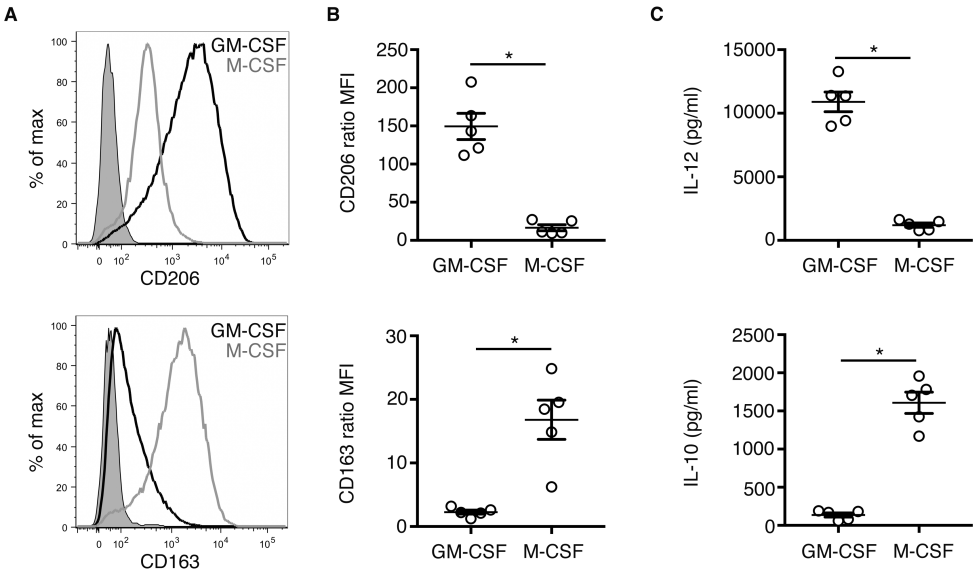
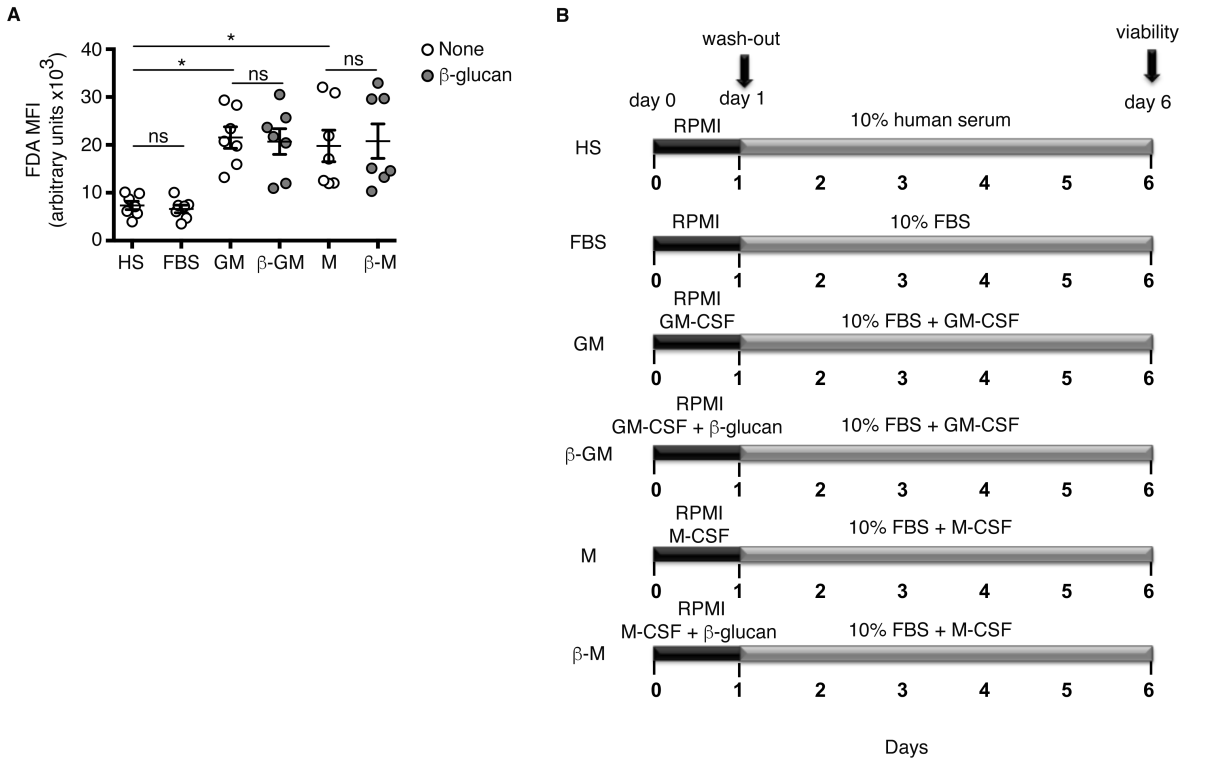
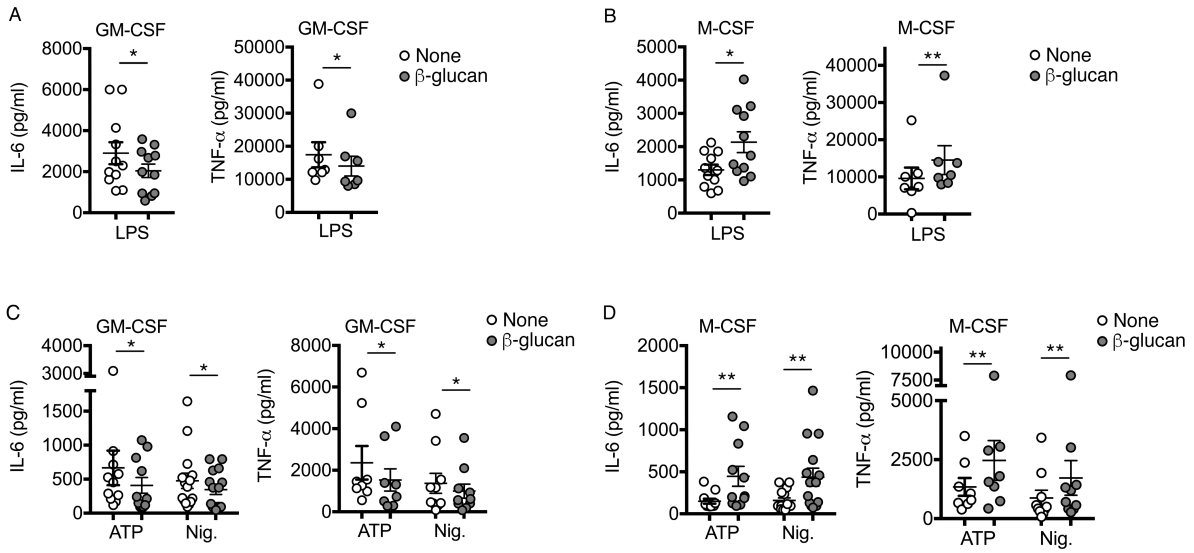


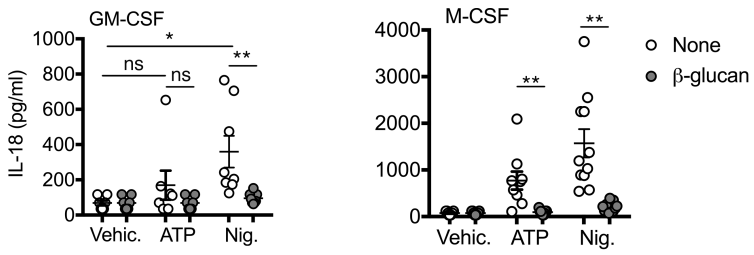
Supplemental Figure 1. Polarization of macrophages in M1-like (GM-CSF) and M2-like (M-CSF) macrophages. Monocytes were differentiated with either GM-CSF or M-CSF to obtain M1-like and M2-like macrophages, respectively. At day 6, macrophages were either stained for flow cytometric analysis (A, B) or stimulated for cytokine secretion analysis (C). (A, B) Macrophages were stained for flow cytometric analysis of GM-CSF-associated (CD206) and M-CSF-associated (CD163) markers. (A) One representative analysis out of five is shown. Histograms show the expression by total cells from GM-CSF (black line) or M-CSF (grey line) differentiated macrophages. Grey filled histograms represent the isotype control. (B) Data are expressed as MFI (Mean Fluorescence Intensity) relative to isotype control. Ratio ± SEM of the analysis of three independent experiments. (C) Macrophages were stimulated with LPS (24h, 50ng/mL) and culture supernatants were collected. Concentration of secreted IL-12 (pro-inflammatory cytokine associated with M1-like macrophages) and IL-10 (anti-inflammatory cytokine associated with M2-like macrophages) was determined by ELISA. Data represent mean±SEM of three independent experiments. (B-C) n=5; *$p<0.05$ (Wilcoxon matched-pairs ranked test).



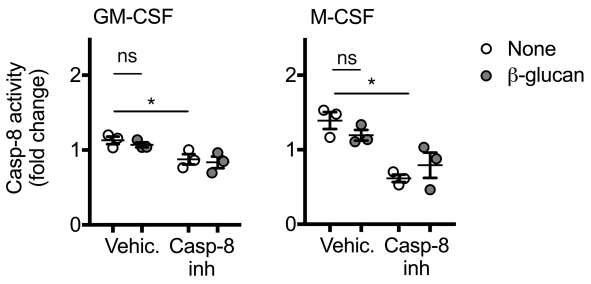
Supplemental Figure 2. Viability of cultured human primary monocytes. (A) Viability of cells was assessed by loading cells with Fluorescein diacetate (FDA) after differentiation of human primary monocytes for 6 days in different conditions as depicted in (B) and in Figure 1. Graphs show the mean ± SEM of at least three independent experiments. n = 7; *$p < 0.05$, (Wilcoxon matched-pairs signed-rank test). (B) Schematic overview of the different culture conditions used in (A). Monocytes were incubated for 24h with RPMI alone (HS and FBS conditions) as previously described (1, 2). In parallel, and as used throughout the present manuscript (Figure 1), monocytes were incubated for 24h with RPMI containing either GM-CSF or M-CSF in the presence (β-GM and β-M conditions respectively) or in the absence (GM and M conditions respectively) of purified β-glucan from *Candida albicans*. After a 24h incubation, cells were washed and differentiated for 5 days in RPMI supplemented with 10% human serum (HS condition), or 10% FBS alone (FBS condition), 10% FBS + GM-CSF (GM condition) or 10% FBS + M-CSF (M condition). The complete medium was changed once at day 3 and the amount of remaining viable cells was evaluated at day 6 by loading cells with Fluorescein diacetate (FDA).



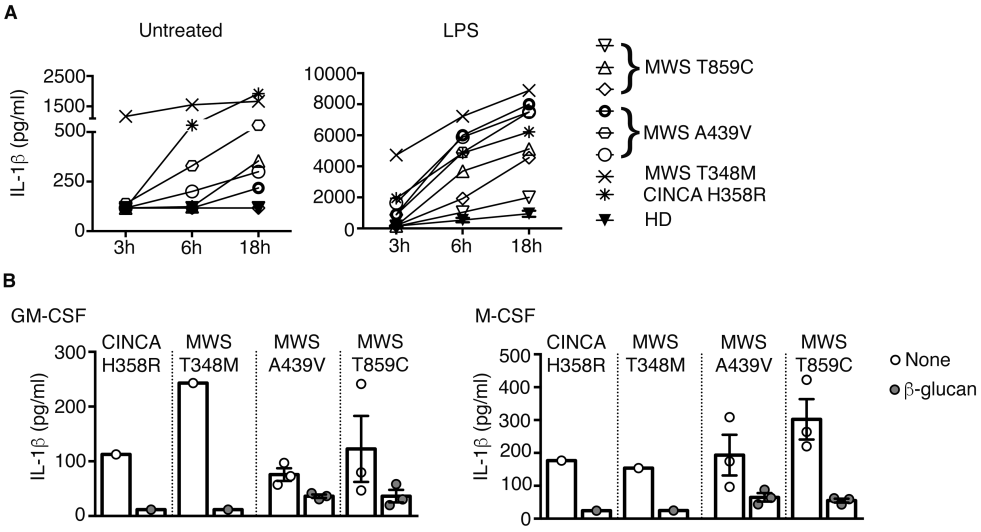
Supplemental Figure 3. Effect of β-glucan pre-incubation on IL-6 and TNFα release by macrophages upon LPS priming and NLRP3 inflammasome activation. Monocytes were pre-incubated or not with β-glucan and differentiated with either GM-CSF (A, C) or M-CSF (B, D). Macrophages were either only primed for 3h with LPS (A, B) or primed with LPS and then stimulated with ATP, or nigericin (C, D). Culture supernatants were collected and concentration of secreted IL-6 or TNFα was determined by ELISA. Graphs show the mean ± SEM of at least three independent experiments. n = 7-11; *p < 0.05, **p < 0.01 (Wilcoxon matched-pairs signed-rank test).



Supplemental Figure 4. Effect of β-glucan on IL-18 release upon NLRP3 inflammasome activation. Monocytes were pre-incubated with β-glucan or left untreated in a medium containing either GM-CSF or M-CSF. After 24h of pre-incubation, the cells were washed and differentiated into macrophages for an additional 5 days with GM-CSF or M-CSF. Macrophages were primed for 3h with LPS and then stimulated for 1h with ATP, nigericin or vehicle, as a control. Culture supernatants were collected and concentration of secreted IL-18 was determined by ELISA. Graphs show the mean ± SEM of at least three independent experiments. n = 8-11; *$p < 0.05$, **$p < 0.01$ (Wilcoxon matched-pairs signed-rank test).



Supplemental Figure 5. Effect of β-glucan on caspase-8 and NLRP3 inflammasome activation. Monocytes were pre-incubated for 24h with or without β-glucan and differentiated into macrophages with either GM-CSF or M-CSF. Macrophages were primed for 3h with LPS and then stimulated for 1h with nigericin. Cells + supernatants were then assayed for caspase-8 activity and normalized for the activity measured in LPS+vehicle-stimulated macrophages. z-IETD-fmk was used as specific caspase-8 inhibitor. Data represent mean values ± SEM of the analysis of 2 independent experiments. n=3; *$p < 0.05$ (Paired 2-tailed Student's test).



Supplemental Figure 6. Effect of β-glucan on IL-1β release by macrophages from CAPS patients according to NLRP3 mutation. (A) Monocytes from healthy donors and from CAPS subjects were left untreated or stimulated with 100 ng/mL of LPS for the indicated time. Culture supernatants were collected and concentration of secreted IL-1β was determined by ELISA. Data were separated according to syndromes and mutations of the patients. Mean values ± SEM are shown. HD, n=8; CINCA H358R, n=1; MWS T348M, n=1; MWS A439V, n=3; MWS T859C, n=3. (B) Monocytes from CAPS patients were pre-incubated with or without β-glucan and then differentiated with either GM-CSF or M-CSF. Macrophages were primed for 3h with LPS and then stimulated for 1h with nigericin. Culture supernatants were collected and concentration of secreted IL-1β was determined by ELISA. Mean values ± SEM are shown. Data were separated according to syndromes and mutations of the patients as in (A).

Supplemental References

1. Quintin J, Saeed S, Martens JHA, Giamarellos-Bourboulis EJ, Ifrim DC, Logie C, et al. Candida albicans infection affords protection against reinfection via functional reprogramming of monocytes. *Cell Host Microbe.* 2012;12(2):223-32.
2. Bekkering S, Blok BA, Joosten LA, Riksen NP, van Crevel R, and Netea MG. In Vitro Experimental Model of Trained Innate Immunity in Human Primary Monocytes. *Clinical and vaccine immunology : CVI.* 2016;23(12):926-33.