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DATA SUPPLEMENT

INDUCTION OF THE INFLAMMATORY REGULATOR A20 BY GIBBERELLIC ACID IN AIRWAY EPITHELIAL CELLS

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Key Words: A20 protein; NF-kappaB; Gibberellic Acid, Airway epithelial cells; Inflammation.
EXPERIMENTAL PROCEDURES

Quantitative real time qPCR

Primers were designed using gene accession numbers and Primer3 open-source PCR primer design software and obtained from Invitrogen Ltd. (Paisley, UK). Primer sequences are given in Table S1.

Table S1: PCR primer sequences.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Accession Number</th>
<th>Sequence</th>
</tr>
</thead>
</table>
| Beta-actin | NM_001101.3 | 5’ctctttcagccttctctcct 3’  
|         |                  | 3’agcacgtggttggcgtacacg 5’          |
| A20    | NM_006290        | 5’gagacgcaatggctgtaa 3’  
|         |                  | 3’tccagttgtgtatcctggtacat 5’      |
| p65    | NM_021975        | 5’ctctggagccagtacgatc 3’  
|         |                  | 3’cactgtcacctggaagcaga 5’          |
| TLR4   | NM_003266.3      | 5’tggacaatttgctagaggg 3’  
|         |                  | 3’gatccacccatctggtct 5’           |
SUPPLEMENTARY RESULTS

Cell Proliferation

[Graph showing cell proliferation with GA3 concentrations]

Figure S1: GA3 does not induce cellular proliferation. 16HBE14o- cells were incubated with GA3 (0-300 µM) for 72h and cell proliferation was determined in using the CellTiter 96® AQueous One Solution Cell Proliferation Assay (Promega, UK) according to the manufacturer’s recommendations. No change in proliferation was observed.

Purity of GA3 preparation - Expression of TLR4 mRNA

[Graph showing TLR4 mRNA expression]

Figure S2: GA3 preparation does not induce TLR4 mRNA expression. To determine if the GA3 preparation used in this study may contain traces of endotoxin, expression of TLR4 and β-actin (housekeeping gene) were assessed by qPCR. LPS alone [10 µg/ml] significantly increased TLR4 mRNA expression in a time dependent manner (p<0.01 LPS 1h vs. LPS 24h, Kruskal Wallis with Dunn’s post hoc test, n=5), but exposure of cells to GA3 [30 µM] did not induce TLR4.