Electronic supplementary information (ESI) †

Aloe-derived Nanovesicles Attenuate Inflammation and Enhance Tight Junction Proteins for Acute Colitis Treatment †

Sang-Hun Choi,‡a Jung-Young Eom,‡b Hyun-Jin Kim,c Wonhyo Seo,d Hyo-Jung Kwun,e Do-Kyun Kim,b Jihoon Kim*,a and Young-Eun Cho*,c

Table S1. Scoring system to calculate the disease activity index (DAI)

<table>
<thead>
<tr>
<th>Score</th>
<th>Weight loss</th>
<th>Stool consistency</th>
<th>Visible blood feces</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>None</td>
<td>Normal</td>
<td>None</td>
</tr>
<tr>
<td>1</td>
<td>1~5%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>6~10%</td>
<td>Loose</td>
<td>Slight bleeding</td>
</tr>
<tr>
<td>3</td>
<td>11~20%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>&lt;20%</td>
<td>Diarrhea</td>
<td>Gross bleeding</td>
</tr>
</tbody>
</table>

The DAI value was calculated as the sum of the scores for weight loss, stool consistency, and occult/gross blood.
Aloe gel + PBS
(200 g + 500 mL)

[Centrifuge]
500 x g, 10 min
2,000 x g, 20 min
10,000 x g, 30 min, 2 times

[Ultracentrifuge]
100,000 x g, 1 h

Suspend in 1 mL PBS

Figure S1. The optimized isolation method of aloe-derived nanovesicles.
**Figure S2.** Total DAI scores with or without VNV, ANV, and SNV administration in DSS-induced acute colitis in mice evaluated at the end of the treatment are shown. **##p < 0.01, #p < 0.05** between DSS vs. VNVs, ANVs, and SNVs groups. Significance of the values for each group was determined using ANOVA and Tukey’s HSD test. Data represent means ± S.E.M.
Figure S3. Cell cytotoxicity of T84 cells using aloe-derived nanovesicles.
Figure S4. Cell cytotoxicity of HT-29 cells using aloe-derived nanovesicles.
Figure S5. VNVs protected the levels of tight junction (TJ) and adherent junction (AJ) proteins in DSS-induced HT-29 cells. Enhancement of TJ protein markers (ZO-1, claudin4, and occludin) in HT-29 cells treated with VNVs. Enhancement of AJ protein markers (γ-catenin, α-tubulin, and E-cadherin) in HT-29 cells treated with VNVs. Various letters in the superscripts indicate significant differences in the specific VNVs concentration (compared to the control; 0 μg/mL) as analyzed using one-way ANOVA followed by Duncan’s multiple range test (p < 0.05)
**Figure S6.** Immunofluorescence imaging of tight junction (TJ) protein enhancement in VNV-treated HT-29 cells.