

Materials and Methods

Materials

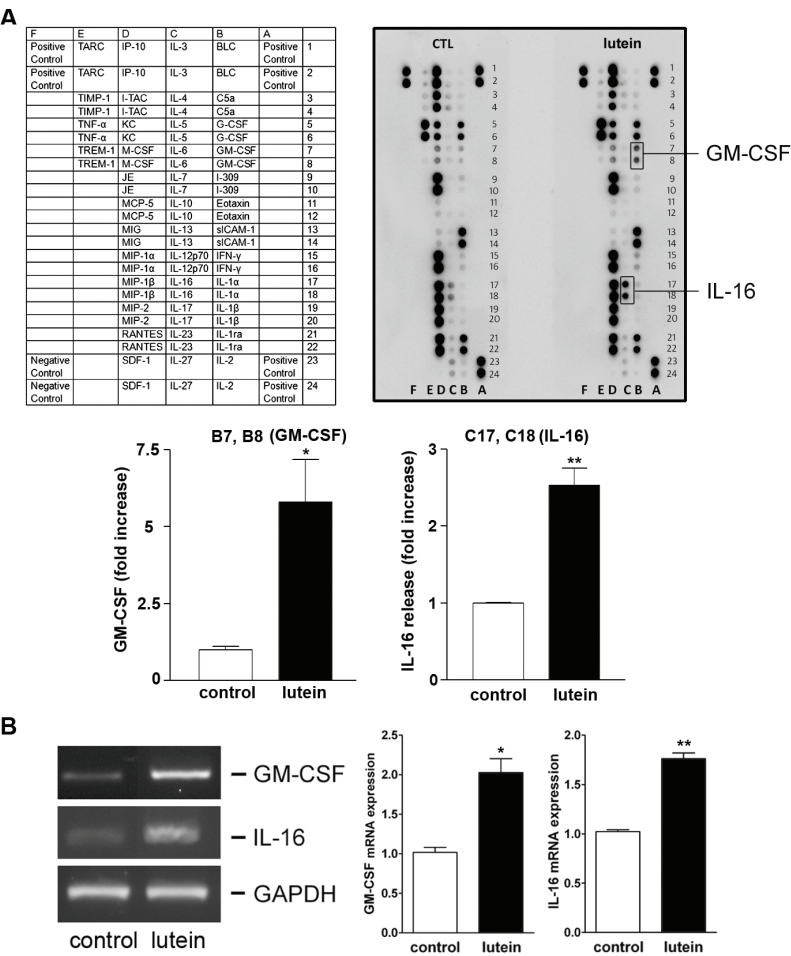
Mouse cytokine array (ARY006) was purchased from R&D systems, Inc. (MN, USA).

All RAR/RXR agonists were from Tocris Bioscience (Bristol, United Kingdom).

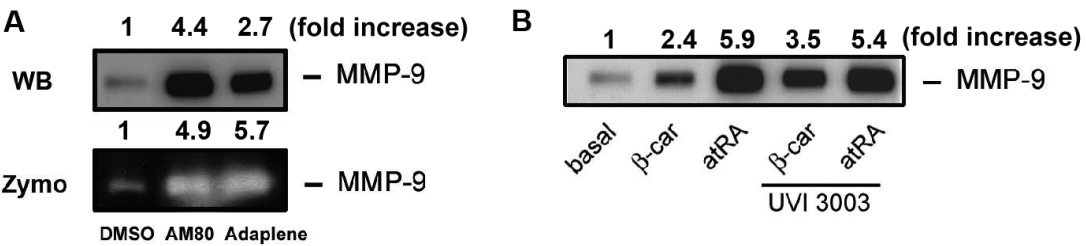
Methods

Cytokine antibody array assay

Cytokine antibody array was performed with a human cytokine array kit (R&D Systems) according to the manufacturer's protocol. Briefly, cell culture supernates were collected. After centrifuged, the membranes precoated with capture antibodies were incubated with supernates. After washed with wash buffer and added with detection antibody, the membranes were by adding streptavidin-HRP and Chemi Reagent Mix. The immunoblot images were captured and visualized using the BioSpectrum Imaging System (Ultra-Violet Products Ltd., Cambridge, UK) and the intensity of each spot in the captured images was analyzed using the ImageQuant 5.0 software (Molecular Dynamics).



Supplementary Figure 1. Effect of lutein on GM-CSF and IL-16 production. (A) Cytokine antibody assay. Left panel: a mouse cytokine antibody array panel showing distribution of 40 cytokine capture antibodies immobilized on membrane. Right panel: Mouse RAW264.7 macrophages were left untreated (control) or treated with lutein (10 μ M) for 24 h. The conditioned media were collected and mouse cytokine array assay were performed. A representative array image was shown. Lower panels: Quantitative analysis of GM-CSF and IL-16 release by densitometry. (B) RT-PCR analysis of GM-CSF and IL-16 mRNA level. RAW264.7 macrophages were treated with lutein (10 μ M) for the indicated time intervals or 8 h. GM-CSF and GAPDH mRNA level were analyzed by RT-PCR. Data were mean $\pm$ S.E.M. (n=2~4). * P < 0.05, ** P < 0.01 versus control.



Supplementary Figure 2. Effect of RAR agonists and RXR antagonist on MMP-9 production. (A) Cells were treated with vehicle, AM80 (RAR α agonist), or adaplene (RAR β/γ agonist) (1 μ M each) for 16 h. (B) Cells were pretreated with vehicle or UVI3003 (1 μ M) for 1 h and then incubated with atRA (0.1 μ M) or β -car (10 μ M) for 16 h. The MMP-9 release in medium was analyzed by zymography or Western blotting. Quantitative analysis of MMP-9 expression was performed by densitometry. The numbers shown above each panel indicate fold increase of basal in this representative blot from three independent experiments.