Decursinol angelate ameliorates dextran sodium sulfate-induced colitis by modulating type 17 helper T cell responses

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Running title: Effect of decursinol angelate on DSS-induced colitis

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Supplemental Table S1. Primer sequences used in the quantitative real-time PCR analysis

<table>
<thead>
<tr>
<th>Gene name</th>
<th>Sequence (5’ to 3’)</th>
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| Actb      | Forward: GGCACCACACCTTCTACAATG  
Reverse: GGGGTGTTGAAGGTCTCAAAC |
| Il17a     | Forward: TTTAACTCCCTTTGGCGAAAA  
Reverse: CTTTCCCTCCGCATTGACAC |
| Rorc      | Forward: TGT CCT GGG CTA CCC TAC TG  
Reverse: GTG CAG GAG TAG GCC ACA TT |
| Tbx21     | Forward: CTTGGATCCTCCTCGCTACCC  
Reverse: ACTTGGACCACAACAGGTGG |
Supplemental Figure Legends

Supplemental Figure S1. Effect of decursinol angelate on Th1, Th2, and Treg cell differentiation: Purified CD4+ T cells were cultured in the presence or absence of DA under Th1 (a), Th2 (b) and Treg (c) polarizing conditions for 5 days. (a & b) Cells were restimulated with PMA and ionomycin for 6 h and analyzed by intracellular cytokine staining. Shown are the representative FACS profiles in the live CD4 T cell gates with the inset numbers indicating the mean (± SD) prevalence of the IFN-γ+ and IL-4+ cells. (c) Treg cells were cultured for 5 d, and FoxP3 expression was measured by flow cytometry. Shown are the representative FACS profiles with the inset numbers indicating the mean (± SD) prevalence of the GITR+ FoxP3+ cells.

Supplemental Figure S2. DA treatment negatively regulates the induction of Treg cells in the colon of the colitis mice. Lymphocytes were isolated from the mesenteric lymph nodes (MLN, a), colonic intraepithelium (colonic IE, b) and lamina propria (colonic LP, c) and stained with the FoxP3 staining kit. Shown are the representative FACS profiles in the TCRβ+ CD4+ gated cells, with the inset numbers indicating the prevalence of the GITR+ FoxP3+ cells.
Thapa et al. Supplemental Figure S1
Thapa et al. Supplemental Figure S2