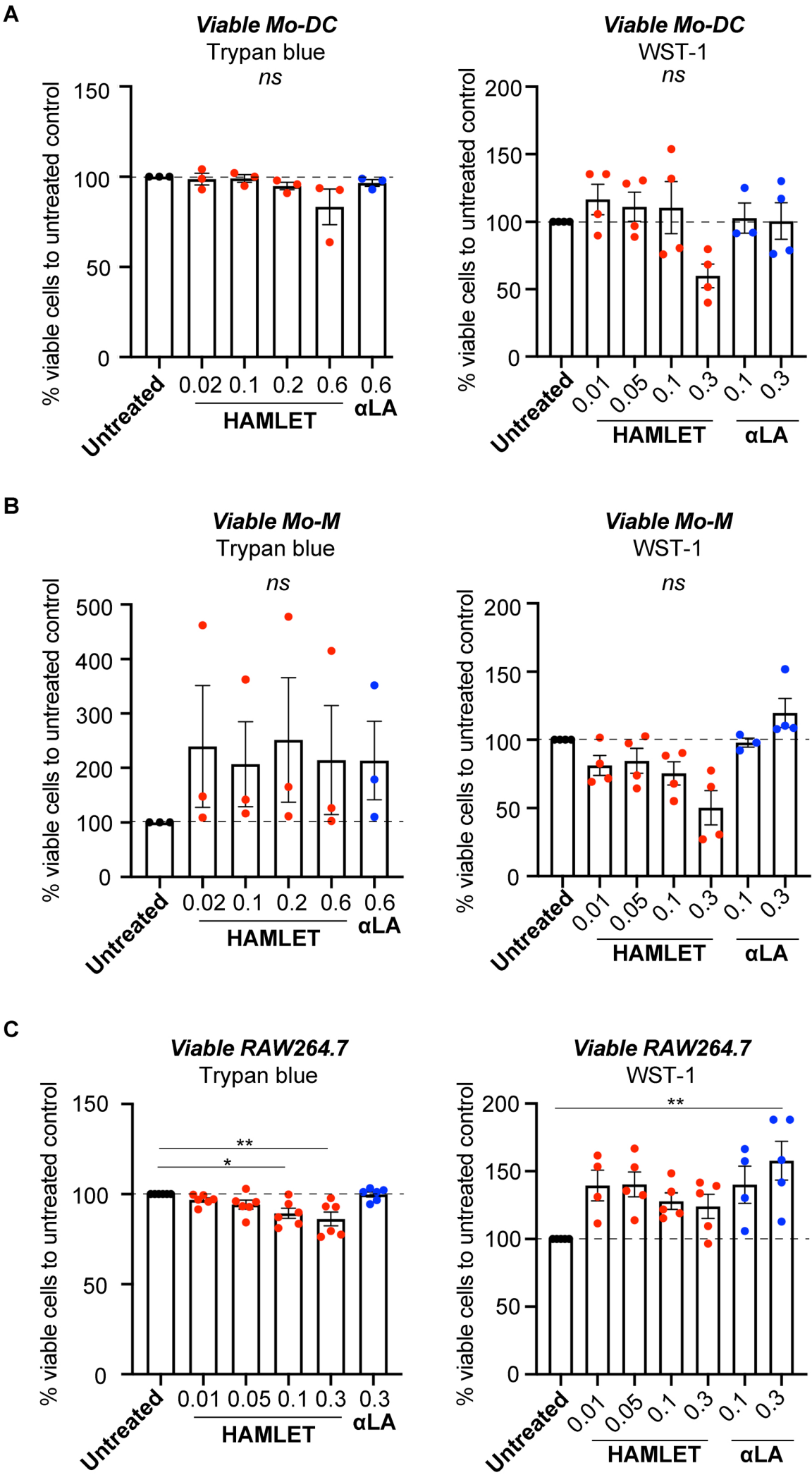
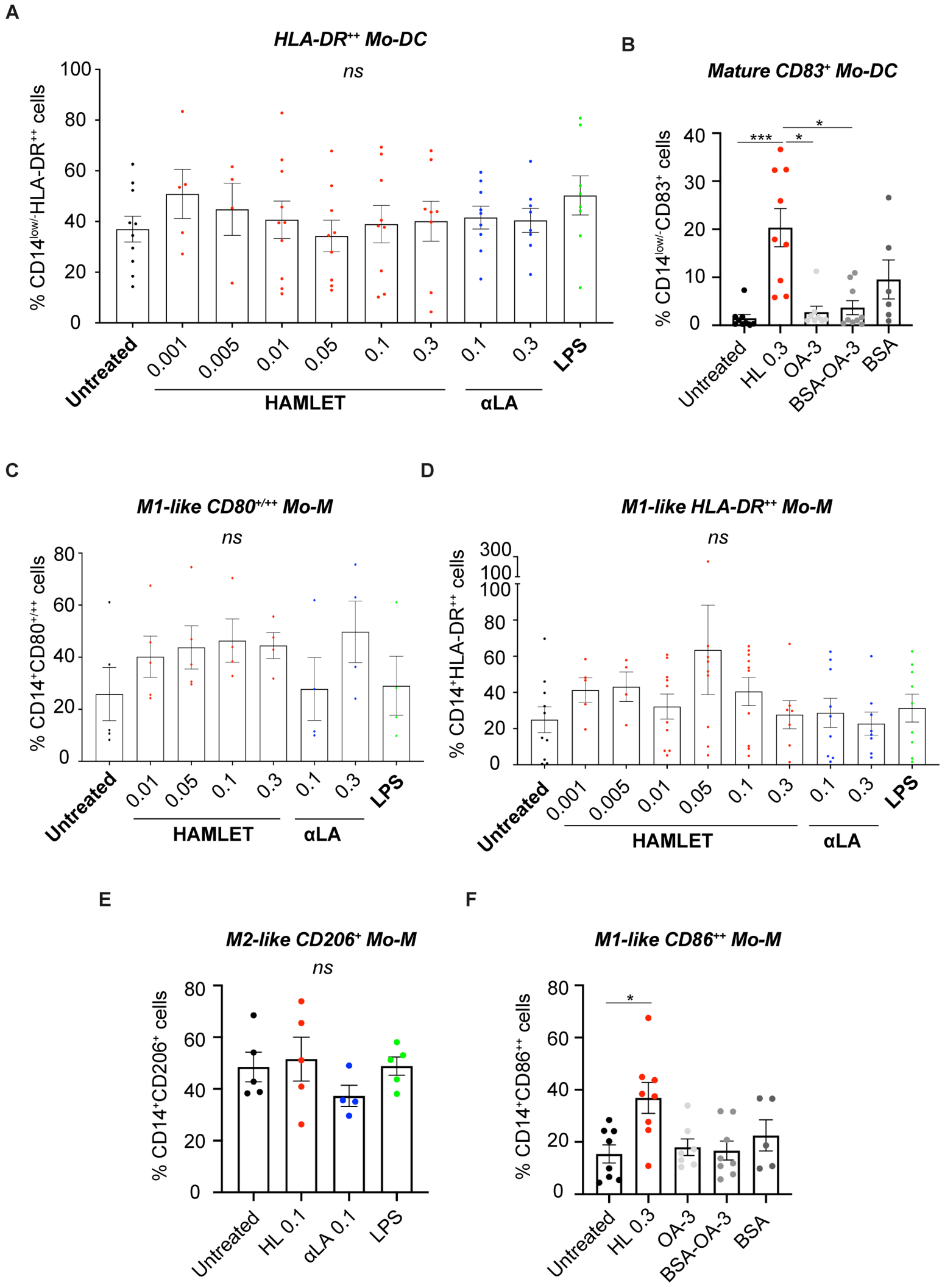
Supplemental Figure 1



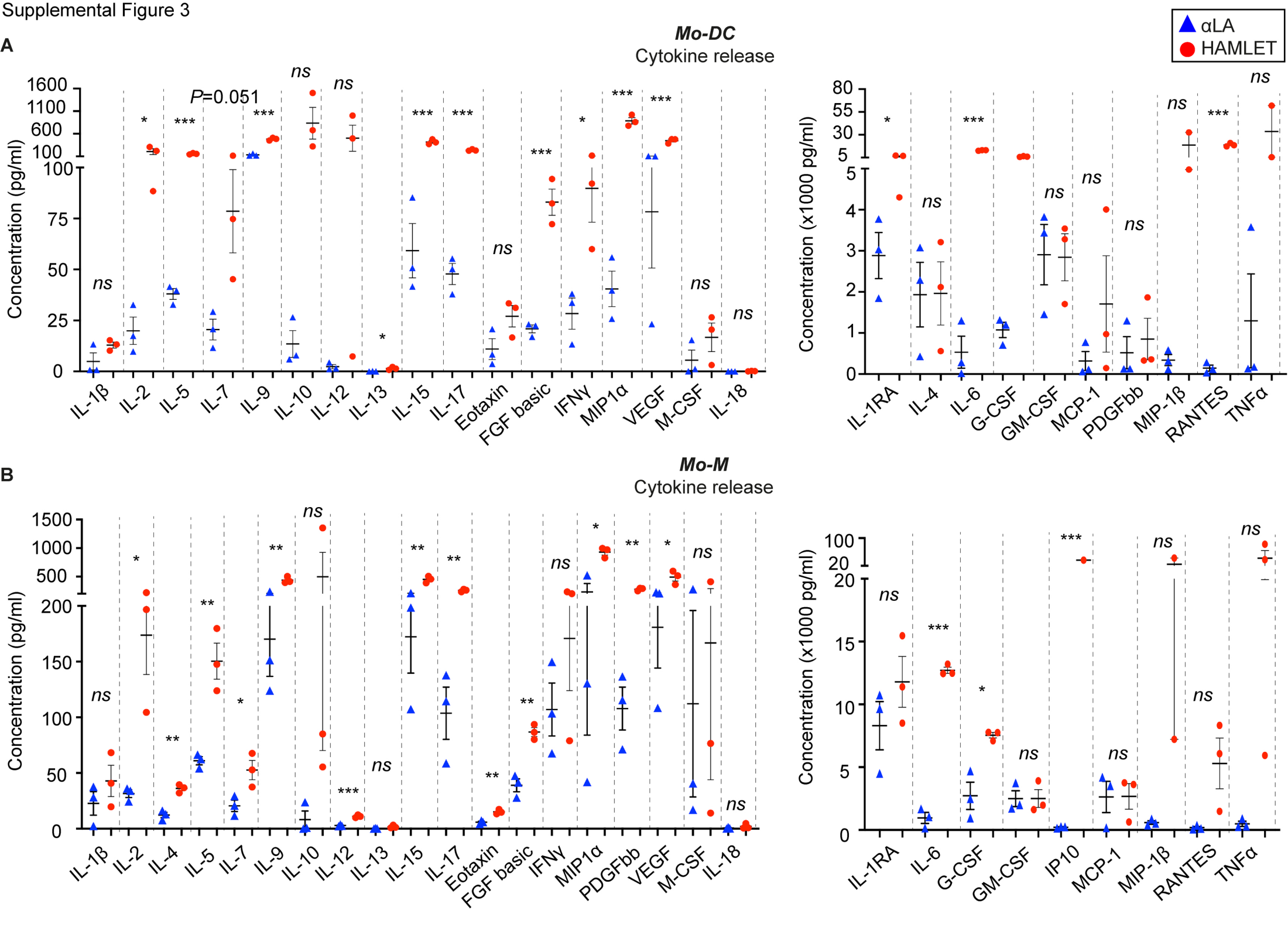
Supplemental Figure 1. Primary human Mo-DC (**A**), Mo-M (**B**) or RAW264.7 murine macrophages (**C**) were treated or not in SFM with indicated concentrations (mg/ml) of HAMLET (*red*) or native αLA (*blue*) for 1-h. The cells were then incubated in fresh medium overnight. The viability was assessed by trypan blue exclusion assay (*left panels*) or WST-1 assay (*right panels*). Individual data points of percentage (%) viable cells compared to untreated control are shown with mean and SEM. N=3-4 for A and B and N=4-6 for C. Statistics by one-way ANOVA with Tukey's multiple comparison test. ns = not significant, * $P<0.05$ and ** $P<0.01$.

Supplemental Figure 2



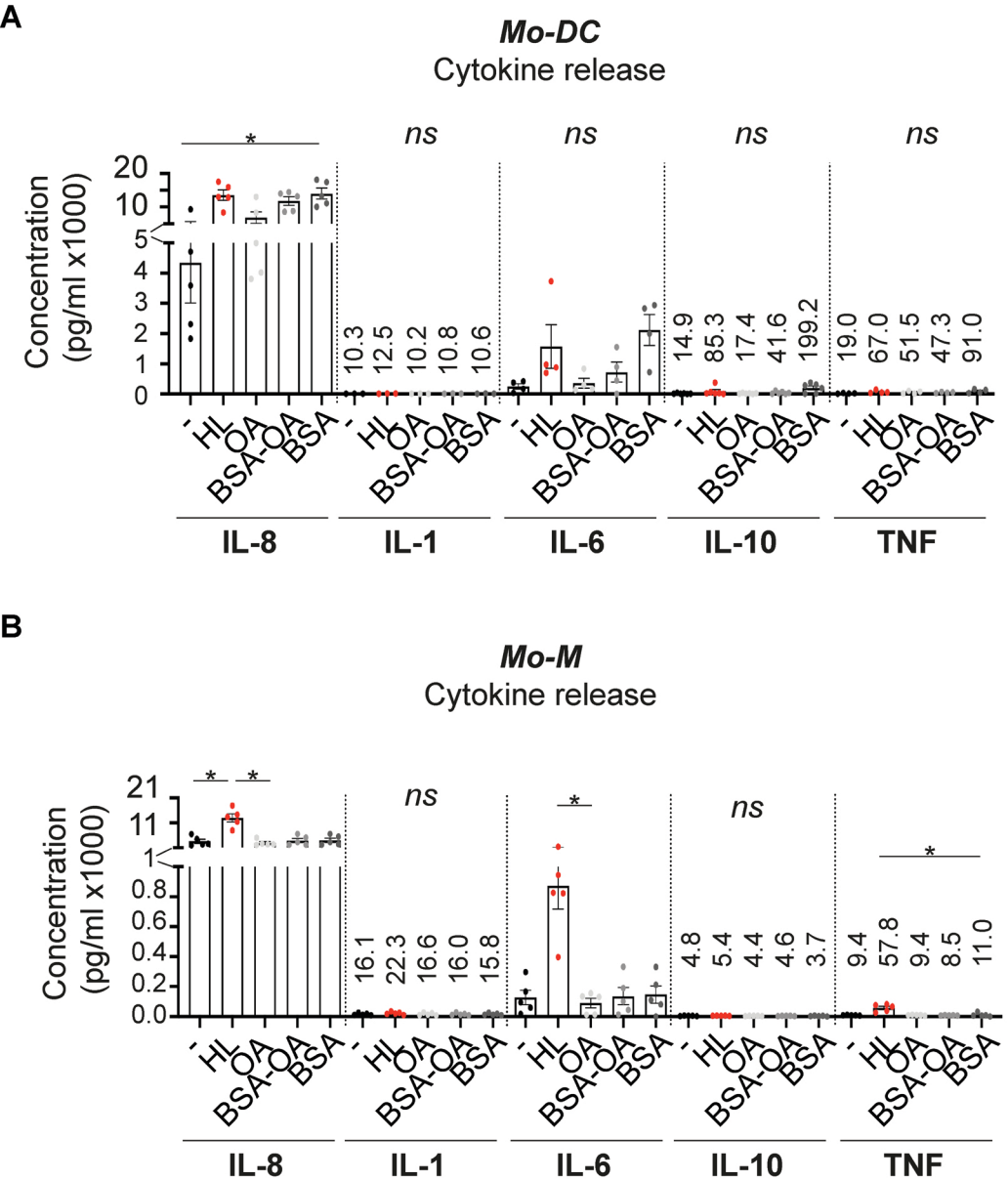
Supplemental Figure 2. Flow cytometric analyses of primary human Mo-DCs and Mo-M treated in SFM for 1-h and grown overnight in fresh differentiation medium. Individual data points of percentages (%) of all viable cells, with mean and SEM shown. (**A**) Mo-DCs were treated with indicated concentration of HAMLET (*red*) or native αLA (*blue*, mg/ml) or with 100 ng/ml LPS (*green*). Graphs depict % $CD14^{low/-}$HLA-DR^{++} of all viable cells. N=4-10. (**B**) Mo-DCs were treated with 0.3 mg/ml HAMLET (*red*) or the corresponding amount of oleic acid (OA-3) in complex or not with BSA (BSA-OA-3) or with BSA alone (BSA). Graphs depict % $CD14^{low/-}CD83^{+}$ cells. N=6-9. (**C-E**) Mo-M were treated with indicated concentrations of HAMLET (*red*) or native αLA (*blue,* mg/ml) or with 100 ng/ml LPS (*green*). Graphs depict % $CD14^{+}CD80^{+/++}$ cells, N=4-5 (C), $CD14^{+}$HLA-DR^{++} cells, N=4-10 (D) or $CD14^{+}CD206^{+}$ cells (E), N=4-5. (**F**) Mo-M were treated with 0.3 mg/ml HAMLET (*red*) or the corresponding amount of oleic acid (OA-3) in complex or not with BSA (BSA-OA-3) or with BSA alone (BSA). Graphs depict % $CD14^{+}CD86^{++}$ cells. N=5-8. All statistics by Kruskal-Wallis with Dunn's multiple comparisons test to the untreated control (A and C-E) or between all samples (B and F). ns = not significant, * $P<0.05$, *** $P<0.001$.

Supplemental Figure 3



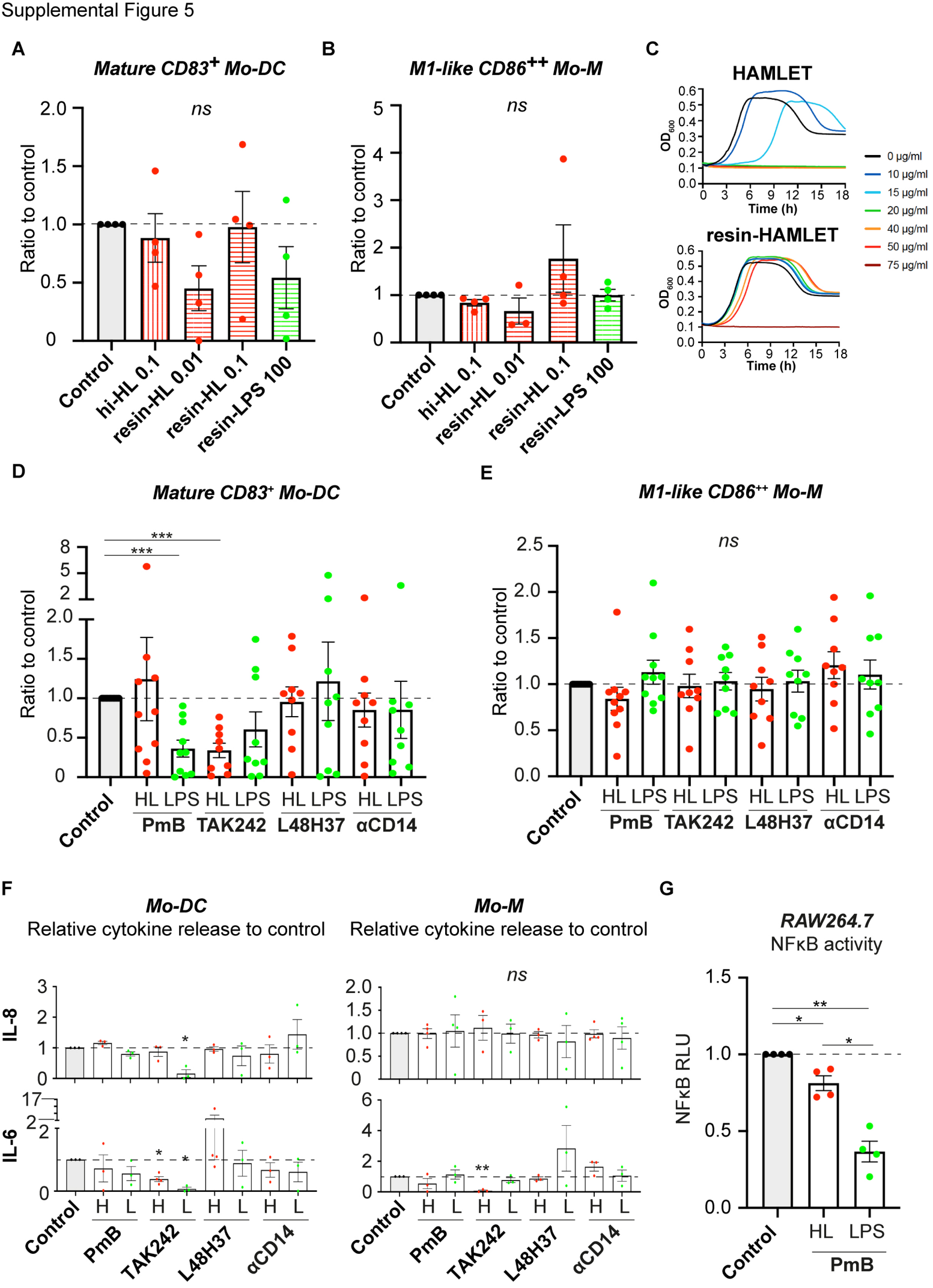
Supplemental Figure 3. Primary human Mo-DCs (**A**) or Mo-M (**B**) were stimulated with 0.1 mg/ml HAMLET (*red* circles) or native αLA (*blue* triangles) for 1-h and grown overnight in fresh differentiation medium. The release of indicated cytokines were analyzed in the supernatants by Bio-plex assay. Individual data points with mean and SEM are shown. N=3. Statistics by unpaired t-test. ns = not significant, * $P<0.05$, ** $P<0.01$ and *** $P<0.001$

Supplemental Figure 4



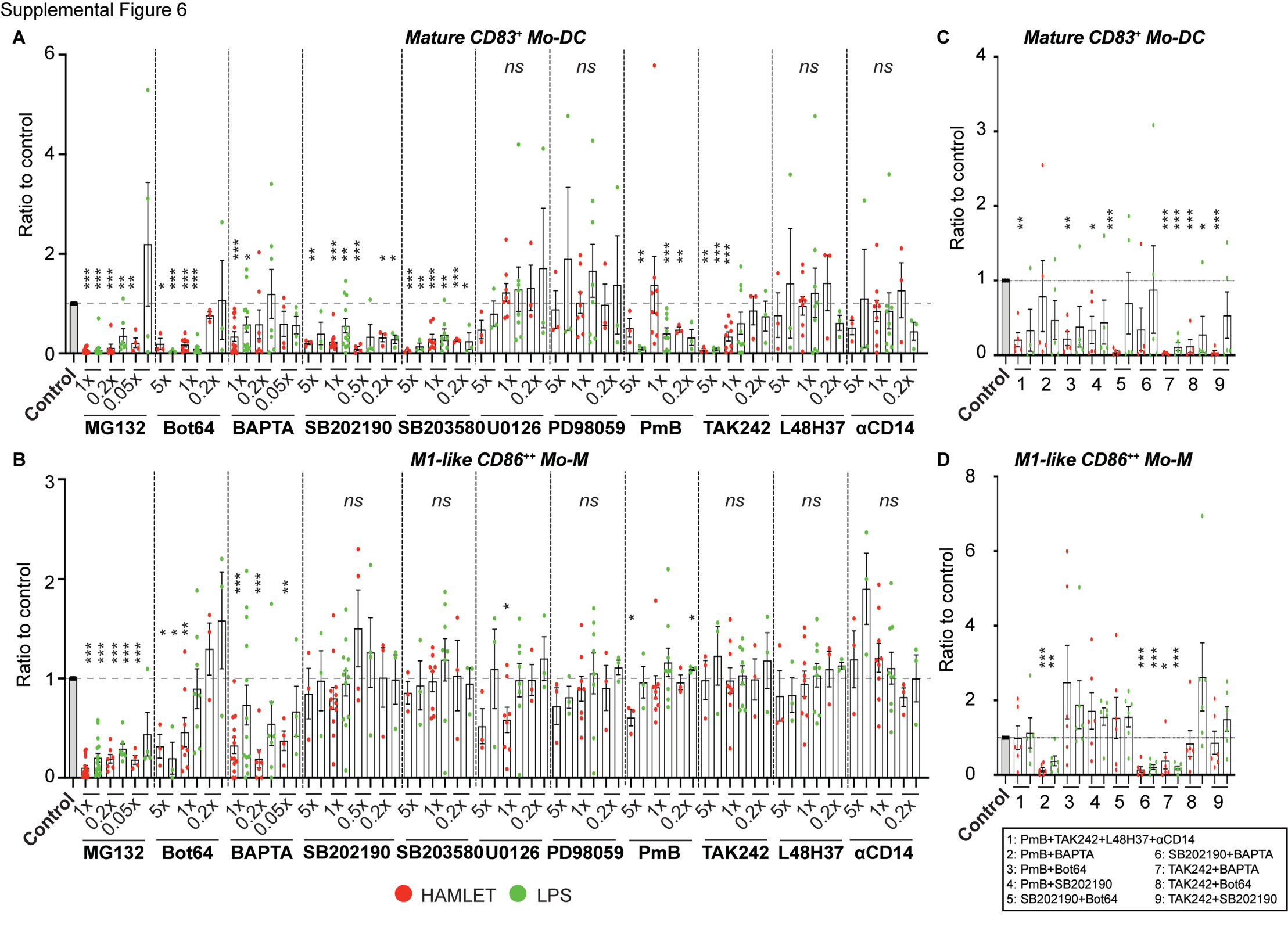
Supplemental Figure 4. Supernatants from primary human Mo-DC (**A**) or Mo-M (**B**) from Supplemental Figure 2B and F, respectively, treated with 0.3 mg/ml HAMLET (*red*) or the corresponding amount of oleic acid (OA-3) in complex or not with BSA (BSA-OA-3) or with BSA alone were analyzed by CBA. Individual data points of respective cytokine concentration (pg/ml) with mean and SEM are shown. Values represent mean concentration. N=3-5 individual experiments. Statistics by Kruskal-Wallis with Dunn's multiple comparison test between all samples. ns= not significant and * $P<0.05$.

Supplemental Figure 5



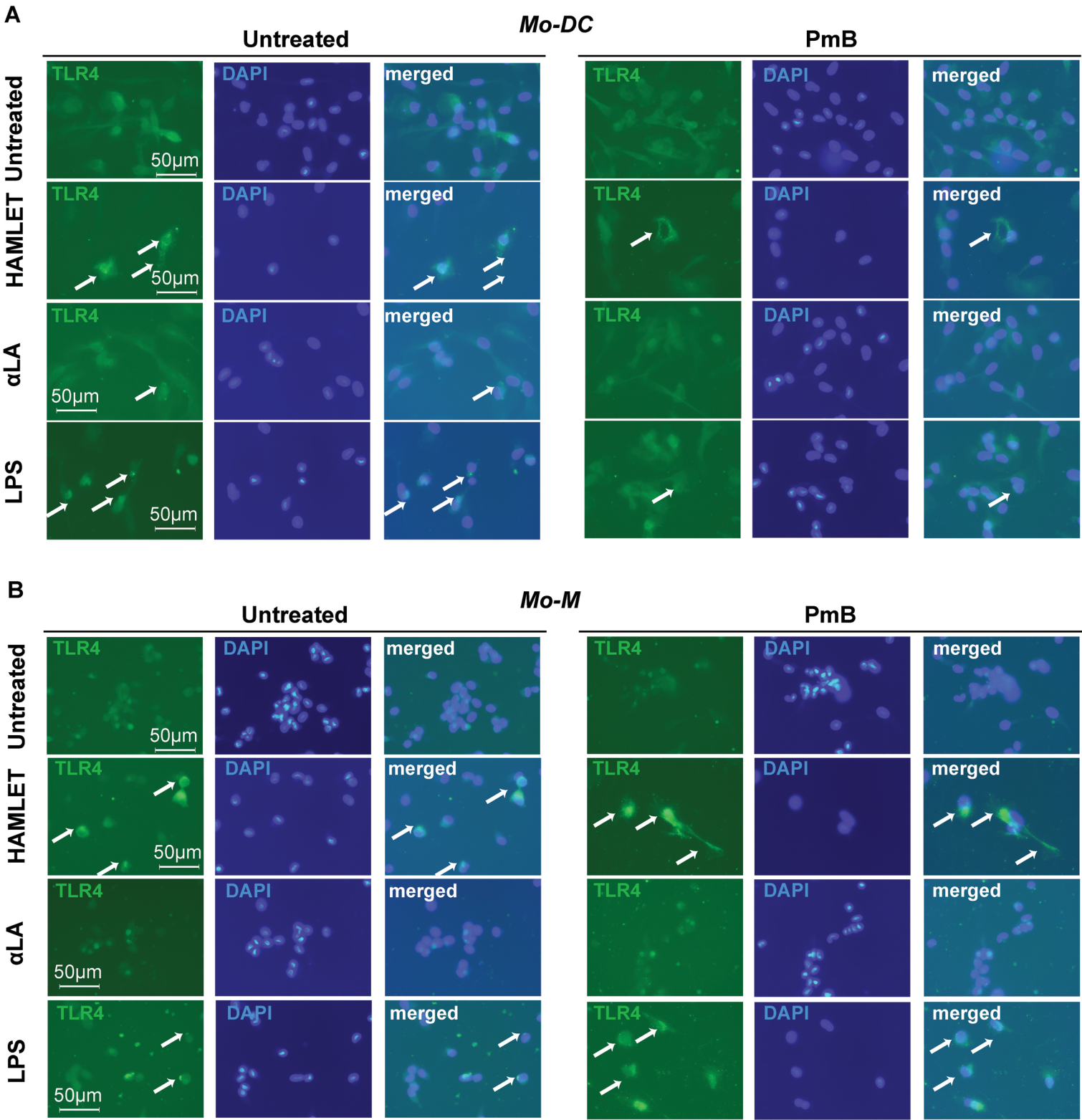
Supplemental Figure 5. (**A-B**) Primary human Mo-DCs (A) and Mo-M (B) were treated or not with 0.1 mg/ml boiled HAMLET (heat-inactivated; hi-HL), 0.01 or 0.1 mg/ml resin-treated HAMLET (resin-HL) or 100 ng/ml resin-treated LPS (resin-LPS) for 1-h, and grown overnight in fresh differentiation medium. Non-processed HAMLET or LPS served as controls (control; *grey*) Individual data points of relative % $CD14^{low/-}CD83^{+}$ mature Mo-DCs, or $CD14^{+}CD86^{++}$ M1-like Mo-M to respective control, with mean and SEM are shown. Individual data points are derived from N=2-3 separate experiments. (**C**) MIC growth curves for *S. pneumoniae* strain D39, treated with indicated concentrations of untreated HAMLET or resin-treated HAMLET for 18-h at 37°C, with the absorbance at OD_{600} measured every 10-minutes. (**D-E**) Primary human Mo-DCs (D) or Mo-M (E) were pre-treated with indicated inhibitor for 2-h, stimulated with 0.1 mg/ml HAMLET (HL; *red*) or 100 ng/ml LPS (*green*) and subsequently grown overnight in fresh differentiation medium. The cells were analyzed by flow cytometry. Individual data points of relative % $CD14^{low/-}$ $CD83^{+}$ Mo-DC or $CD14^{+}CD86^{++}$ M1-like Mo-M to respective control (HL or LPS without inhibitor, but with PBS, ethanol or DMSO), with mean and SEM shown. N=9-10 individual experiments. (**F**) Supernatants from primary human Mo-DCs (*left*) or Mo-M (*right*) from panel D-E were analyzed by CBA. Individual data points of the relative release of IL-8 (*upper panel*) or IL-6 (*lower panel*) to respective control, with mean and SEM are shown. N=3 separate experiments. (**G**) Dual luciferase NFκB promoter assay using murine RAW264.7 macrophages stimulated with 0.1 mg/ml HAMLET (HL) or 100 ng/ml LPS with or without 10 μg/ml PmB. Individual data points of relative promoter activity (RLU) to control (HL or LPS only) with mean and SEM are shown. N=2 experiments. All statistics by paired t-test. ns = not significant, * $P<0.05$ and ** $P<0.01$.

Supplemental Figure 6



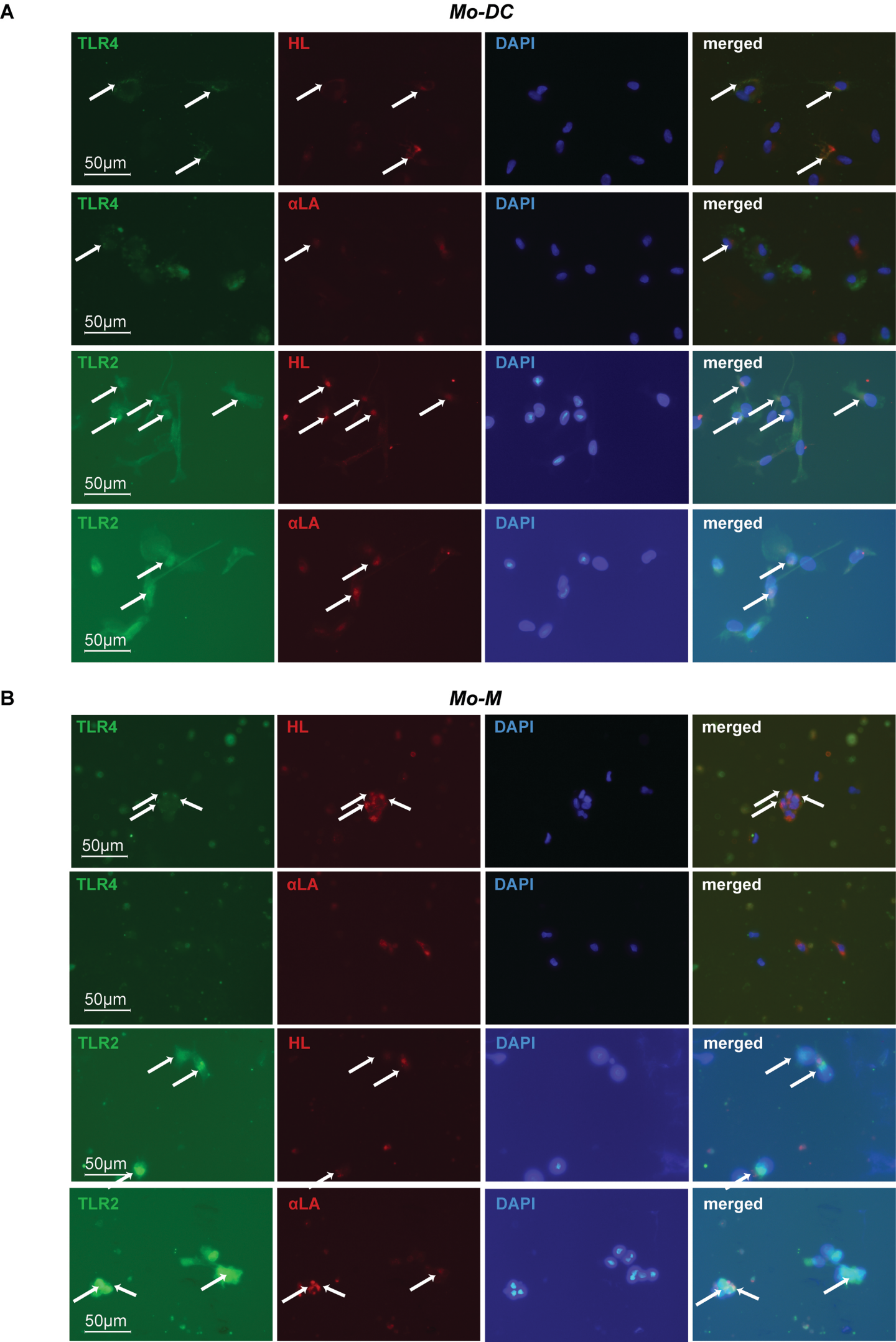
Supplemental Figure 6. Primary human Mo-DCs (A, C) or Mo-M (B, D) were pre-treated with indicated inhibitors at various concentrations for 2-h alone (**A-B**) or in combinations (**C-D**), stimulated with 0.1 mg/ml HAMLET (HL; *red*) or 100 ng/ml LPS (*green*) for 1-h and subsequently grown overnight in fresh differentiation medium. (C-D) 1: 1x PmB + 1x TAK242 + 1x L48H37, 1x αCD14 neutralizing antibody, 2: 1x PmB + 0.2x BAPTA-AM, 3: 1x PmB + 1x BOT-64, 4: 1x PmB + 0.5x SB202190, 5: 0.5x SB202190 + 1x BOT-64, 6: 0.5x SB202190 + 0.2x BAPTA-AM, 7: 1x TAK242 + 0.2x BAPTA-AM, 8: 1x TAK242 + 1x BOT-64, 9: 1x TAK242 + 0.5x SB202190. The cells were analyzed by flow cytometry. Individual data points of relative % $CD14^{low/-}$ $CD83^{+}$ Mo-DC or $CD14^{+}CD86^{++}$ M1-like Mo-M to respective control (HL or LPS without inhibitor, but with PBS, ethanol or DMSO), with mean and SEM shown. N=3-16 individual experiments. All statistics by paired t-test to the respective control. * $P<0.05$, ** $P<0.01$, *** $P<0.001$.

Supplemental Figure 7



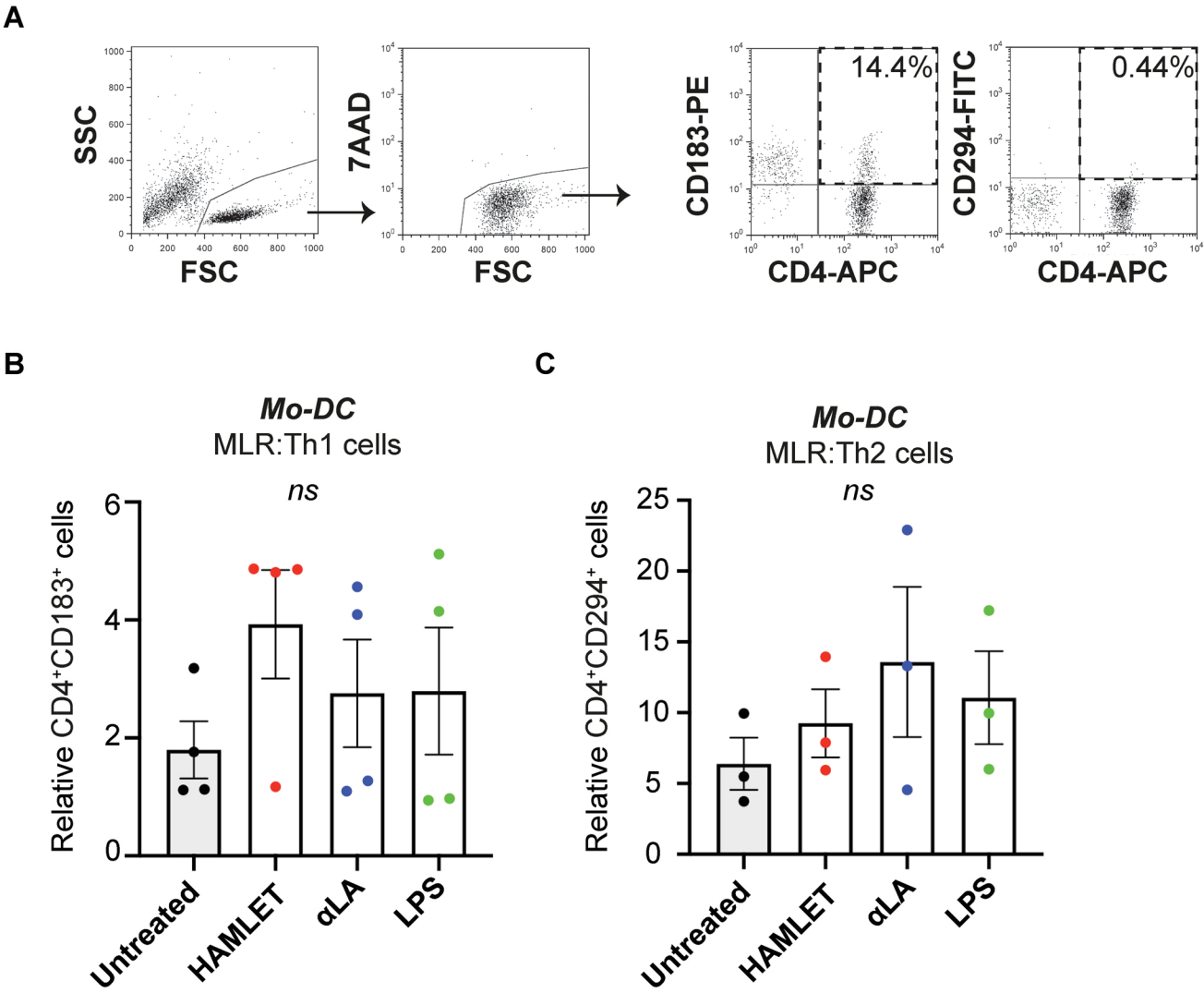
Supplemental Figure 7. Primary human Mo-DC (**A**) and Mo-M (**B**) were pre-treated with 10 μg/ml PmB for 2-h and stimulated with HAMLET, αLA or LPS. The cells were analyzed 18-h post-stimulation by immunofluorescence for TLR4 localization. White arrows indicate differences in localization pattern of TLR4. Vectashield DAPI was used as nuclear stain for all experiments. Images are representative of N=3 individual experiments.

Supplemental Figure 8



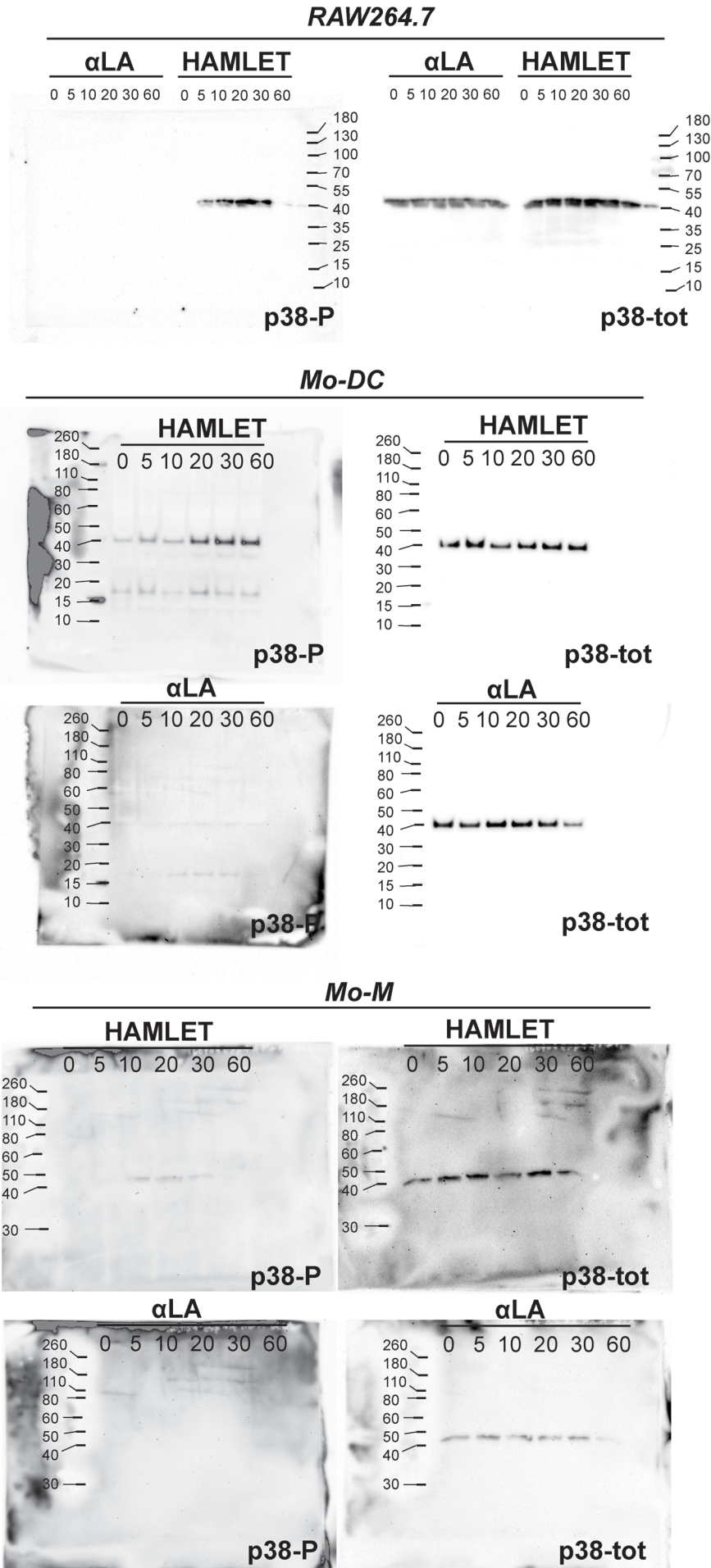
Supplemental Figure 8. Primary human Mo-DC (**A**) and Mo-M (**B**) were treated with Alexa-Fluor-568 labelled HAMLET (HL-568) or αLA (αLA-568) for 2-h and then co-stained with TLR4 or TLR2. White arrows indicate sites of potential co-localization. Vectashield DAPI was used as nuclear stain for all experiments. Images are representative of N=3 individual experiments.

Supplemental Figure 9



Supplemental Figure 9. (**A-C**) Primary human Mo-DCs were stimulated or not with 0.1 mg/ml HAMLET, 0.1 mg/ml native αLA or 100 ng/ml LPS for 1-h and grown over night in fresh differentiation medium. The cells were re-seeded with allogeneic T-lymphocytes and co-cultured for 5 days in a mixed lymphocyte reaction (MLR) and cells were analyzed by flow cytometry. (A) Representative dot plots and gating strategies for $CD4^+CD183^+$ Th1 cells and $CD4^+CD294^+$ Th2 cells (dashed boxes). (B-C) Relative percentage of Th1 cells (B) and Th2 cells (C) to unstimulated T-cell control. Individual data points with mean and SEM are shown. N=3-4. Statistics by paired t-test. ns = not significant.

Supplemental Figure 10



Supplemental Figure 10. Raw data of Western blots from Figure 4 F-H.