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Supplemental Information

Dual-Functionalized MSCs that Express CX3CR1 and IL-25 Exhibit Enhanced Therapeutic Effects on Inflammatory Bowel Disease

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The supplemental material contains supplemental figures and legends.

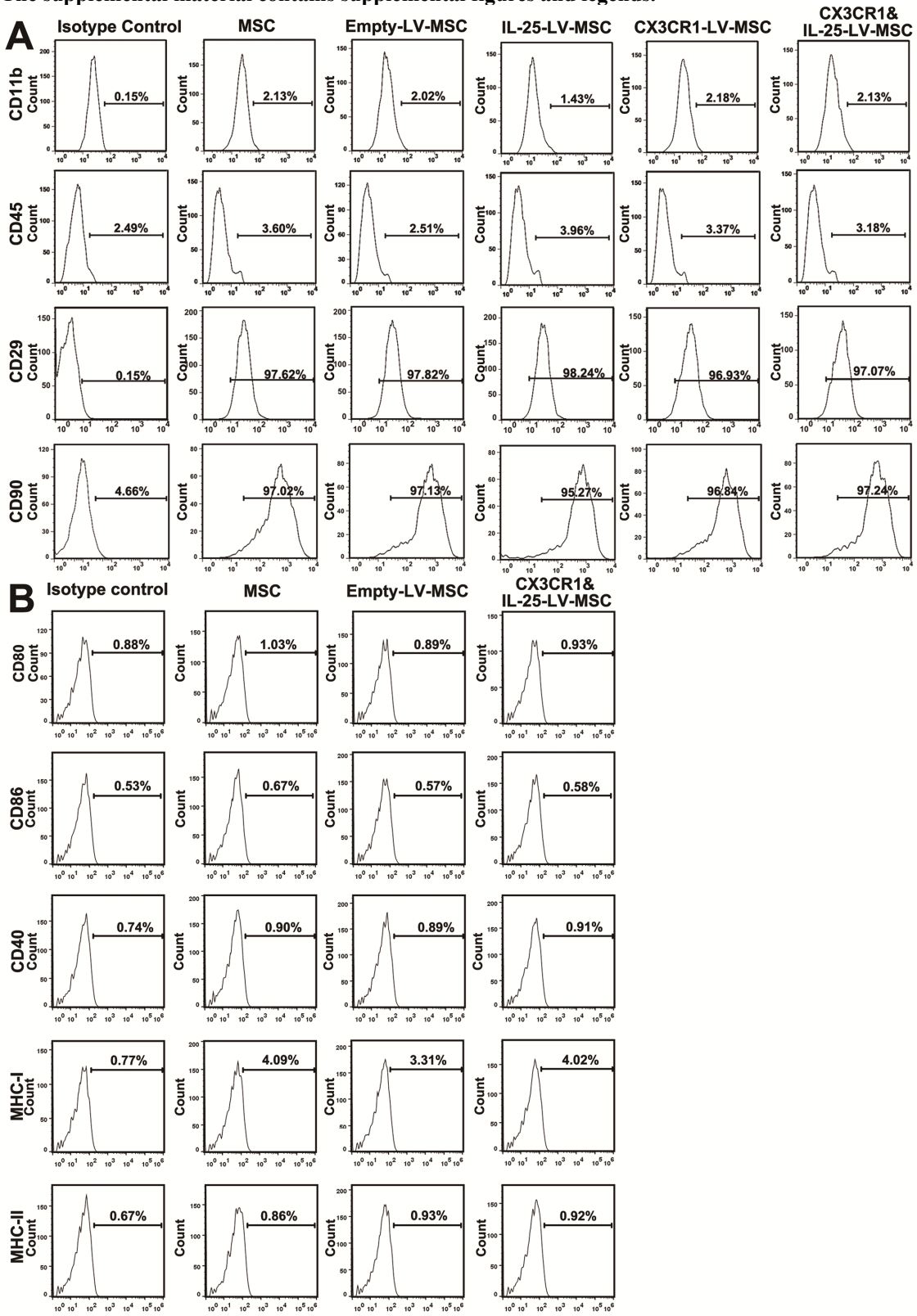


Figure S1 Phenotypic characterization and immunogenicity analysis of MSCs following lentivirus infection. (A) Flow cytometry analysis of immune cell markers (CD11b and CD45) and stem cell markers (CD29 and CD90) in MSCs treated with lentivirus infection. (B) Flow cytometry analysis of immunogenicity markers (CD80, CD86, CD40, MHC-I and MHC-II) in MSCs treated with lentivirus infection. For cell experiments, five samples were analyzed per condition, and the experiments were performed in triplicate.

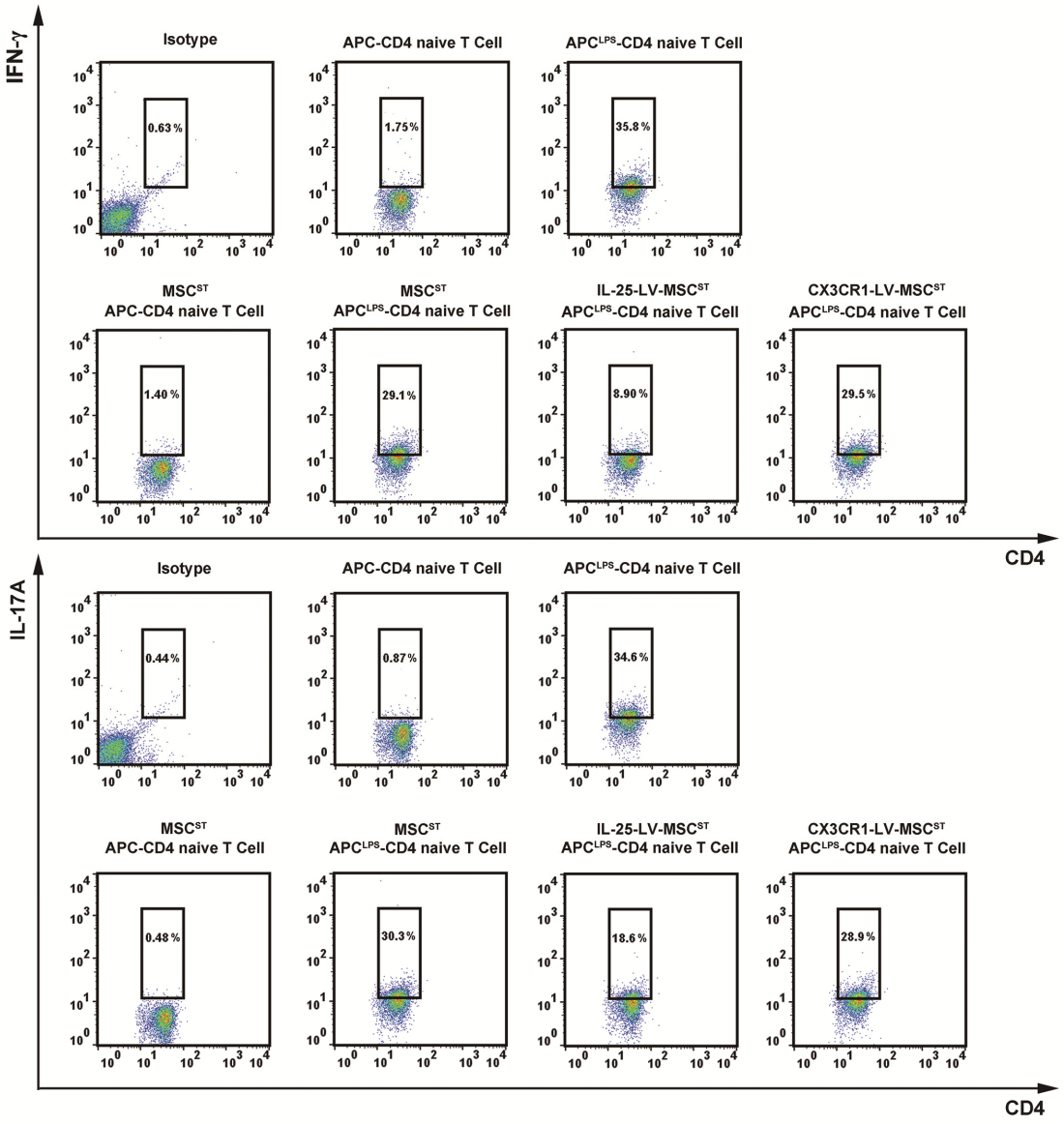


Figure S2 Flow cytometry analysis of naive CD4$^+$ T cells magnetically purified from spleens harvested from C57BL/6 mice and cultured with medium from LPS-treated APCs incubated with supernatants from MSCs infected with different lentiviruses infection for 72 h. For cell experiments, five samples were analyzed per condition, and the experiments were performed in triplicate. ST, supernatant.

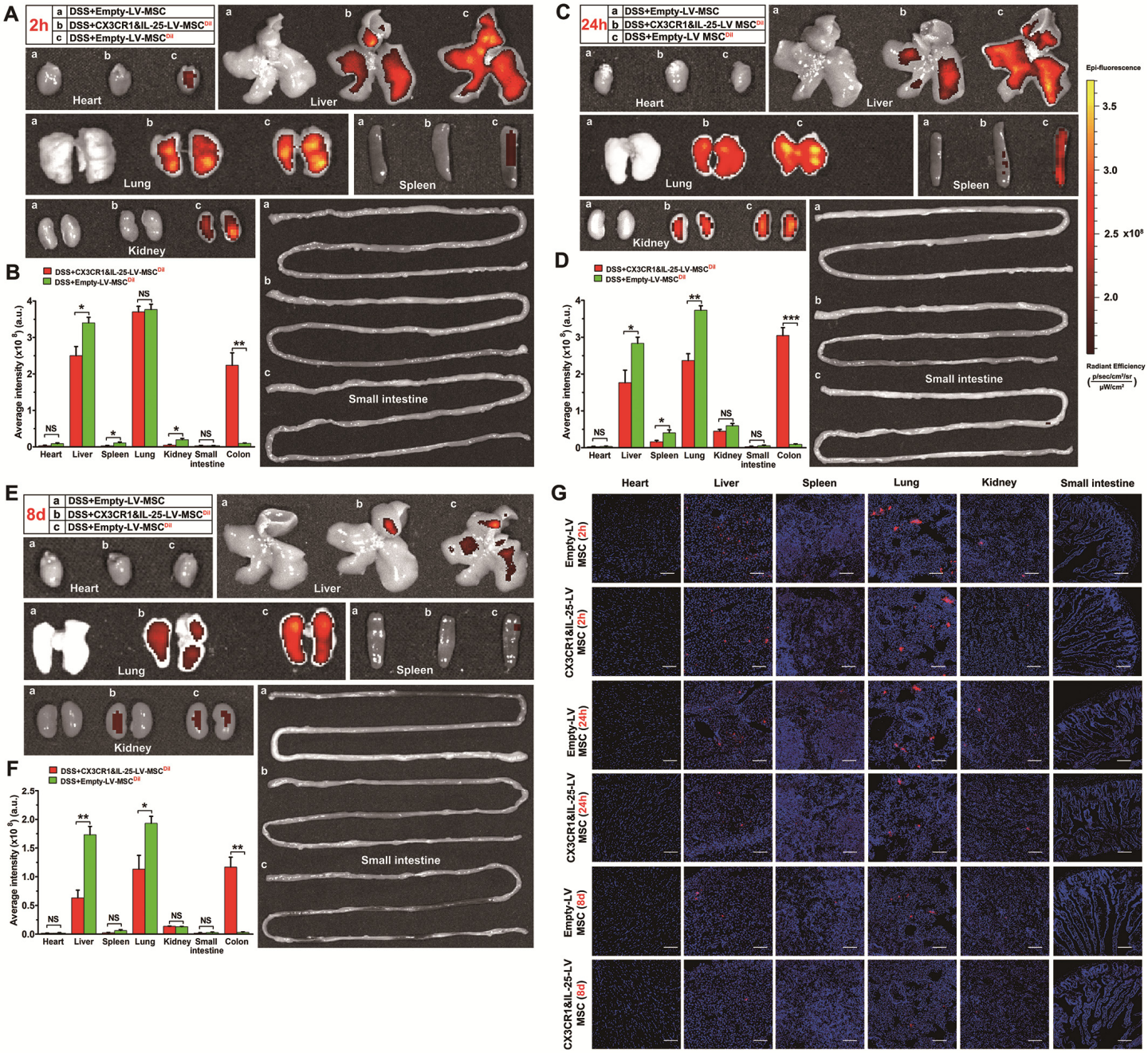


Figure S3 The biodistribution of engineered MSCs. (A-F) Fluorescence images and fluorescence quantitative analysis of the heart, liver, spleen, lungs, kidneys and small intestine at 2 h, 24 h and 8 d after the administration of different modified MSCs (96 h post infection, empty-LV- or CX3CR1&IL-25-LV-treated MSCs). (G) Fluorescently labeled MSCs were observed in frozen tissue sections of different organs at 2 h, 24 h and 8 d after the administration of different modified MSCs (red, MSCs; blue, DAPI nuclear staining). Scale bar, 100 μm. Values are expressed as the mean ± SEM (n = 7 mice per group). *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. NS, no significant change.

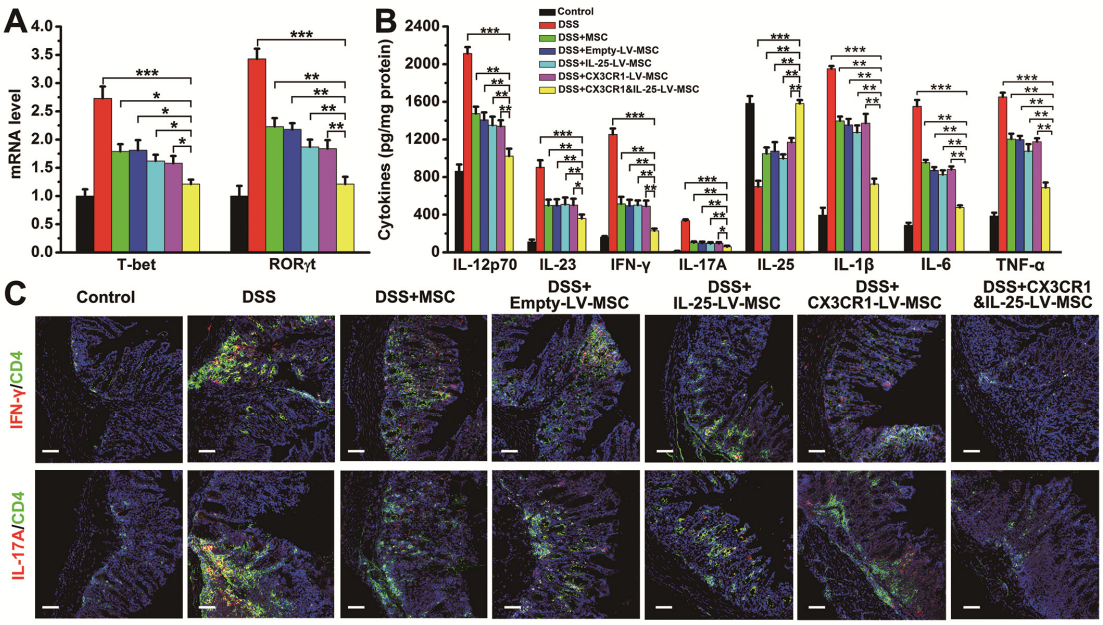
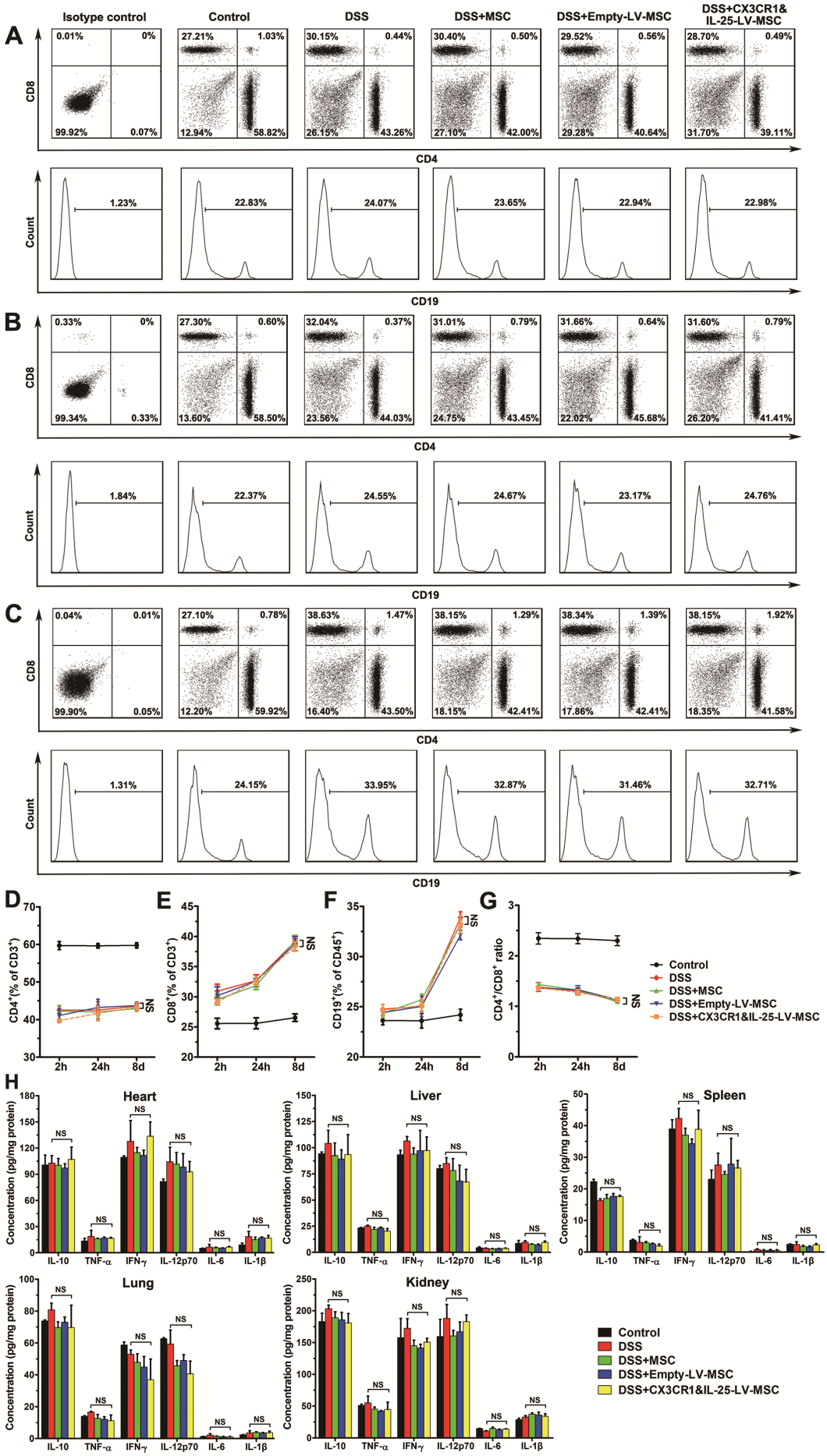


Figure S4 The effects of engineered MSCs on colonic Th1/Th17 responses during the colitis process. (A) At day 16 after model induction, the levels of T-bet and RORγt in colonic $CD4^+$ T cells were examined by qRT-PCR, and (B) the levels of colonic cytokines (IL-12p70, IL-23, IFN-γ, IL-17A, IL-25, IL-6, IL-1β and TNF-α) were measured by ELISA kits. (C) The influences of different populations of treated MSCs on Th1/Th17 cell differentiation in the colon were further examined by immunofluorescence costaining and imaging (red, IFN-γ/IL-17A; green, CD4; and blue, DAPI nuclear staining). Scale bar, 100 μm. Values are expressed as the mean ± SEM (n = 7 mice per group). $*p < 0.05$, $**p < 0.01$, and $***p < 0.001$.


Isotype control
Control
DSS
DSS+MSC
DSS+Empty-LV-MSC
DSS+CX3CR1&
IL-25-LV-MSC
A
B
C
CD8
CD4
Count
CD19
0.01%
0%
99.92%
0.07%
27.21%
1.03%
12.94%
58.82%
30.15%
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43.26%
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24.15%
33.95%
32.87%
31.46%
32.71%
D
E
F
G
CD4+(% of CD3+)
CD8+(% of CD3+)
CD19+(% of CD45+)
CD4+/CD8+ ratio
SN
2h
24h
8d
Control
DSS
DSS+MSC
DSS+Empty-LV-MSC
DSS+CX3CR1&IL-25-LV-MSC
H
Heart
Liver
Spleen
Lung
Kidney
Concentration (pg/mg protein)
NS
IL-10
TNF-α
IFN-γ
IL-12p70
IL-6
IL-1β

Figure S5 The evaluation of immune rejection responses of engineered rat MSCs in DSS-induced mice. (A-C). The representative flow cytometry data of $CD4^+$, $CD8^+$ T cells (gated on $CD3^+$ cells) and $CD19^+$ B cells (gated on $CD45^+$ cells) in the peripheral blood from DSS colitis mice at 2 h, 24 h and 8 d after the injection of engineered MSCs. The ratio of peripheral blood (D) $CD4^+$ T cells, (E) $CD8^+$ T cells and (F) $CD19^+$ B cells was determined. (G) The CD4/CD8 ratio was calculated. (H) The cytokine levels (IL-10, TNF-α, IFN-γ, IL-12p70, IL-6 and IL-1β) in various tissues (heart, liver, spleen, lung and kidney) from DSS colitis mice at 8 d after the injection of engineered MSCs were measured by ELISA kits. Values are expressed as the mean ± SEM (n = 7 mice per group). NS, no significant change, compared between DSS group and DSS+ CX3CR1&IL-25-LV-MSC group.

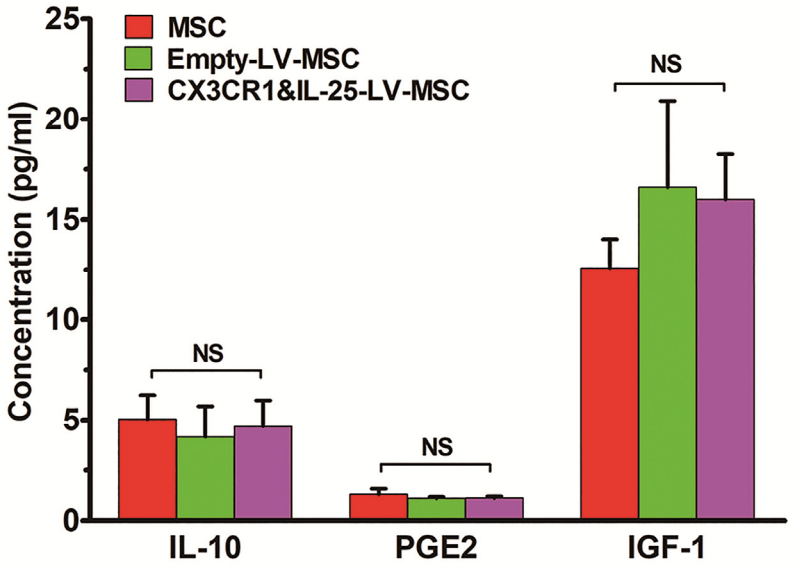

Figure S6 The examination of paracrine factors from MSCs following lentivirus infection. The levels of IL-10, IGF-1 and PGE2 in the supernatant from MSCs with different treatments were examined by ELISA kits. For cell experiments, five samples were analyzed per condition, and the experiments were performed in triplicate. Values are expressed as the mean ± SEM (n = 7 mice per group). NS, no significant change.