

**25-hydroxycholesterol enhances cytokine release and toll-like
receptor 3 response in airway epithelial cells**

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Additional file 1

Figure legends

Additional Figure 1. Effect of NF- κ B and MAPK inhibitors on cell viability in 25-HC-treated HBEpC. Cells were treated with 10 μ M 25-HC or vehicle in the presence of an NF- κ B inhibitor, caffeic acid phenethyl ester (CAPE)(A), an I κ B α inhibitor, BAY 11-7085 (B), an IKK-2 inhibitor, SC-514 (C), a JNK inhibitory peptide, L-JNKi1 (D), a p38 MAPK inhibitor, SB203580 (E) or a MEK1/2 inhibitor, U0126 (F). After 24 h, the cell viability was assessed by MTT assay. Cell viability is calculated as a percentage of the viable cells in the vehicle treated group. The data are expressed as mean values \pm SEM for four separate experiments with HBEpC from two donors. BAY = BAY 11-7085. N.S. = not significant.

Additional Figure 2. Effect of NF- κ B inhibitors on cell viability in 25-HC and poly(I:C)-treated cells. (A) Effect of an NF- κ B inhibitor, CAPE on cell viability in poly(I:C)-treated HBEpC. Cells were treated with various concentrations of CAPE

30 min prior to the treatment with 10 µg/ml poly(I:C). After 24 h, cell viability was assessed by MTT assay. (B-D) Effect of the NF-κB inhibitor on cell viability in 25-HC and poly(I:C)-treated cells. Various concentrations of the NF-κB inhibitor, CAPE (B), an IκBα inhibitor, BAY 11-7085 (C) or an IKK-2 inhibitor, SC-514 (D) were added before 10 µM 25-HC treatment, and the cells were then cultured in the presence of 10 µg/ml poly(I:C). After 24 h, cell viability was assessed by MTT assay. Cell viability is calculated as a percentage of the viable cells in the vehicle treated group. The data are expressed as mean values ± SEM for three to four separate experiments with HBEpC from two donors. BAY = BAY 11-7085. N.S. = not significant.