

SUPPLEMENTARY DATA:

**Table S1.** TaqMan® gene expression assays

Species	Gene	Assay ID	Provider
Mouse	<i>HPRT</i>	Mm03024075_m1	Life technologies
Mouse	<i>Il1b</i>	Mm00434228_m1	Life technologies
Mouse	<i>Il1α</i>	Mm00439620_m1	Life technologies
Mouse	<i>Cxcl1</i>	Mm04207460_m1	Life technologies
Mouse	<i>Cxcl2</i> (=MIP-2)	Mm00436450_m1	Life technologies
Mouse	<i>Tnf</i>	Mm00443258_m1	Life technologies
Mouse	<i>Csf2</i> (=GM-CSF)	Mm01290062_m1	Life technologies
Mouse	<i>Il6</i>	Mm00446190_m1	Life technologies
Mouse	<i>Ccl3</i> (= MIP-1α)	Mm00441258_m1	Life technologies
Mouse	<i>Ccl4</i> (=MIP-1β)	Mm00443111_m1	Life technologies

**Table S2:** Histopathological findings

Groups	Presence of inflammation and location	Type of inflammatory infiltrate	Inflammation intensity	Perivascular oedema
Control group (7 mice)	14.3% (1/7) Perivascular and bronchiolitis in 1 case (+ subpleural lymphoid tissue)	Lymphocytes and plasma-cells	- Mild: 14.3% (1/7) - Moderate: 0% - Severe: 0%	Absence
Spore group (7 mice)	87.5% (6/7) Bronchiolitis, alveolitis and perivascular in 6 cases	Lymphocytes, plasma-cells and rare neutrophils/eosinophils	- Mild: 14.3% (1/6) - Moderate: 71.4% (4/6) - Severe: 14.3% (1/6)	100% (7/7)

**Table S3.** Summary of inflammatory mediators increases in BAL of mice exposed to spores compared to controls, with fold increases and p-values.

Inflammatory mediators	Fold increase	<i>p-value</i>
TNF- $\alpha$	1.33	0.0087
CXCL-1	2	0.0155
IL-1 $\beta$	1.5	0.0052
IL-6	5	<0.0001
IL-4	12	0.0006
IL-5	14	0.0012
IL-9	2	0.0261
IL-10	2.33	0.0157
IL-12(p70)	4	0.0004
IL-13	1.77	0.0277
IL-17A	2.55	0.0023
Eotaxin	5	0.0061
G-CSF	4.7	0.0041
IFN- $\gamma$	2	0.0495
MCP-1	1.5	0.0064
MIP-1 $\alpha$	6.6	0.0070
MIP-1 $\beta$	36	0.0006

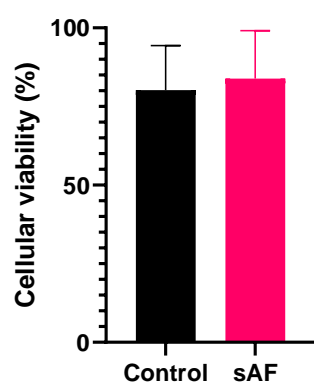
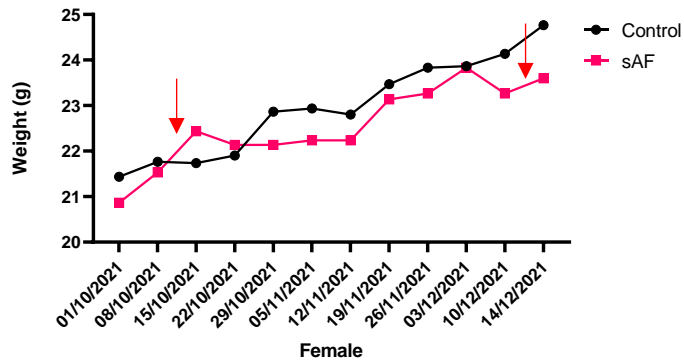
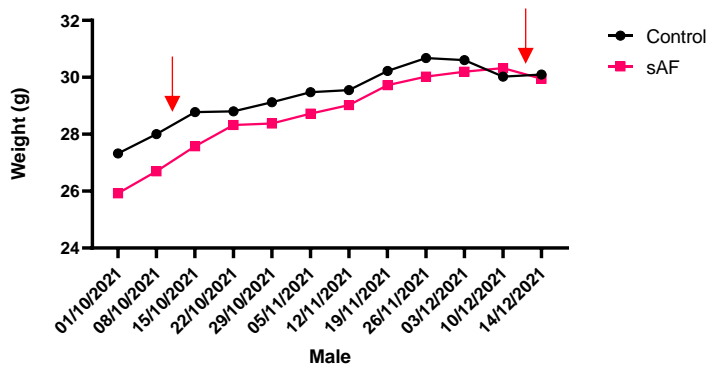


Figure S1: Percentage of cellular viability on co-cultured cells. No statistical difference between control cells and cells challenged with AFsp ( $p=0.7326$ ).



**A**



**B**

Figure S2: Weight of female (A) and male (B) mice after exposure to *Aspergillus fumigatus* spores. No difference between control and challenged mice in body weight gain or lose. Times where instillations were made are indicated with red arrows.

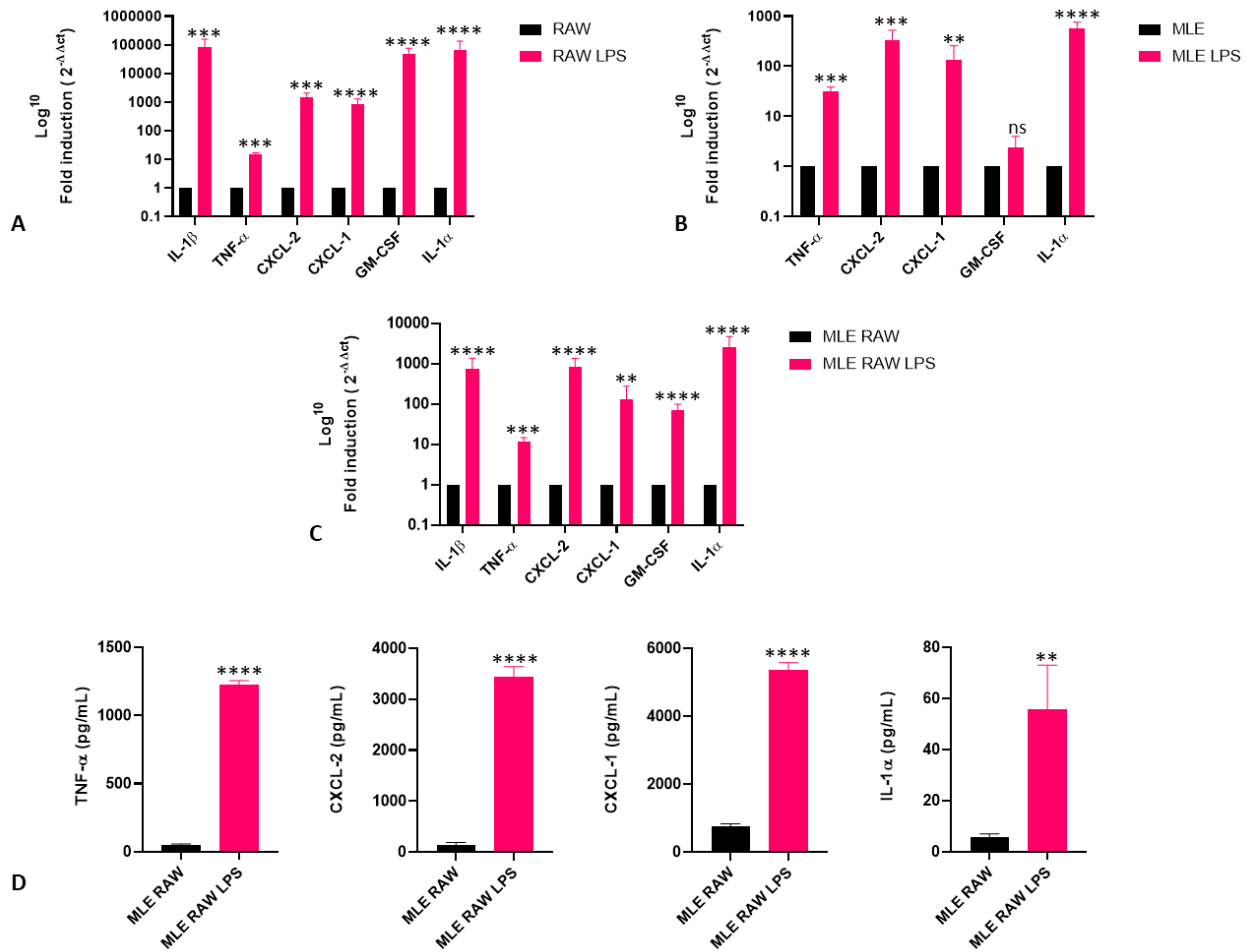


Figure S3: Gene expression and secretion of defined inflammatory mediators after a 4-hour exposure of RAW 264.7, MLE-15 and co-cultured MLE RAW cells to LPS at 1µg/mL (A) Quantification of the gene expression of inflammatory markers in RAW 264.7 cells by RT-qPCR. (B) Quantification of the gene expression of inflammatory markers in MLE-15 cells by RT-qPCR. (C) Quantification of the gene expression of inflammatory markers in co-cultured MLE-15/RAW 264.7 cells by RT-qPCR. (D) Cytokine secretion in the supernatants. Data are represented by mean with SD. (\*\*\*\*  $p<0.0001$ , \*\*\* $p<0.001$ , \*\* $p<0.01$  versus untreated cells,  $n=3$  for monocultures and  $n=4$  for co-culture)