Expanded View Figures

Figure EV1. LIF exhibits therapeutic effects in the wild-type mouse colitis model and shows minor effect on Th1-, Th2-, or Treg-cell differentiation.

A, B Quantitative expression of *Lifr and gp130* mRNA in colon tissue from DSS-challenged or control mice (n = 6 per group) (A) or in IECs following stimulation by LPS (2.5 μg/ml), PGN (3 μg/ml), or LTA (2 μg/ml) for 2 h (B).

C Macroscopic changes in the colon of WT and *Stat4*-KO mice on day 14 of colitis induction.

D FACS staining of LPLs isolated on day 10 from the colon of WT and *Stat4*-KO colitis mice receiving PBS or LIF (n = 4 per group). The percentage of IL-17A$^+$ (left) and IFNγ$^+$CD4$^+$ (right) T cells *in vivo* was analyzed.

E FACS staining of WT and *Stat4*-KO naïve CD4$^+$ T cells treated or not treated with LIF on day 4 of induction into Th1-, Th2-, or Treg-cell subsets.

F, G qPCR analysis of Th1, Th2, or Treg signature gene expression in WT and *Stat4*-KO naïve CD4$^+$ T cells on day 4 of induction into the indicated subsets in the absence or presence of LIF. The data are representative of three independent experiments.

Data information: *P < 0.05, **P < 0.01, and ***P < 0.001, ns: not significant (Student's t-test). Error bars represent the SEM.

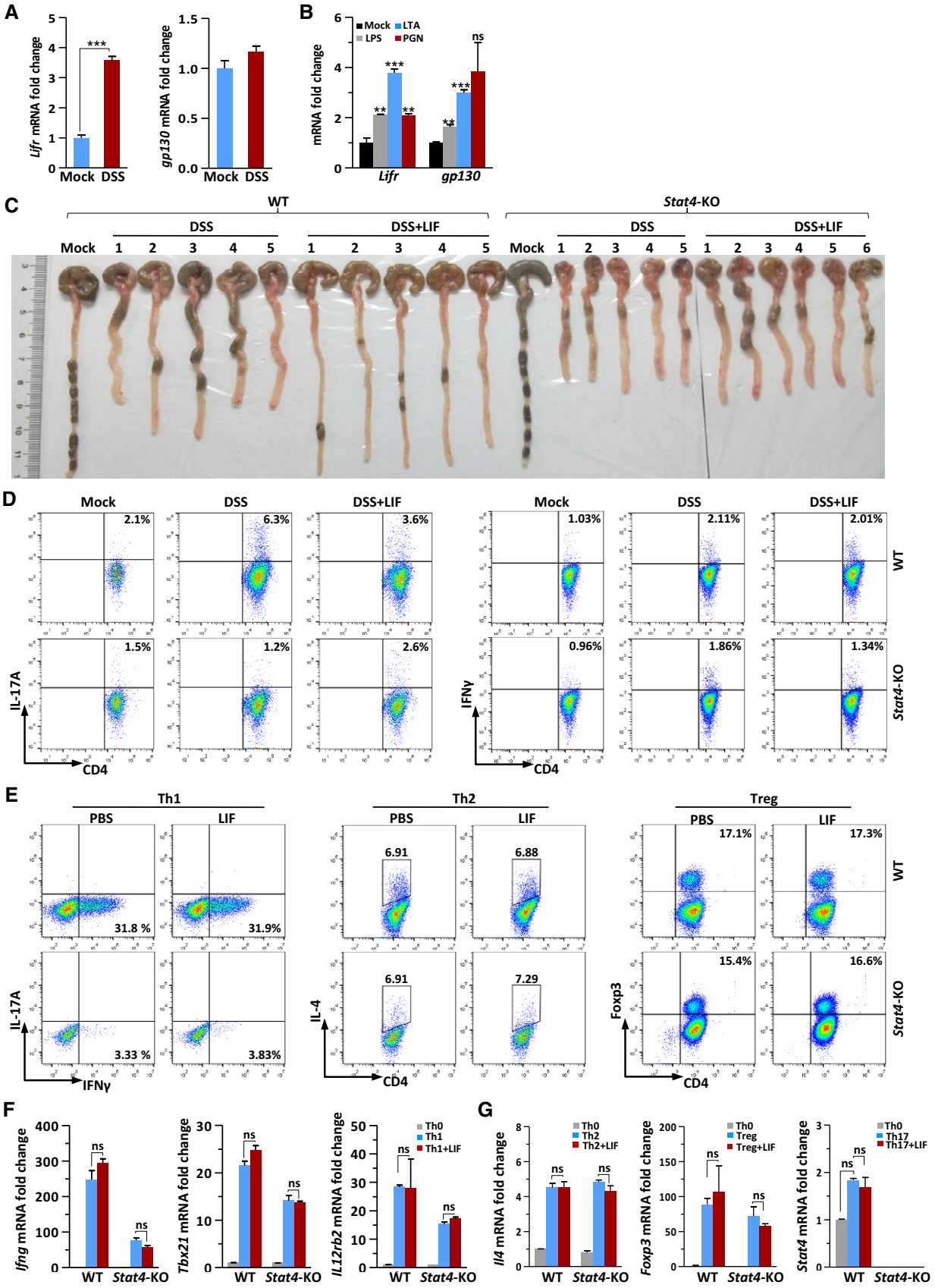

Figure EV1.

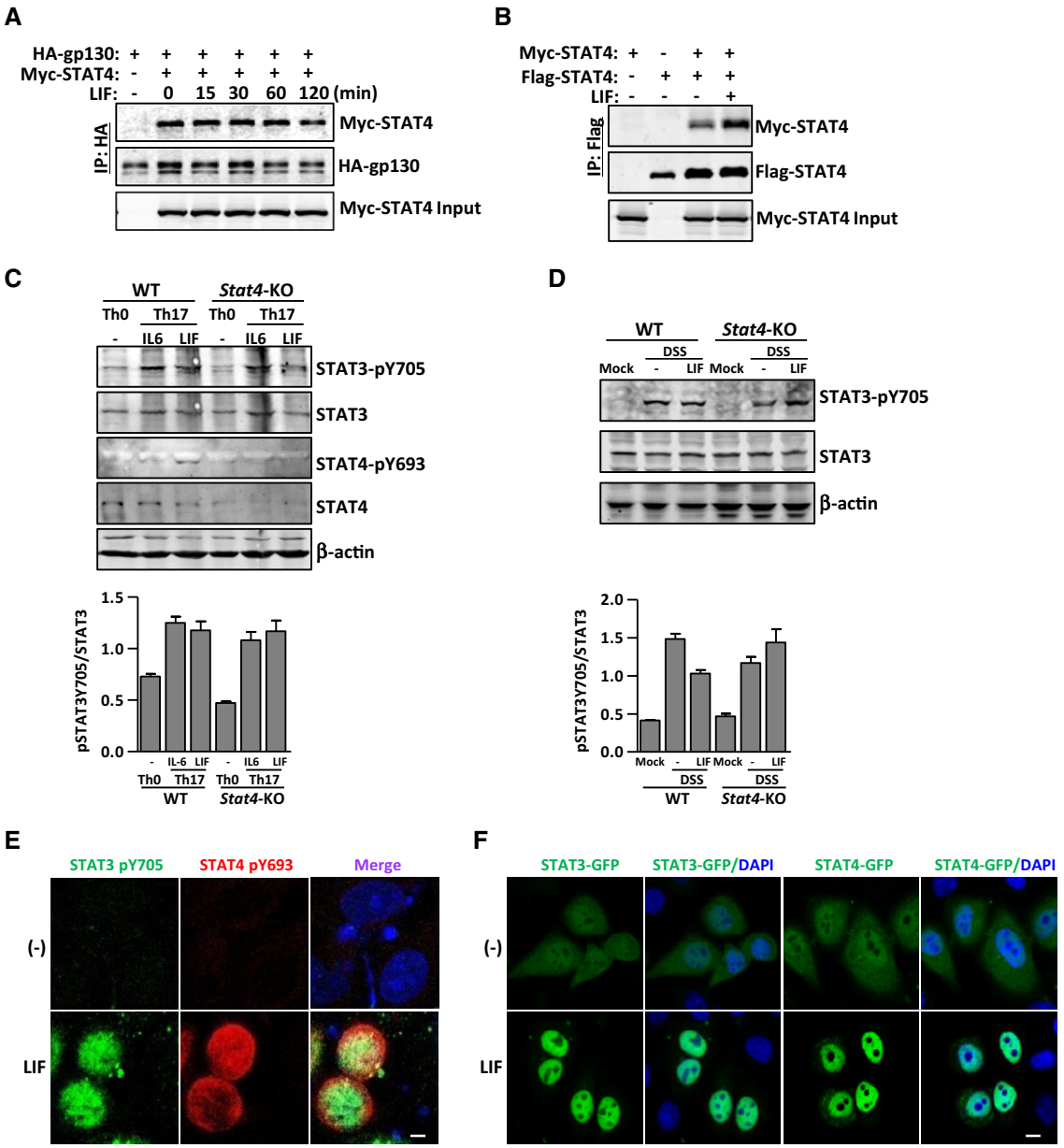

Figure EV2. STAT4 does not alter LIF-induced STAT3 activation.

A, B Coimmunoprecipitation and immunoblot analysis of LIF-treated HEK293T cells cotransfected with the indicated constructs.

C, D Immunoblot analysis of the expression and modification of the indicated proteins in WT or *Stat4*-KO naïve $CD4^+$ T cells on day 4 of induction into Th0 or Th17 cells in the presence or absence of LIF (50 ng/ml) (C) or in spleen extracts from WT or *Stat4*-KO colitis mice treated with or without LIF (50 ng/ml) (D). The bottom panel shows the intensity analysis of the bands from three independent experiments. The data are representative of two independent experiments. Error bars represent the SEM.

E Immunostaining of cultured HeLa cells transfected with STAT4 and STAT3 after LIF treatment with STAT4 and STAT3 phosphorylation antibody. Scale bar, 20 μm.

F Cultured HeLa cells transfected with GFP-tagged STAT4 and RFP-tagged STAT3 were exposed to LIF and imaged 30 min later. Scale bar, 50 μm.

Source data are available online for this figure.

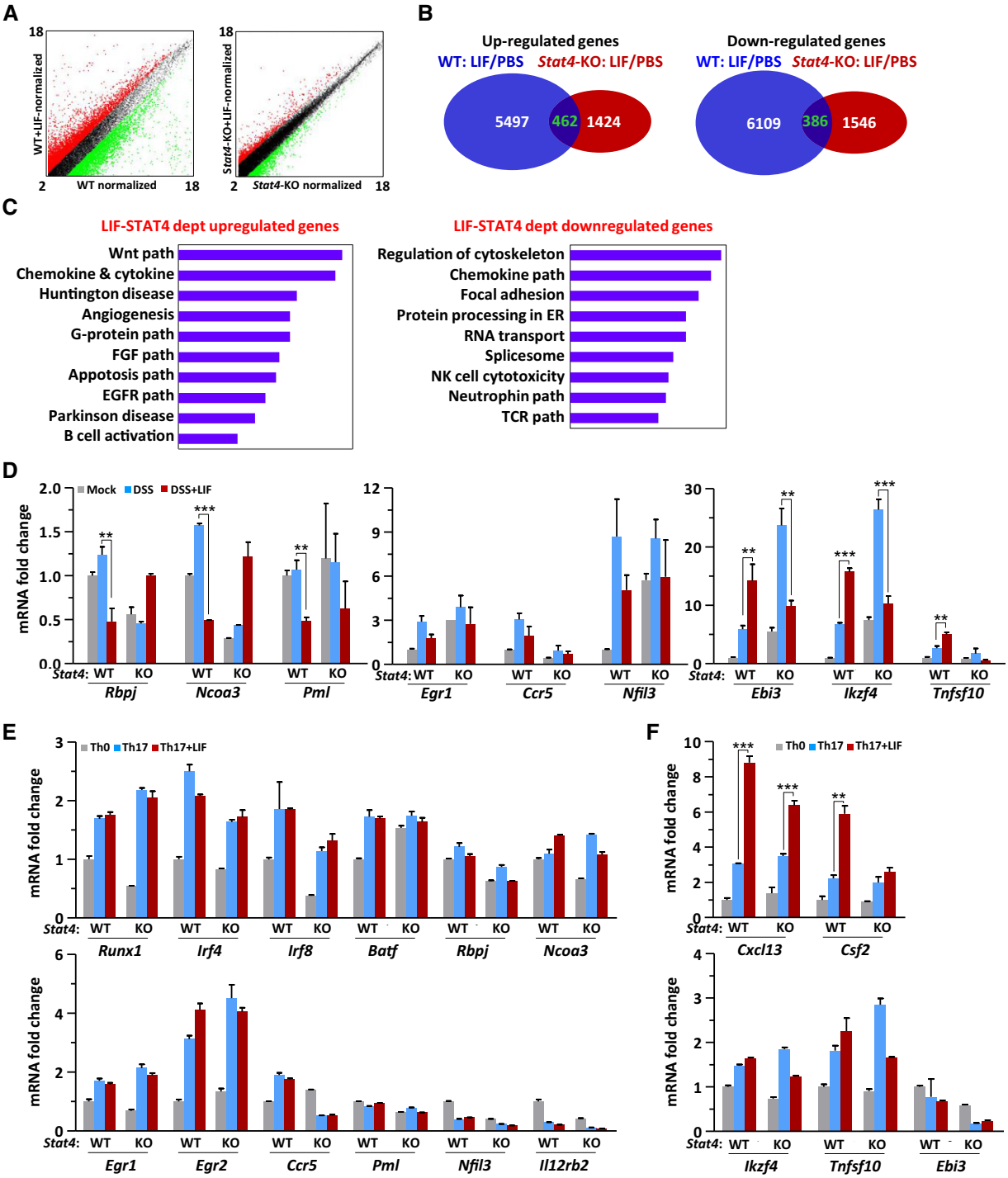

Figure EV3. LIF shows minimal effect on Th17 regulatory genes expression *in vitro*.

A, B Gene chip analysis of LPLs purified from colitis mice treated as described in Fig 2A. A scatter plot (A) and the statistical analysis (B) of the average fragments per kilobase of transcript per million mapped reads in WT and *Stat4*-KO mice receiving PBS or LIF.

C Functional pathway analysis of LPLs isolated on day 10 from the colon of WT or *Stat4*-KO colitis mice receiving injections of PBS or LIF (n = 4 per group).

D–F qPCR analysis of the indicated genes in LPLs from colitis mice treated as described in Fig EV3A (D) or in WT or *Stat4*-KO naïve $CD4^+$ T cells on day 4 of induction into Th17 subsets in the absence or presence of LIF (E, F). The data are representative of two experiments. $^{**}P < 0.01$ and $^{***}P < 0.001$ (Student's t-test). Error bars represent the SEM.

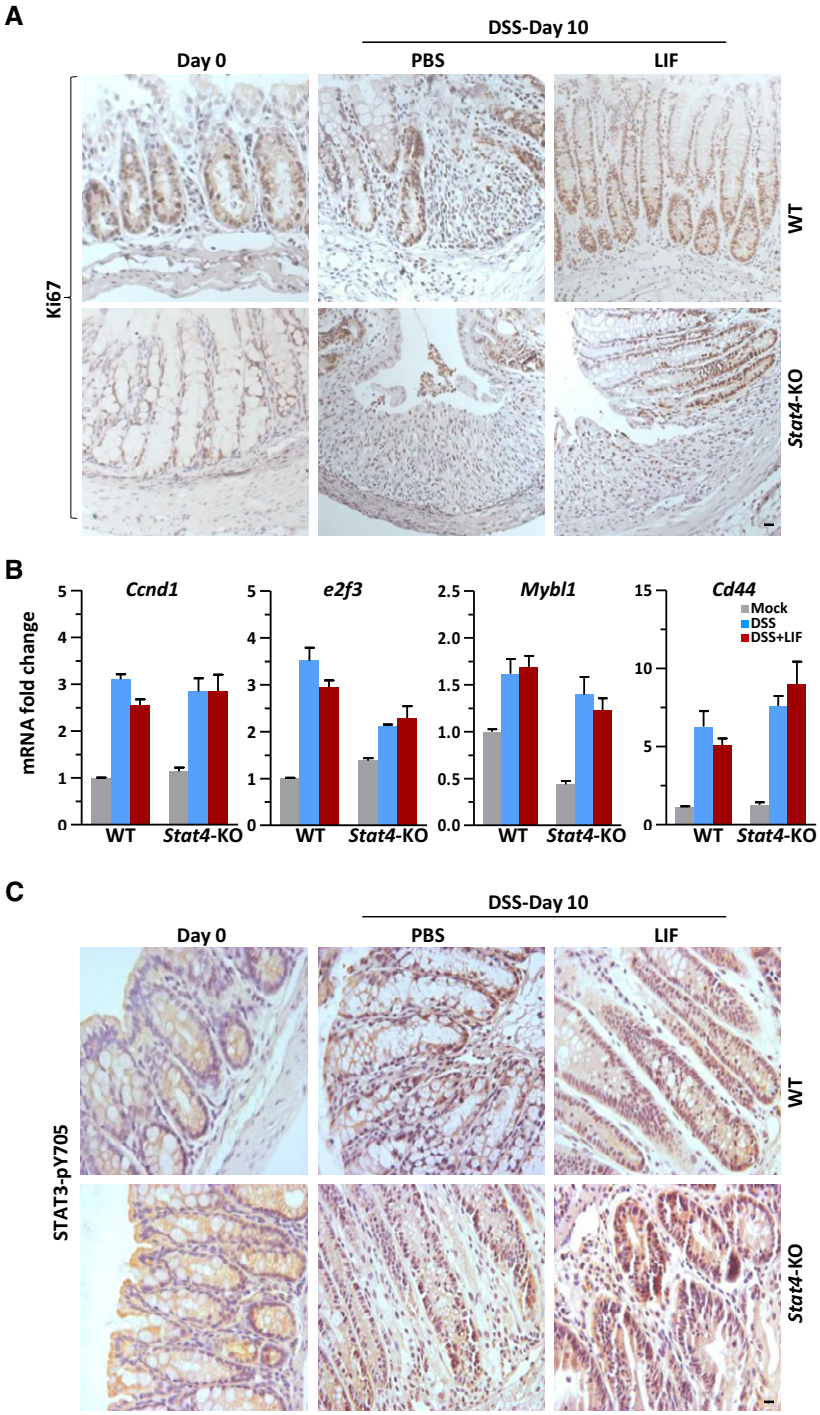

Figure EV4. STAT4 does not regulate the expression of proliferation-related genes in IECs.

A Ki67 staining of representative colons on day 10 of colitis induction as described in Fig 2A. Scale bar, 20 μm.

B Quantitative mRNA expression analysis of the indicated genes in colon tissue from WT or *Stat4*-KO colitis mice treated as described in Fig 2A (n = 3 per group). Error bars represent the SEM.

C Colons obtained from WT or *Stat4*-KO mice on day 10 of the colitis model induced as in Fig 2A were immunostained with an antibody against STAT3 phosphorylated on Y705. Scale bar, 20 μm.

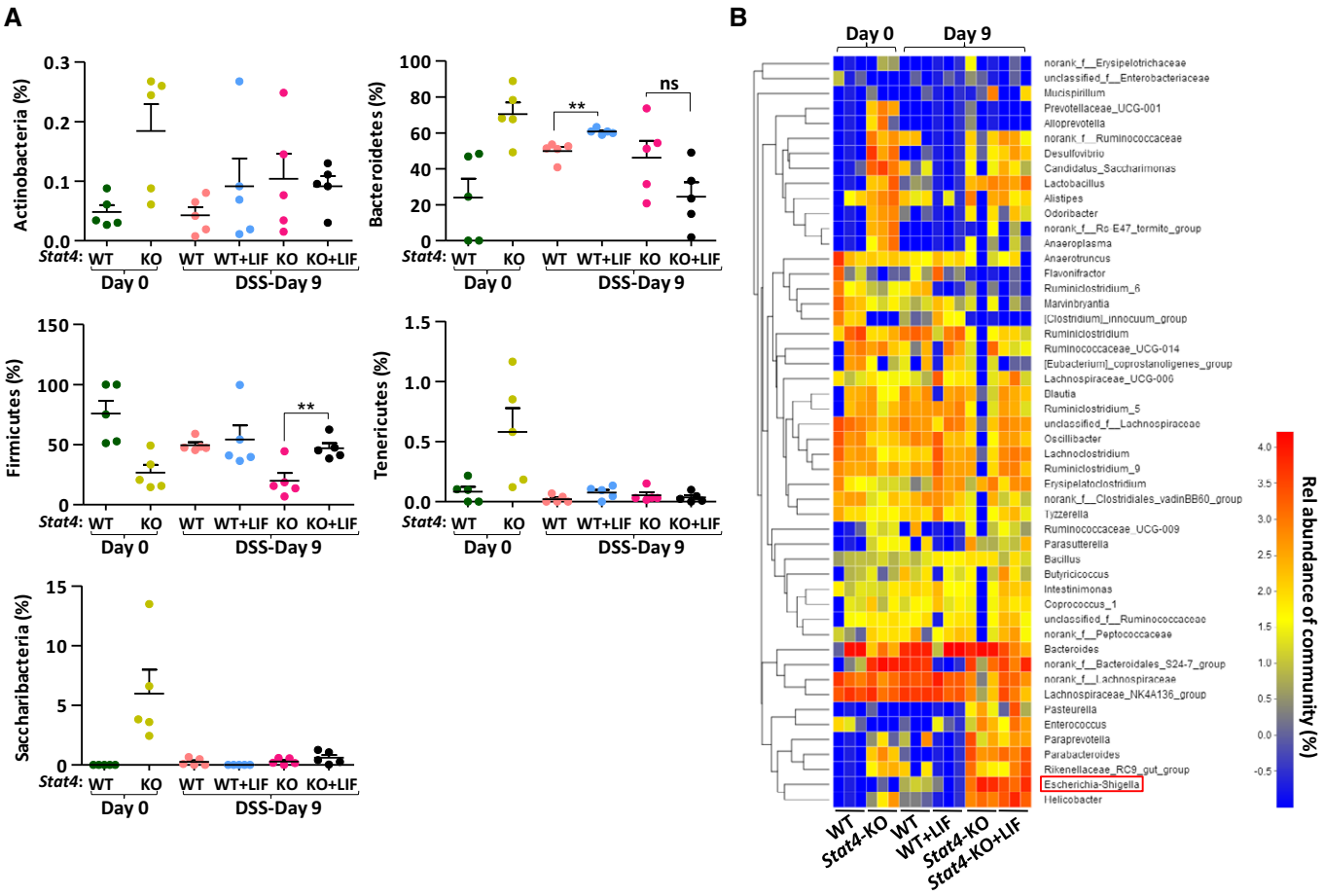

Figure EV5. Escherichia-Shigella but not other bacteria are affected by LIF in the amelioration of colitis.

A 16S rRNA sequencing analyses of the microbial distribution at the phylum level in feces from WT and *Stat4*-KO colitis mice treated as described in Fig 2A (n = 5 per group). Error bars represent the SEM. $^{**}P < 0.01$ (Student's t-test).

B Heatmap depicting the relative microbial abundance at the genus level in the fecal specimens described in (A) (n = 3 per group).