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## Supporting Information

## for

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**Bromodomain inhibitor I-BET151 suppresses immune responses during fungal–immune interaction**

| Compound Number | Cytokine | Mean pIC50 | SD | Range | Number of curves (n) | Mean Hill Slope | Mean asymptote max |
|---|---|---|---|---|---|---|---|
| GSK1210151A | TNFα | 6,5 | 0,43 | 5.9 - 7.8 | 68 | 1,3 | 105 |
| GSK1210151A | IL-6 | 6,1 | 0,36 | 5.5 - 7.8 | 116 | 1,1 | 98 |

| % Inhibition | [GSK1210151A] M | |
|---|---|---|
| | TNFα | IL-6 |
| 1,25 | 1,10E-08 | 1,50E-08 |
| 2,5 | 1,89E-08 | 2,84E-08 |
| 5 | 3,28E-08 | 5,46E-08 |
| 10 | 5,83E-08 | 1,08E-07 |
| 20 | 1,09E-07 | 2,25E-07 |
| 30 | 1,65E-07 | 3,68E-07 |
| 40 | 2,31E-07 | 5,49E-07 |
| 50 | 3,16E-07 | 7,94E-07 |
| 60 | 4,32E-07 | 1,15E-06 |
| 70 | 6,07E-07 | 1,72E-06 |
| 80 | 9,19E-07 | 2,80E-06 |
| 90 | 1,71E-06 | 5,85E-06 |
| 95 | 3,05E-06 | 1,15E-05 |
| 97,5 | 5,30E-06 | 2,22E-05 |

**Figure S1. Target engagement plots for GSK1210151A (I-BET151).**

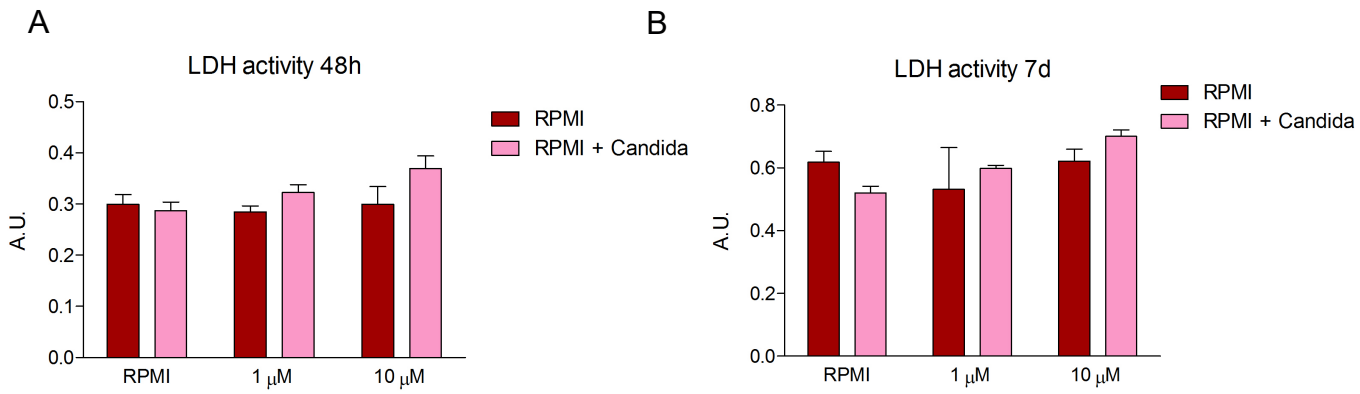


**Figure S2. Cytotoxicity assay for I-BET151 treatment**. Detection of cell death by lactate dehydrogenase (LDH) assay in PBMCs treated with control medium, 1 μM or 10 μM I-BET151 and stimulated with RPMI or $10^6$ heat-killed *C. albicans* conidia during 48h (A) or 7 days (B) (mean ± SEM, n =3 (from 3 different individual donors)).

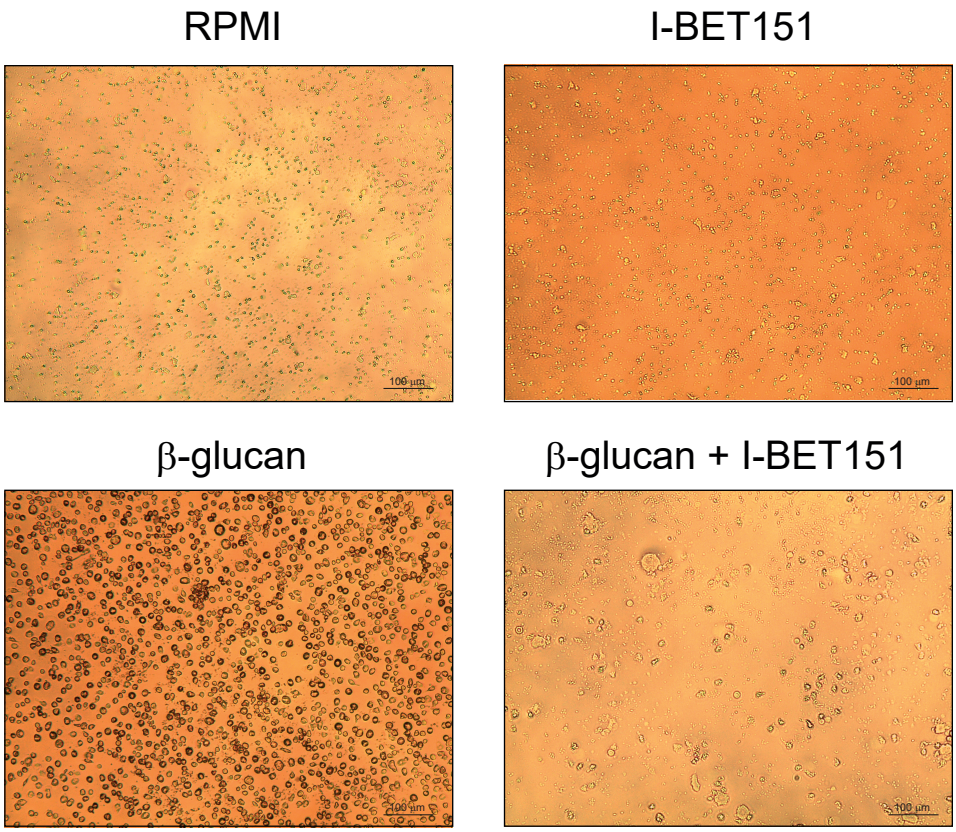


**Figure S3. Morphological changes induced by trained immunity.**

Morphology of cells after 24 h of training and 6 days of rest when the cells were trained with RPMI (negative control), 10 µM I-BET151, 1 mg/ml β-glucan or 10 µM I-BET151 + 1 mg/ml β-glucan. Pictures were taken at day 6, magnification x10.

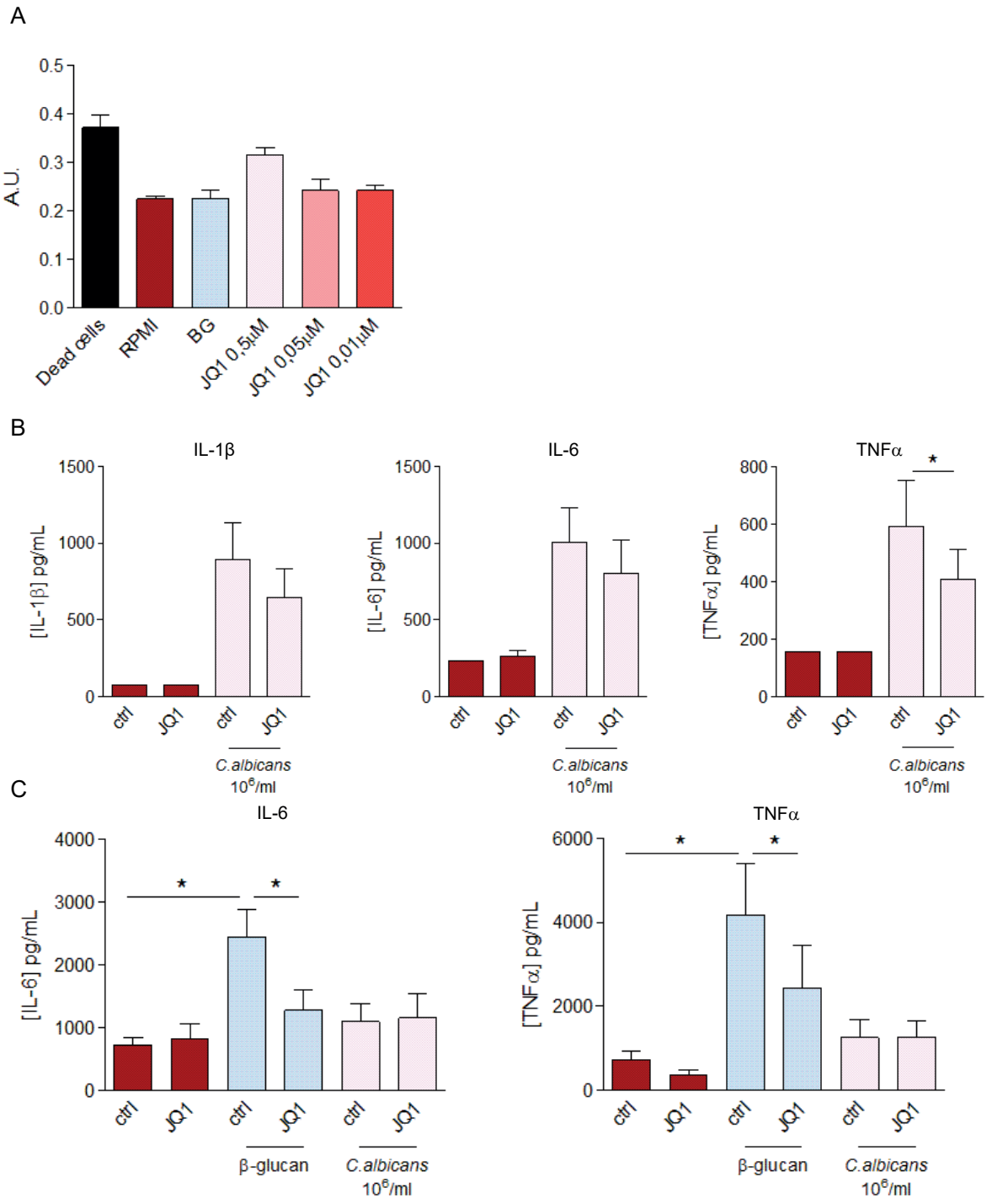


**Figure S4. Effects of JQ1 in the induction of trained immunity.**

(A) Cytotoxicity assay for the treatment of cells with three different concentrations of JQ1. Dead cells were used as a positive control. (B) IL-1β, IL-6 and TNFα production by human monocytes treated or not with 0.05 µM JQ1 and stimulated with $10^6$/ml heat-killed *C. albicans* conidia for 24 h. (C) IL-6 and TNFα production by human monocytes treated or not with 0.05 µM JQ1 and stimulated with RPMI, 1 µg/mL β-glucan or $10^6$/ml heat-killed *C. albicans* conidia, following a similar protocol displayed in Figures 3A and 3D. Cells were re-exposed to 10 ng/ml LPS after 6 days, and cytokines were measured 24 h later. Mean ± SEM, n=6 (from 6 different individual donors); pooled from 2 independent experiments with 3 individual donors each. *p<0.05, Wilcoxon signed-rank test.

**Table 1. Primer pairs used for epigenetic study.**

| | | |
|---|---|---|
| *IL6.1* | FW | TCGTGCATGACTTCAGCTTT |
| *IL6.1* | RV | GCGCTAAGAAGCAGAACCAC |
| *IL6.2* | FW | AGGGAGAGCCAGAACACAGA |
| *IL6.2* | RV | GAGTTTCCTCTGACTCCATCG |
| *TNFA.1* | FW | AGAGGACCAGCTAAGAGGGA |
| *TNFA.1* | RV | AGCTTGTCAGGGGATGTGG |
| *TNFA.2* | FW | GTGCTTGTTCCTCAGCCTCT |
| *TNFA.2* | RV | ATCACTCCAAAGTGCAGCAG |
| *TNFA.3* | FW | TGTCTGGCACACAGAAGACA |
| *TNFA.3* | RV | CCCTGAGGTGTCTGGTTTTC |
| *TNFA.4* | FW | AGCCAGCTGTTCCTCCTTTA |
| *TNFA.4* | RV | TTAGAGAGAGGTCCCTGGGG |
| *TNFA.5* | FW | TGATGGTAGGCAGAACTTGG |
| *TNFA.5* | RV | ACTAAGGCCTGTGCTGTTCC |
| *TNFA.6* | FW | CAGGCAGGTTCTCTTCCTCT |
| *TNFA.6* | RV | GCTTTCAGTGCTCATGGTGT |

## Supplementary Methods

### LPS stimulated human blood assay

Blood was collected into 10 IU / mL sodium heparin and incubated for 30 min at 37ºC in a concentration response (10 µM-0.51nM) of I-BET151, prior to the addition of 200 ng/mL LPS. Supernatants were removed after 22h incubation 37ºC and analysed for IL-6 and TNFα using MesoScaleDescovery (MSD) technology. ICxx calculations were determined using the formula ICxx = ((% inhibition / (100-% inhibition))^(1/Hill Slope))*IC50).

### Viability assays

Cell viability was assessed using CytoTox 96® Non-Radioactive Cytotoxicity Assay (Promega). Released LDH in culture supernatants is measured with a 30-minute coupled enzymatic assay. The amount of color formed is proportional to the number of lysed cells.