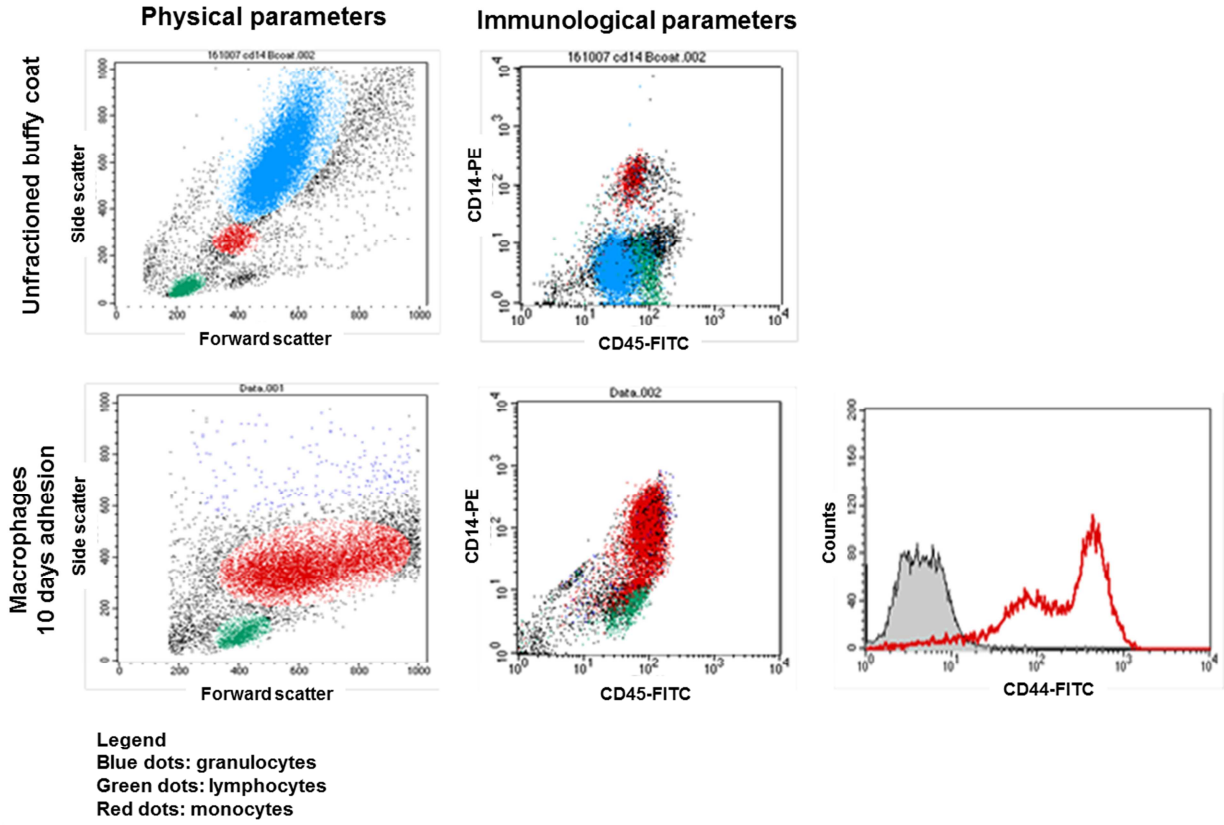
Serafino *et al.* "Thymosin α1 activates complement receptor-mediated phagocytosis in human monocyte-derived macrophages." SUPPLEMENTARY FIGURES



Supplementary Fig. S1. Evaluation of the purity and maturation of macrophage cultures tested by flow cytometry. The lymphocytic/monocytic cellular fraction was isolated from buffy coats of healthy donors by density gradient centrifugation using Lympholyte-H, then seeded on flasks and maintained at 37°C in 5% CO_2, to obtain adhering macrophages. After 1 hr of culture, non-adhering cells were removed and the residual adhering MDMs were maintained in culture. Unfractioned buffy coat and the monocyte-derived macrophages after 10 days of grown in adhesion were analysed for physical (forward and side scattering) and immunological parameters (CD14, CD45 and CD44 expression). The antibodies used for the immunological assessment were:

- FITC-conjugated mouse monoclonal antibody against human CD45
- PE-conjugated mouse monoclonal antibody against human CD14
- FITC-conjugated mouse monoclonal antibody against human CD44

All antibodies were purchased by BD Pharmingen (San Diego, CA, USA).

The MDM culture resulted almost exclusively constituted by the CD45+ and CD14+ monocytic fraction (red dots), and expressed high levels of CD44 on cell surface, features of mature macrophages

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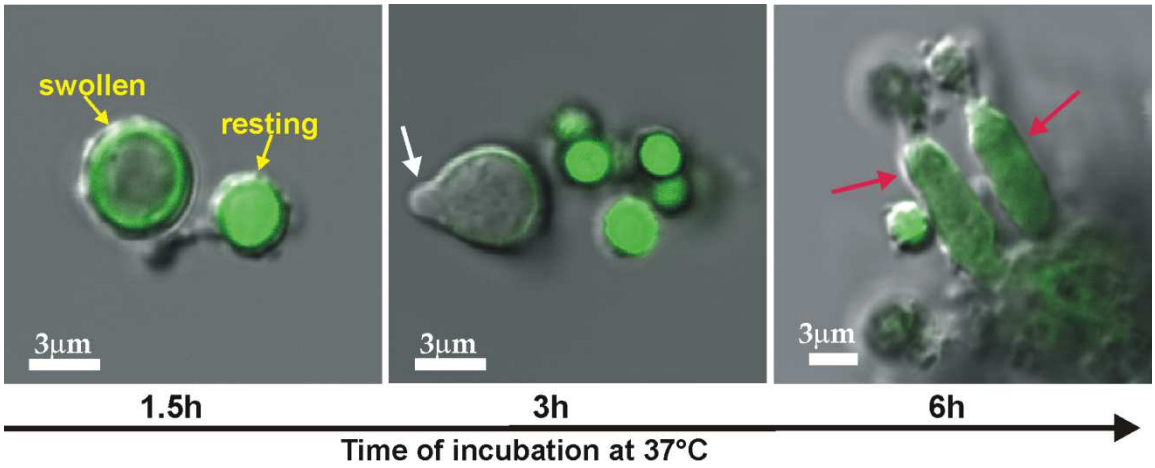
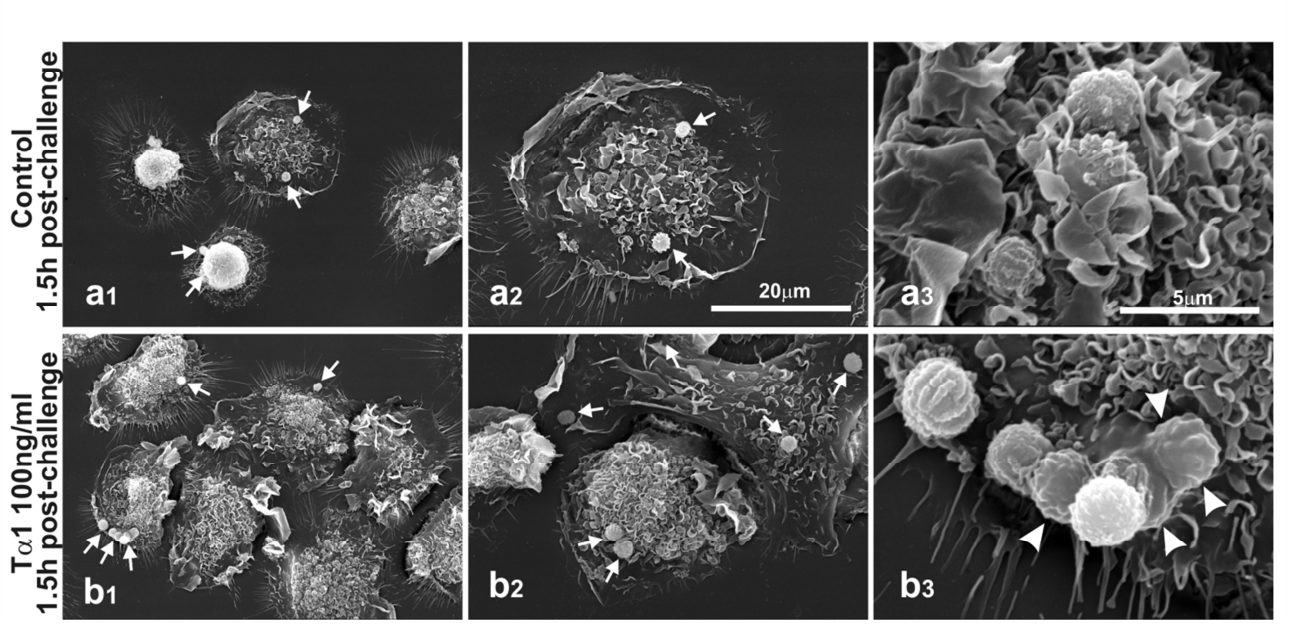


Supplementary Fig. S2. *Aspergillus* conidia viability tested after staining with Alexa Fluor 488 succinimidyl ester. After 3 hrs and 6 hrs of incubation at 37°C, the conidia retained their germination ability, producing both germ tube (white arrow) and hyphae (red arrows).



Supplementary Fig. S3. Effect of Tα1 on adhesion of *Aspergillus niger* conidia on human MDMs. Scanning electron microscopy of untreated (**a**) and Tα1-treated MDMs (**b**) 1.5 hrs post-challenge, showing interaction with *Aspergillus* conidia (arrows). In **(b$_3$)**, attached and internalized conidia associated with the same MDM are shown; arrowheads point to conidia completely surrounded by cellular protrusions indicative of phagocytosis. Bars = **a$_1$**, **b$_1$**, **a$_2$** and **b$_2$**: 20μm; **a$_3$** and **b$_3$**: 5μm.

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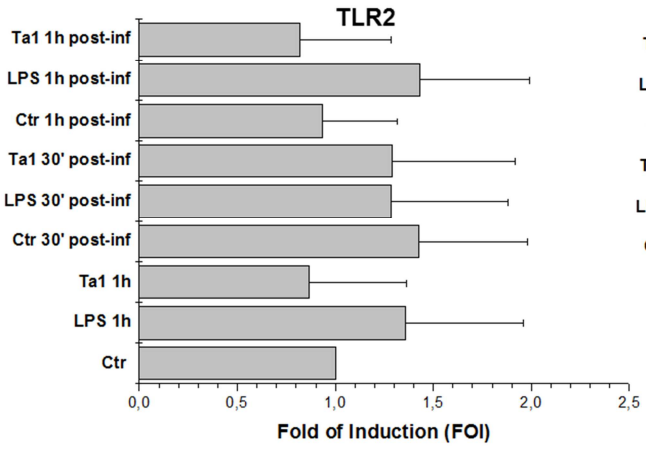
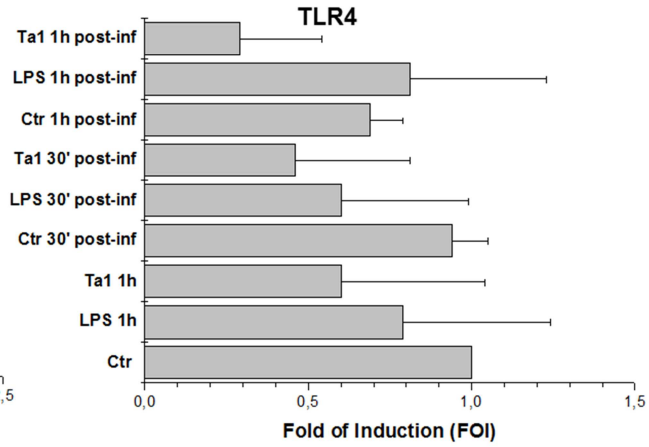
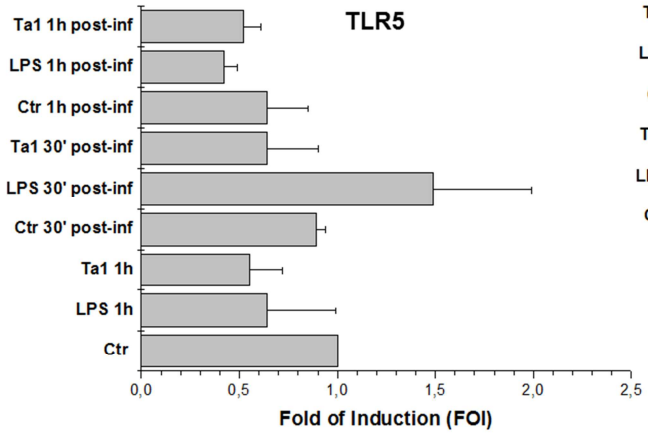
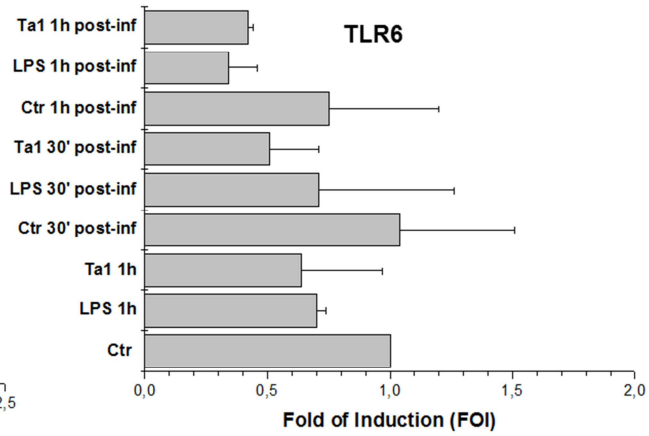
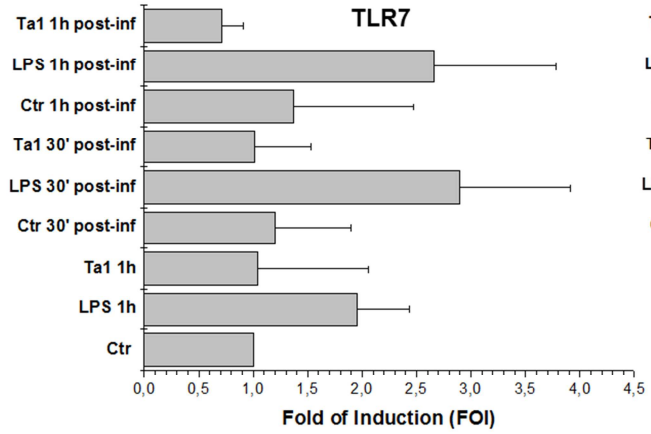
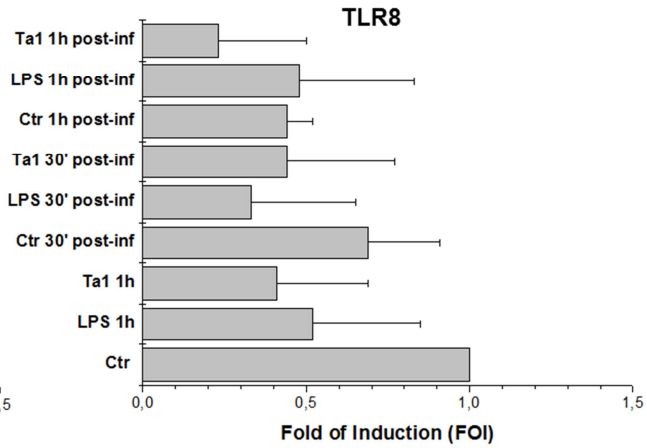


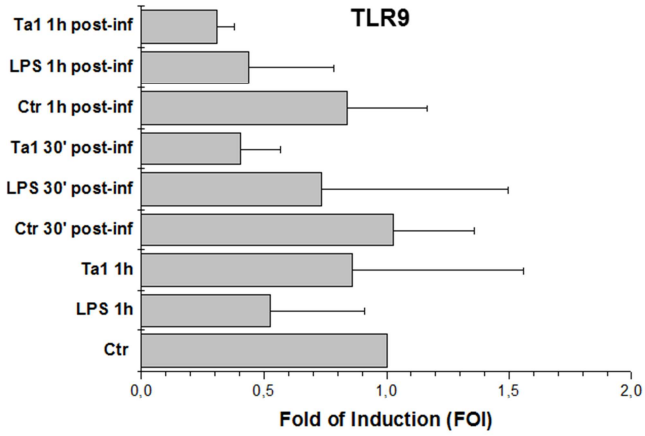




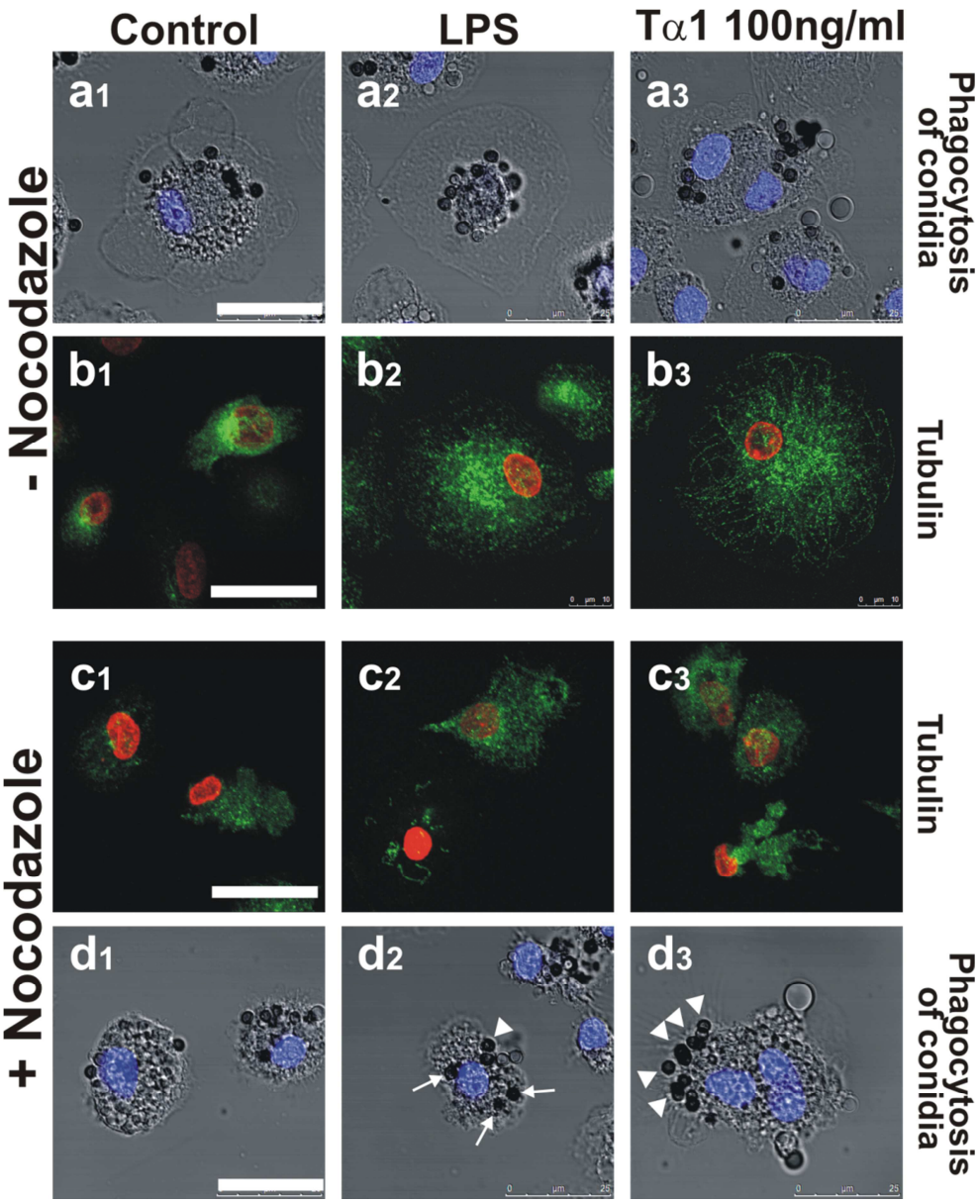








Supplementary Fig. S4. Effect of Tα1 treatment, before and after challenging with *A. niger* conidia, on the transcription levels of TLRs (TLR 2,TLR 4, TLR 5, TLR 6, TLR 7, TLR 8, TLR 9) by RT-PCR; results represent the mean fold of induction (FOI) from four independent experiments using macrophages obtained from four different donors in each experimental setting. Significance, calculated *vs* untreated control (for pre-treatments) or *vs* uninfected controls for each time point post-challenging, was $P<0.05$



Supplementary Fig. S5. Effect of microtubule destabilization by nocodazole on phagocytic activity of Tα1-stimulated human MDMs. **a**, **d** Confocal microscopy images showing the *Aspergillus* conidia uptake in control (**a_1**, **d_1**), LPS-stimulated (**a_2, d_2**) and Tα1-stimulated MDMs (**a, d_3**), in absence (**a**) or in presence (**d**) of nocodazole (2μg/ml for 30min); cell and conidia morphology were visualized by CLSM in bright field modality; cell nuclei were counter-stained with Hoechst (*blue* hue); arrows point to internalized conidia, arrowheads point to adhering conidia. **b**, **c** Confocal microscopy images, showing the microtubular network (*green* hue) in nocodazole-treated and untreated cells: **b_1, c_1**: controls; **b_2, c_2**: LPS pre-treated MDMs; **b_3, b_3**: Tα1-stimulated MDMs; cell nuclei were counter-stained with PI (*red* hue). Bars = 25μm.