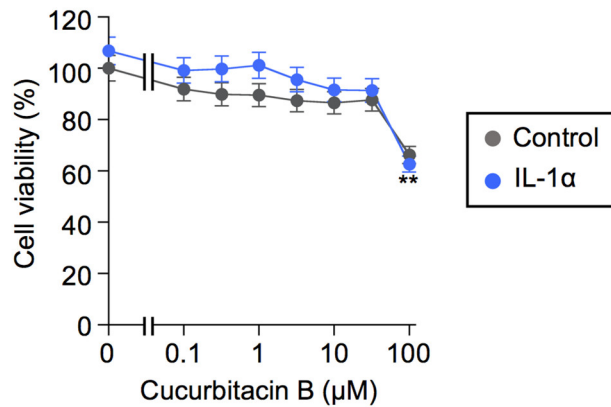
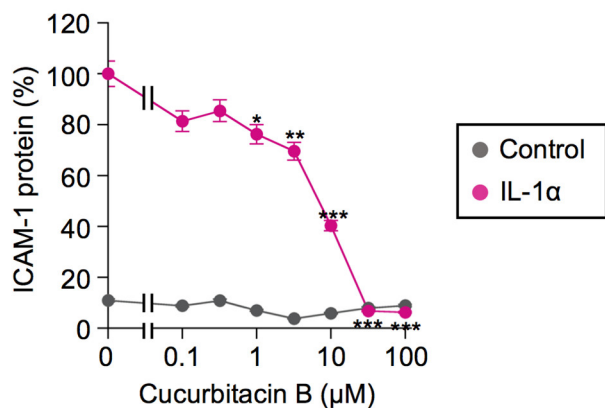


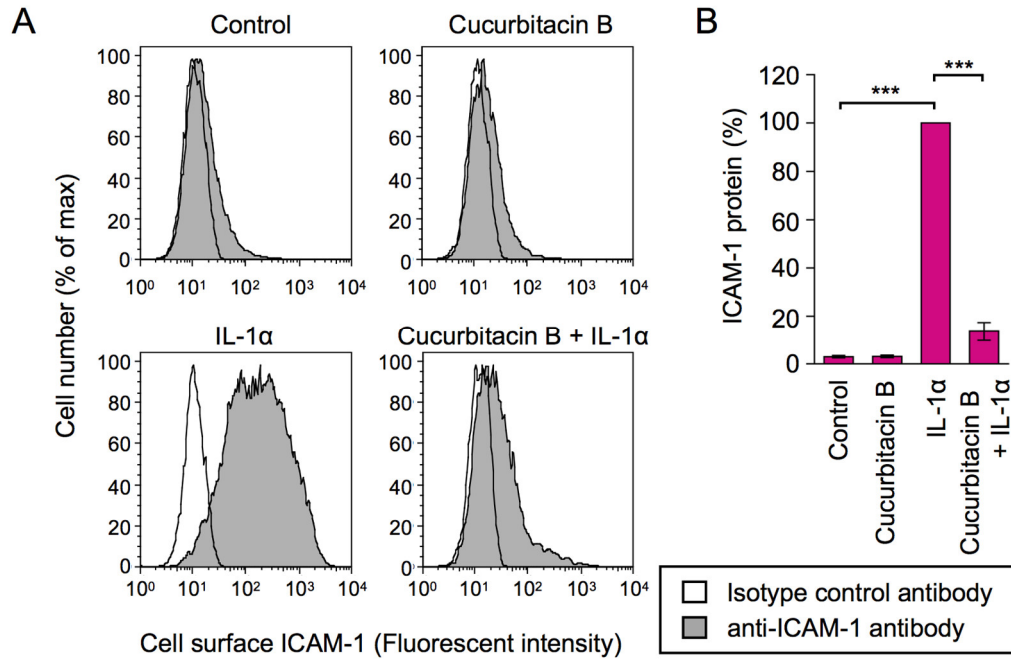
## Supplementary Materials



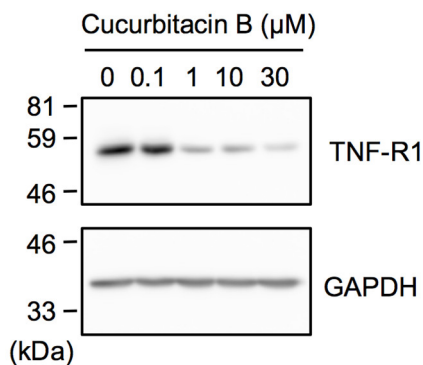
**Figure S1.** Cucurbitacin B did not markedly affect cell viability at concentrations up to 30  $\mu\text{M}$ . A549 cells were pretreated with serial dilutions of cucurbitacin B for 1 h, and were then treated with (blue circles) or without (gray circles) IL-1 $\alpha$  (0.25 ng/mL) in the presence of cucurbitacin B at the indicated final concentrations for 6 h. Cell viability (%) is shown as the mean  $\pm$  S.E. of three independent experiments. \*\* $P < 0.01$ .



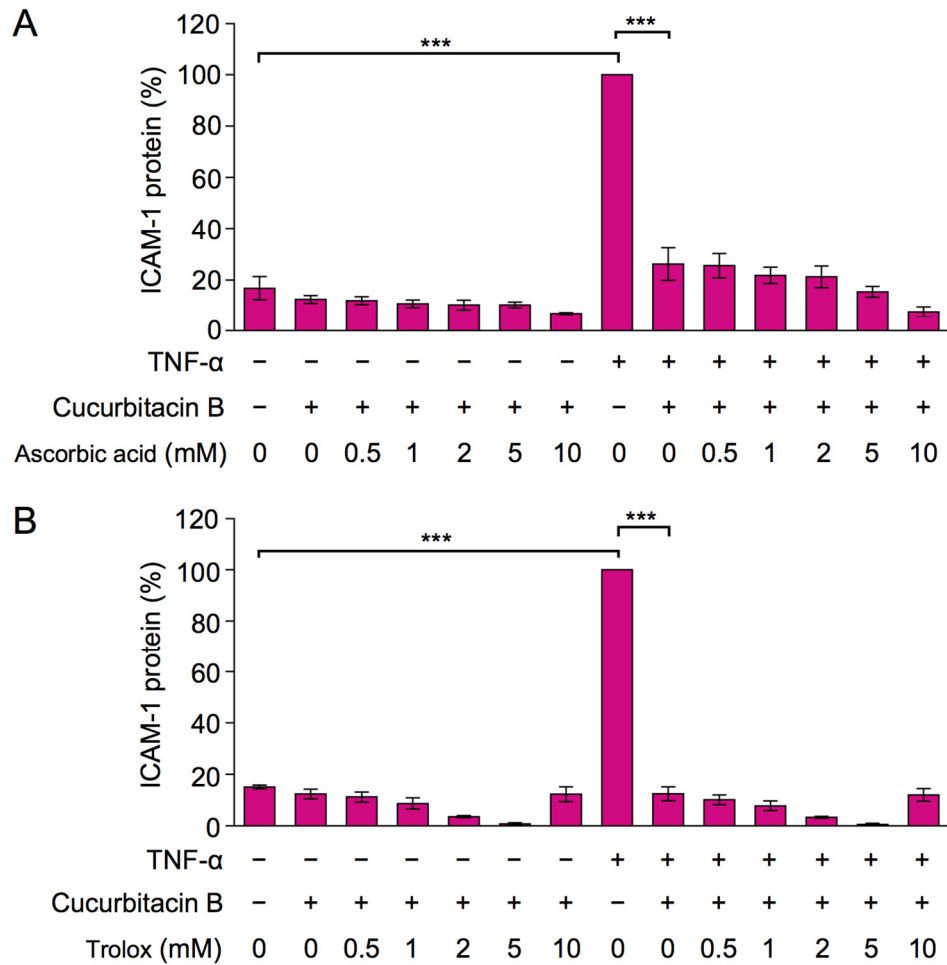
**Figure S2.** Cucurbitacin B inhibited the IL-1 $\alpha$ -induced expression of ICAM-1 protein. A549 cells were pretreated with serial dilutions of cucurbitacin B for 1 h, and were then treated with (red circles) or without (gray circles) IL-1 $\alpha$  (0.25 ng/mL) in the presence of cucurbitacin B at the indicated final concentrations for 6 h. ICAM-1 protein (%) is shown as the mean  $\pm$  S.E. of three independent experiments. \* $P < 0.05$ , \*\* $P < 0.01$ , and \*\*\* $P < 0.001$ .



**Figure S3.** Cucurbitacin B inhibited IL-1 $\alpha$ -induced cell-surface ICAM-1 expression. (**A** and **B**) A549 cells were incubated with cucurbitacin B for 1 h, and were then treated with or without IL-1 $\alpha$  (0.25 ng/mL) in the presence or absence of cucurbitacin B (30  $\mu$ M) for 6 h. Histograms are representative of three independent experiments (**A**). Cell-surface ICAM-1(%) is shown as the mean  $\pm$  S.E. of three independent experiments (**B**). \*\*\* $P$  < 0.001.



**Figure S4.** Cucurbitacin B down-regulated the expression of TNF-R1. A549 cells were treated with or without cucurbitacin B at the indicated concentrations for 1 h, followed by the preparation of cytoplasmic fractions. TNF-R1 was detected by the anti-TNF-R1 antibody H5, which recognized an epitope in the CRD. Blots are representative of two independent experiments.



**Figure S5.** The inhibitory effect of cucurbitacin B was not attenuated by ascorbic acid or Trolox.

(**A and B**) A549 cells were pretreated with serial dilutions of ascorbic acid (**A**) and Trolox (**B**) for 1 h, then with (+) or without (-) cucurbitacin B (10  $\mu$ M) for 1 h, and then with (+) or without (-) TNF- $\alpha$  (2.5 ng/mL) for 6 h in the presence of absence of compounds at the indicated final concentrations. Cell-surface ICAM-1 (%) is shown as the mean  $\pm$  S.E. of three independent experiments. \*\*\* $P < 0.001$ .