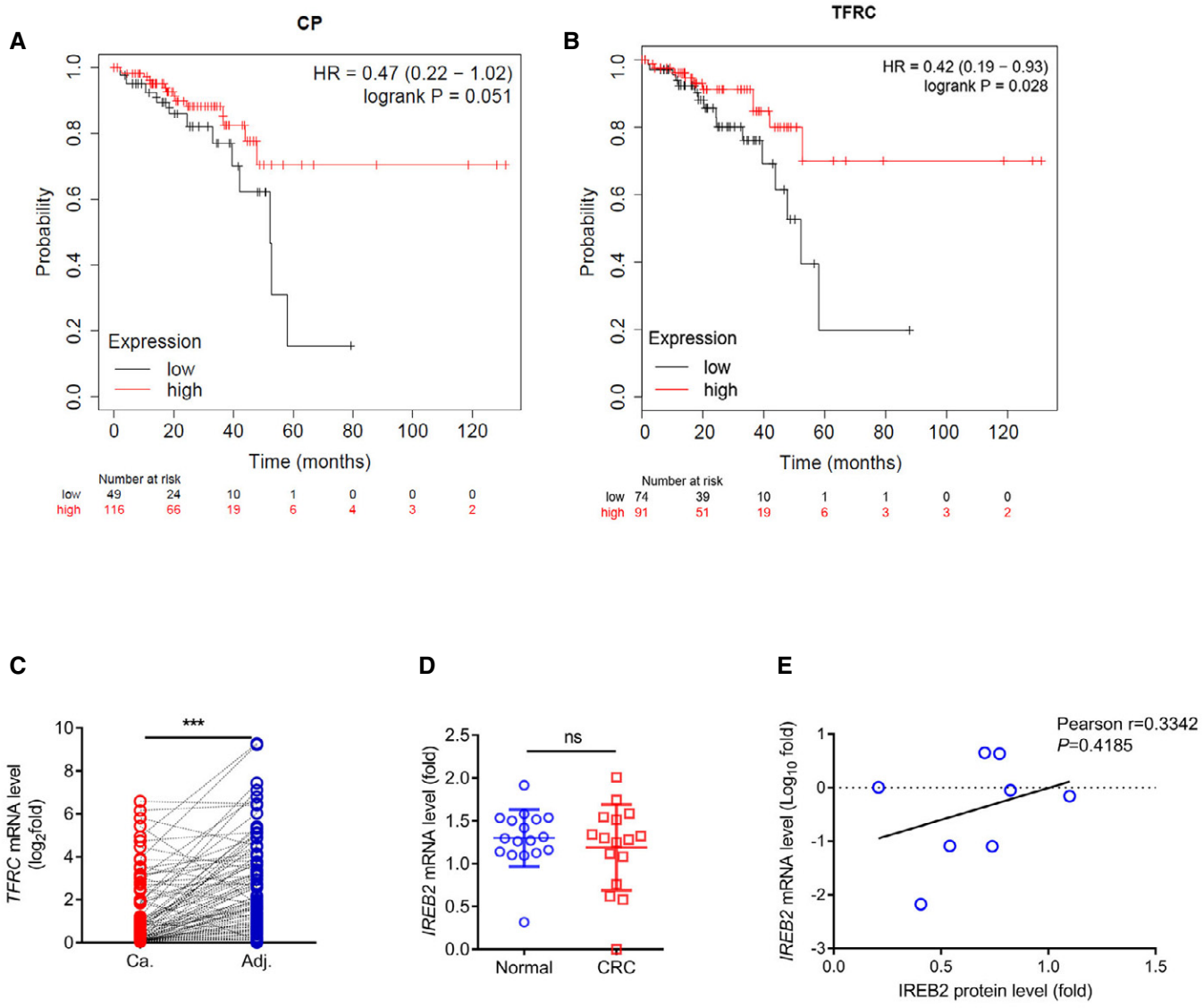


Figure EV1. Iron transportation signaling is impaired in colorectal cancers.

A, B Kaplan–Meier survival curves for patients with colon cancer containing low or high expression of *CP* (low expression of *CP*, $n = 49$ patients; high expression of *CP*, $n = 116$ patients) and *TFRC* (low expression of *TFRC*, $n = 74$ patients; high expression of *TFRC*, $n = 91$ patients) mRNA in the GSE dataset.

C RT–qPCR analysis of *TFRC* mRNA levels in colon tumors and matched adjacent normal tissues ($n = 101$ human samples, $^{***}P < 0.001$, two-tailed paired Student's *t*-test).

D The mRNA level of IREB2 in normal ($n = 17$) or colorectal cancer ($n = 16$) tissues from the GEO database (GDS4382) (mean ± s.e.m., ns, not significant ($P > 0.05$), two-tailed unpaired Student's *t*-test).

E The relationship of protein level and mRNA level of IREB2 in colon cancer ($n = 8$ samples, $P = 0.4185$, two-tailed Pearson correlation analysis).

Figure EV2. OTUD1 deubiquitinases and stabilizes IREB2.

A Confocal examination of OTUD1 and IREB2 colocalization in HEK293T cells ectopically expressing GFP-tagged OTUD1. The scale bars represent 10 μm.

B HEK293T cells were co-transfected with IREB2-FLAG and S-tagged OTUD1 and its truncations. At 24 h later, cell lysates were immunoprecipitated with S-protein agarose and analyzed by immunoblot with anti-FLAG antibody.

C Co-immunoprecipitation analysis of IREB2-FLAG together with OTUD1-GFP with treatment of hemin (100 μM) or DFO (100 μM).

D *In vivo* ubiquitination assay of IREB2. HEK293T cells were co-transfected with indicated plasmids and subjected to immunoprecipitation with anti-FLAG antibody followed by Western blot analysis.

E *In vitro* ubiquitination assay of IREB2. IREB2 was enriched by anti-FLAG beads and incubated with purified OTUD1 and OTUD1^{C320S} protein in deubiquitination buffer followed by Western blot analysis.

F Half-life analysis of IREB2 in the presence or absence of OTUD1 in CT26 cells. Cells were treated with CHX for indicated times and analyzed by Western blot (up), and the relative protein level of IREB2 was assessed by ImageJ software (down) (n = 3 biological replicates, mean ± s.e.m., ***P < 0.001, two-tailed unpaired Student's t-test).

G Analysis of endogenous IREB2 and TFRC level in wild-type (WT) or *Ireb2*$^{-/-}$ CT26 cells with or without OTUD1 overexpressing.

H Flow cytometric analysis of TFRC expression in wild-type (WT) or *Ireb2*$^{-/-}$ CT26 cells with or without OTUD1 overexpression treated with AFC (50 μM). MFI, mean fluorescence intensity. (n = 4 biological replicates, mean ± s.e.m., ns, not significant (P > 0.05), ***P = 0.0001, two-tailed unpaired Student's t-test).

Source data are available online for this figure.

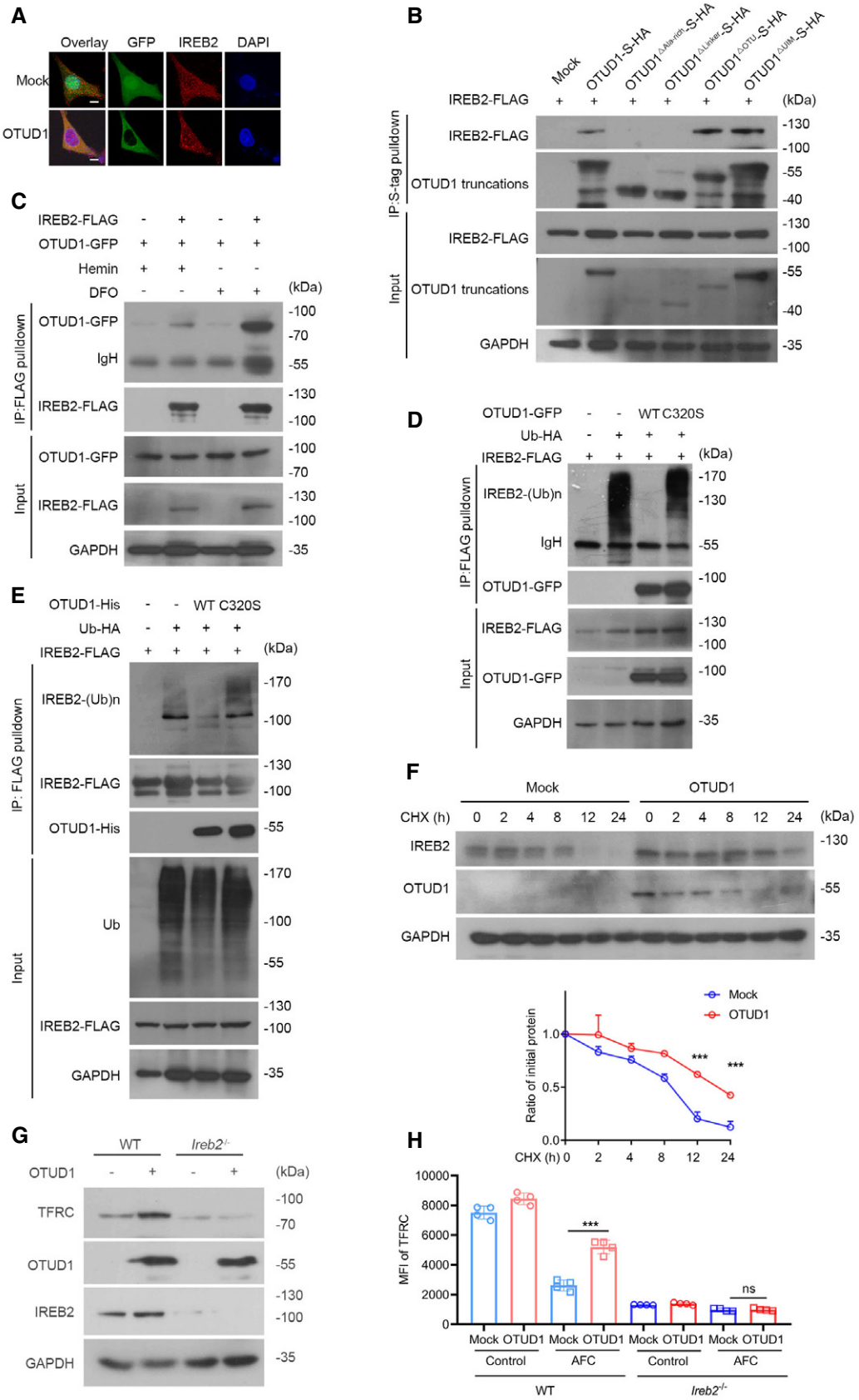
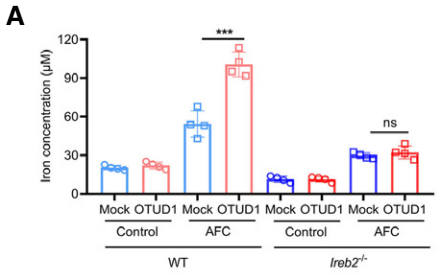
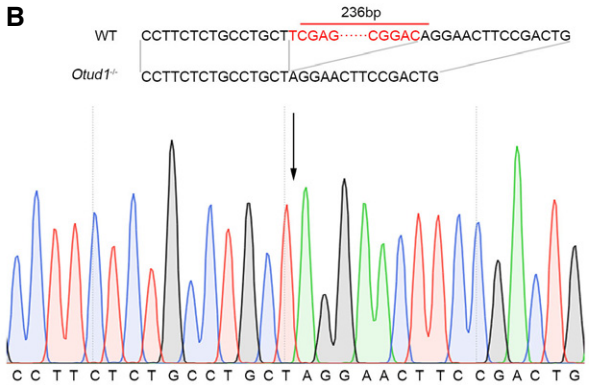
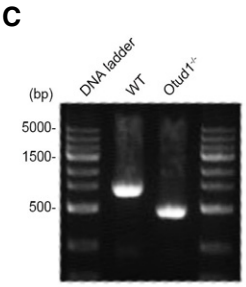


Figure EV2.







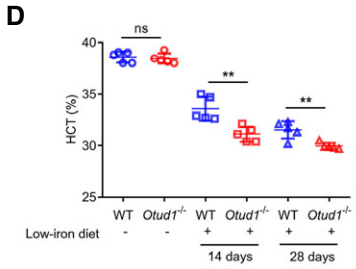


Figure EV3. Strategy of generation of *Otud1*$^{-/-}$ mice.

A Intracellular iron concentration was measured in wild-type (WT) and *Ireb2*$^{-/-}$ CT26 cells with or without OTUD1 overexpression treated with AFC (50 μM) (n = 4 biological replicates, mean ± s.e.m., ns, not significant (P > 0.05), ***P = 0.0006, two-tailed unpaired Student's t-test).

B Targeting strategy for generation of *Otud1*$^{-/-}$ mice (up) and sequence for *Otud1*$^{-/-}$ mice genomic DNA (down).

C Validation of *Otud1*$^{-/-}$ mice by PCR.

D Hemoglobin and hematocrits (HCTs) in wild-type (WT) and *Otud1*$^{-/-}$ mice with or without low-iron diets (n = 5 biological replicates, mean ± s.e.m., ns, not significant (P > 0.05), **P < 0.01, two-tailed unpaired Student's t-test).

Figure EV4. OTUD1 promotes tumor cell ferroptosis.

A Proliferation of mock- or OTUD1-expressing CT26 cells was measured by CCK-8 (n = 3 biological replicates, mean ± s.e.m., ns, not significant (P > 0.05), two-tailed unpaired Student's t-test).

B, C Macroscopic evaluation (B) and tumor volume (n = 6 biological replicates, mean ± s.e.m., ns, not significant (P > 0.05), two-tailed unpaired Student's t-test) (C) of mock- and OTUD1-expressing CT26 tumors in NOD-SCID mice.

D, E The particle size (D) and zeta-potential (E) of vitamin E nanoparticles (NP-VE) were determined by PSS ZPW388-NICOMP Particle Sizing System.

F Mock- and OTUD1-expressing CT26 cells treated with indicated regents (RSL3, 10 μM; NP-VE, 20 mM) for 12 h and LDH releasing level was detected (n = 4 biological replicates, mean ± s.e.m., ns, not significant (P > 0.05), **P = 0.0029, two-tailed unpaired Student's t-test).

G ATP leaking in NCM460 cells treated with several cell death inducers including Erastin (10 μM), RSL3 (5 μM), TNFα (100 ng/ml), CHX (2 μg/ml), Z-VAD (25 μM), and H_2O_2 (100 μM) was detected (n = 4 biological replicates, mean ± s.e.m., ns, not significant (P > 0.05), *P = 0.0214, ***P < 0.001, two-tailed unpaired Student's t-test).

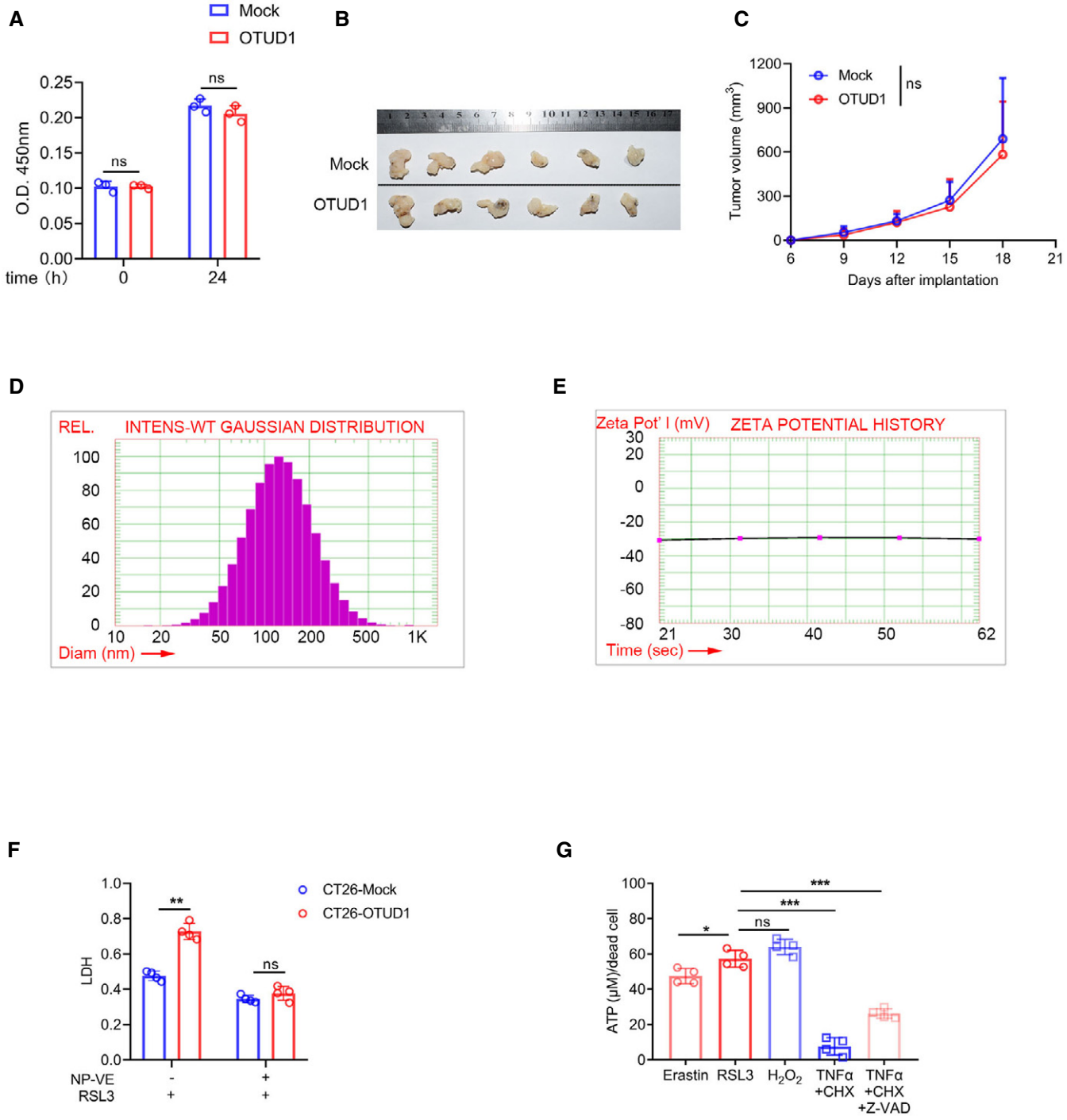
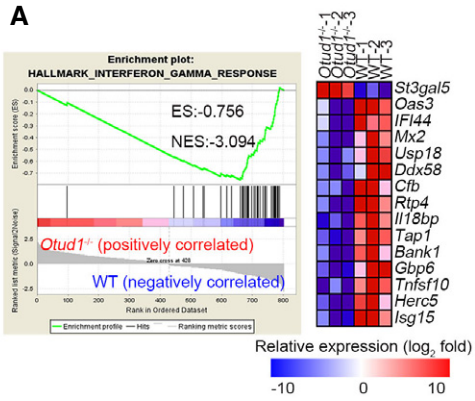
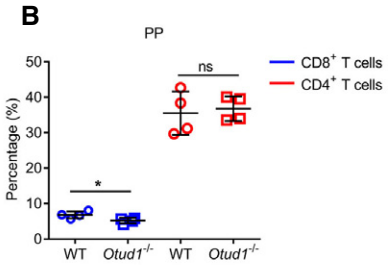


Figure EV4.





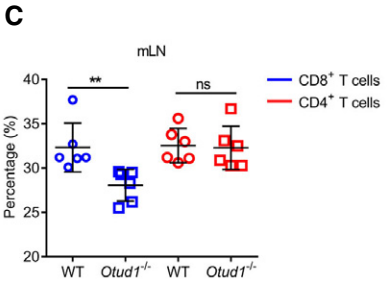


Figure EV5. Loss of OTUD1 impedes host antitumor immunity.

A GSEA of genes expressed in colon tissues from wild-type (WT) and *Otud1*$^{-/-}$ mice treated with AOM/DSS (n = 3 mice). ES, enrichment score; NES, normalized enrichment score.

B, C Flow cytometric analysis of frequency of T-cell subsets in Peyer's patches (PP) (n = 4 mice, mean ± s.e.m., ns, not significant (P > 0.05), *P = 0.0406, two-tailed unpaired Student's t-test) (B) and mesenteric lymph node (mLN) (n = 6 mice, mean ± s.e.m., ns, not significant (P > 0.05), **P = 0.0098, two-tailed unpaired Student's t-test) (C) isolated from wild-type (WT) and *Otud1*$^{-/-}$ mice treated with AOM/DSS.