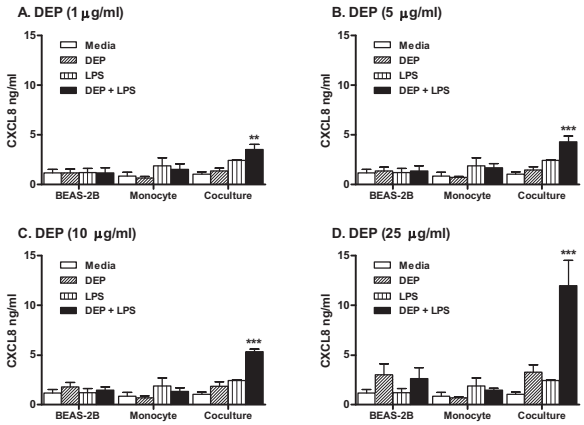
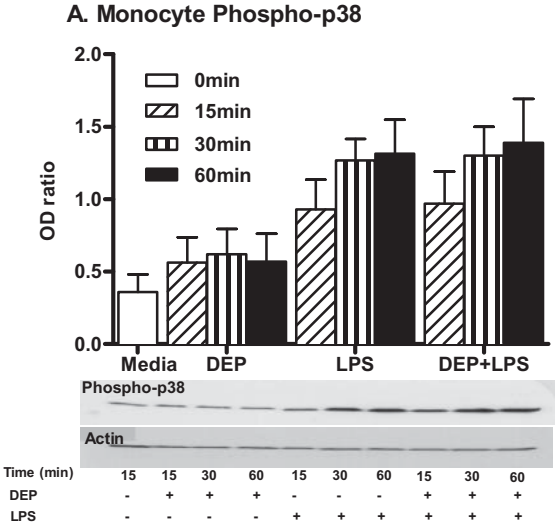
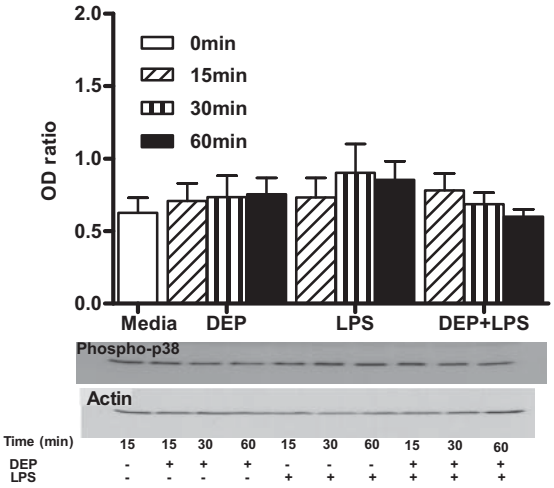
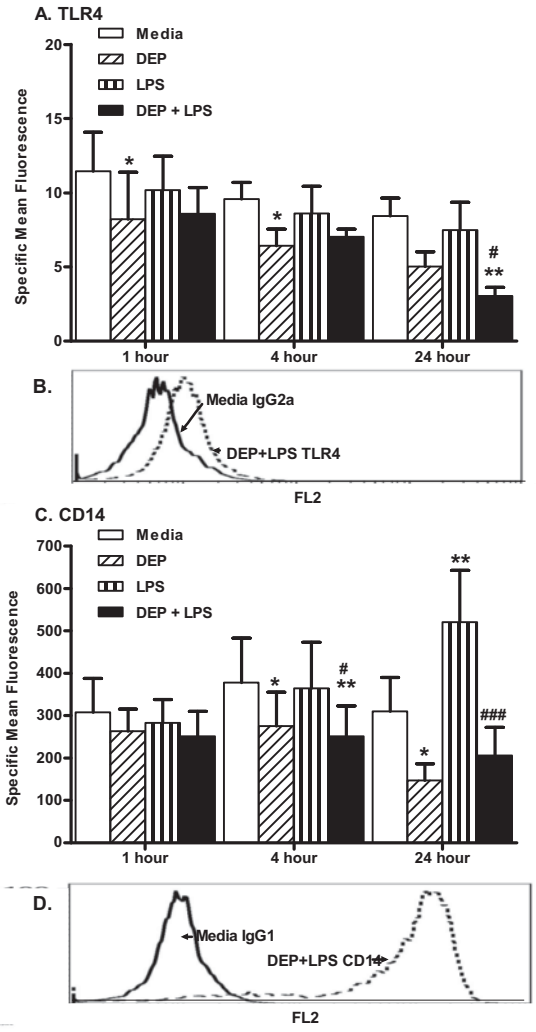
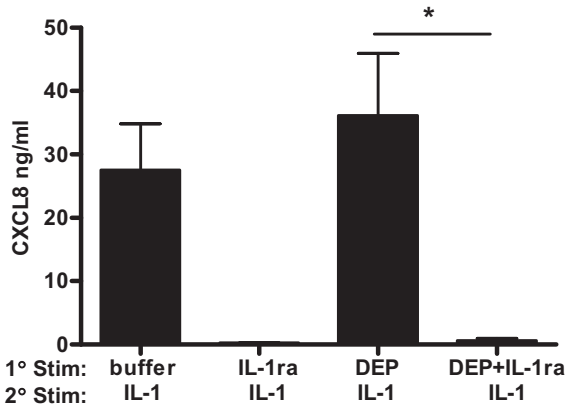
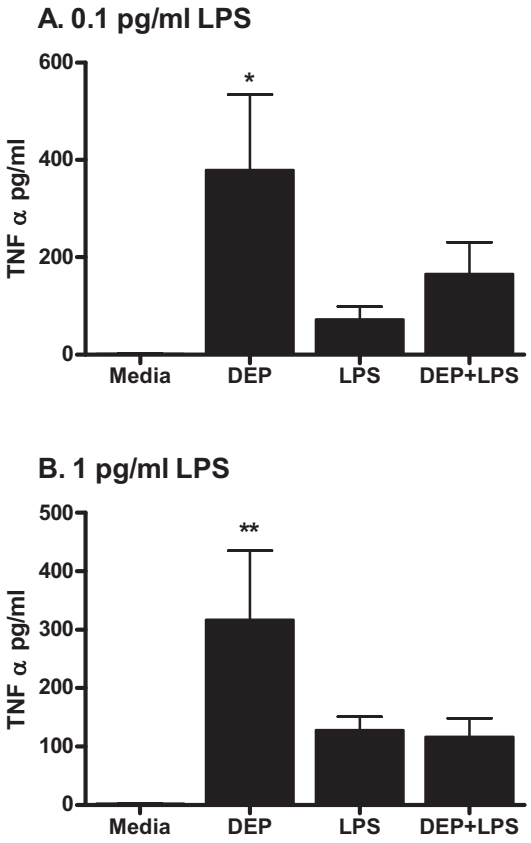

A. DEP (1 μg/ml)
B. DEP (5 μg/ml)
C. DEP (10 μg/ml)
D. DEP (25 μg/ml)
Media
DEP
LPS
DEP + LPS
CXCL8 ng/ml
15
10
5
0
**
***
BEAS-2B
Monocyte
Coculture


A. Monocyte Phospho-p38
OD ratio
2.0
1.5
1.0
0.5
0.0
0min
15min
30min
60min
Media
DEP
LPS
DEP+LPS
Phospho-p38
Actin
Time (min) 15 15 30 60 15 30 60 15 30 60
DEP - + + + - - - + + +
LPS - - - - + + + + + +


B. Epithelial Cell Phospho-p38
OD ratio
2.0
1.5
1.0
0.5
0.0
0min
15min
30min
60min
Media
DEP
LPS
DEP+LPS
Phospho-p38
Actin
Time (min) 15 15 30 60 15 30 60 15 30 60
DEP - + + + - - - + + +
LPS - - - - + + + + + +


A. TLR4
Media
DEP
LPS
DEP + LPS
Specific Mean Fluorescence
20
15
10
5
0
1 hour
4 hour
24 hour
B.
Media IgG2a
DEP+LPS TLR4
FL2
C. CD14
Media
DEP
LPS
DEP + LPS
Specific Mean Fluorescence
700
600
500
400
300
200
100
0
1 hour
4 hour
24 hour
D.
Media IgG1
DEP+LPS CD14
FL2


*
50
40
30
20
10
0
CXCL8 ng/ml
1° Stim:
2° Stim:
buffer
IL-1
IL-1ra
IL-1
DEP
IL-1
DEP+IL-1ra
IL-1


A. 0.1 pg/ml LPS
600
400
200
0
TNF α pg/ml
*
Media
DEP
LPS
DEP+LPS
B. 1 pg/ml LPS
500
400
300
200
100
0
TNF α pg/ml
**
Media
DEP
LPS
DEP+LPS

Supplementary Figure 1. Lower concentrations of DEP potentiates the proinflammatory response of LPS epithelial cell and monocyte cocultures.

Epithelial cell/monocyte cocultures were established as described in Figure 1. Cells were stimulated with varied concentrations of DEP (as shown) in the presence or absence of 0.01 pg/ml LPS. After 24 hours, levels of CXCL8 in the supernatant were determined by ELISA. Data shown are mean ± SEM of n=3 replicates. Each replicate was performed at a separate passage with freshly prepared monocytes from different donors. Significant differences in CXCL8 are indicated by **$p<0.01$ and ***$p<0.001$ compared to DEP or LPS alone, as measured by 2 way ANOVA and Bonferroni's post test.

Supplementary Figure 2. DEP does not enhance p38 phosphorylation in monocytes or epithelial cells.

Monocytes (A) or BEAS-2B cells (B) were stimulated in 12 well plates with 50 µg/ml DEP, 1 pg/ml LPS or both DEP and LPS for 15, 30 and 60 minutes. Cell lysates were analysed for phospho-p38 and actin expression by western blot. Quantitative signals were derived by densitometric analysis using NIH image 1.62 and data displayed as ratio compared to actin loading control. Data shown are mean ± SEM of n=3 replicates. Each replicate was performed at a separate passage or on freshly prepared monocytes from a different donor. Significant differences are indicated by **$p<0.01$ and ***$p<0.001$ compared to media, measured by 2-way ANOVA and Bonferroni's post test.

Supplementary Figure 3. Effects of DEP on TLR4 and CD14 expression in monocytes.

Monocytes were stimulated in 96 flexi-well plates, to minimise adherence, with 50 μg/ml DEP, 1 pg/ml LPS or both DEP + LPS and incubated for 1, 4 and 24 hours. Surface protein expression was assessed using PE-conjugated anti-human TLR4 (A) or CD14 (C) antibodies with appropriate isotype controls. TLR4 and CD14 fluorescence was detected in the FL-2 channel of a FACSCalibur flow cytometer. Data shown are representative of n=4 (each from a separate donor), with representative flow cytometry histograms also shown (B and D). Significant differences are indicated by *$p<0.05$, **$p<0.01$ and ***$p<0.001$ compared to media at the same time point and #$p<0.05$, and ###$p<0.001$ compared to LPS at the same time point, measured by 1-way ANOVA and Dunnett's post test for each time point.

Supplementary Figure 4. IL-1ra incubated with DEP retains biological activity.

Epithelial cells were stimulated for 3 hours (1° Stim) with buffer, 10 μg/ml IL-1ra, 50 μg/ml DEP or both IL-1ra and DEP. Cells were then stimulated for a further 21 hours (2° Stim) with 1 ng/ml IL-1. Data shown are mean ± SEM of n= 3 experiments. Each experiment was performed at a separate passage of BEAS-2B cells. Significant differences are indicated by *$p<0.05$ as measured by 1-way ANOVA and Tukey's post test.

Supplementary Figure 5. DEP causes release of TNFα in epithelial cell and monocyte cocultures.

Epithelial cell/monocyte cocultures were established as described in Figure 1. Cells were stimulated with 50 µg/ml DEP, LPS or combined DEP+LPS. After 24 hours, levels of TNFα in the supernatant were determined by ELISA. Data shown are mean ± SEM of n=6 replicates. Each replicate was performed at a separate passage with freshly prepared monocytes from different donors. Significant differences compared to media are indicated by *$p<0.05$ and **$p<0.01$, as measured by 2 way ANOVA and Bonferroni's post test.