Electronic Supplementary Information

Orlistat increases arsenite tolerance in THP-1 derived macrophages through the up-regulation of ABCA1

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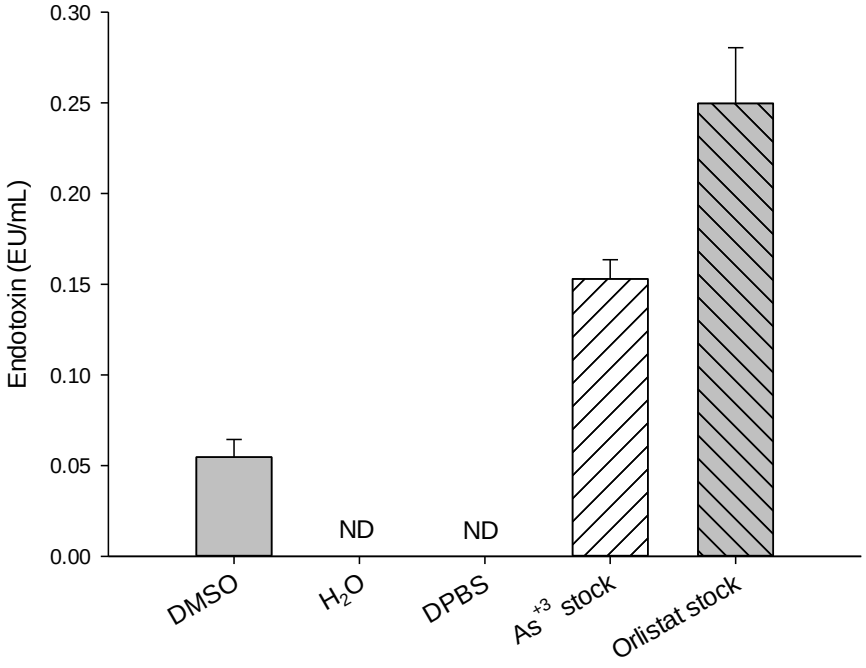

Figure S1. Detection of endotoxins in As^{+3} and orlistat stock solutions. Endpoint Chromogenic LAL Assays (Cat. No. 60402ES32, Yeasen, detection range: 0.01-0.1 (T1) and 0.1-1 (T2) EU/mL) was used to detect the endotoxins. The experiment was performed according to the user manual provided by the manufacturer with slight modifications. The bacteria endotoxin standards were prepared at 0.5, 0.25, 0.125 and 0.0625 EU/ml. The DMSO (BioPerformance certified, Sigma, used for the preparation of orlistat stock), H_2O (DNase/RNase-Free, Sterile, Beyotime, used for the prepatation of As^{+3} stock), DPBS (Cat. No. C0221D, Beyotime, used to dilute As^{+3} and orlistat), 0.1 M As^{+3} stock solution and 0.5 M orlistat solution were tested for endotoxins. The calculation was based on the absorbance values acquired on a Synergy Lx microplate reader (BioTek) at 545 nm. Results are mean ± SD. ND, Not detectable (below the detection limit of the assay).

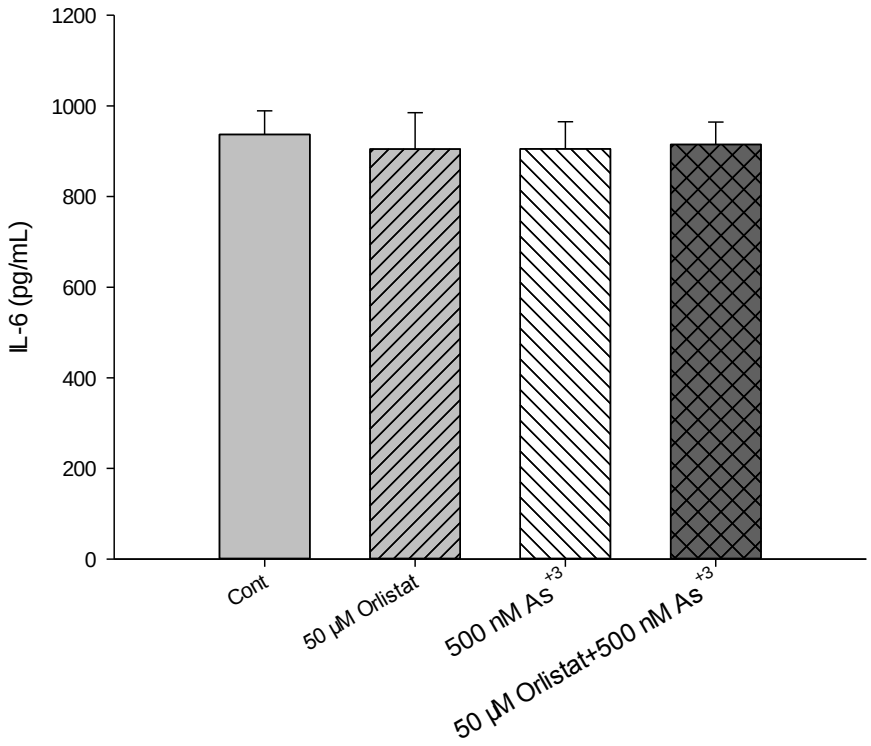

Figure S2. IL-6 secretion of THP-1 derived macrophages treated with As^{+3}, orlistat and their combinations. THP-1 derived macrophages were treated with 50 μM orlistat, 500 nM As^{+3}, and their combinations for 24 h with the stimulation of 10 ng/mL LPS. IL-6 levels in the cell supernatants were quantified by Human IL-6 ELISA Kit (Cat. No. PI330, Beyotime). The results were calculated from the kit standards with the absorbance values at 450 nm, which were acquired on a Synergy Lx microplate reader (BioTek). Results are mean ± SD.

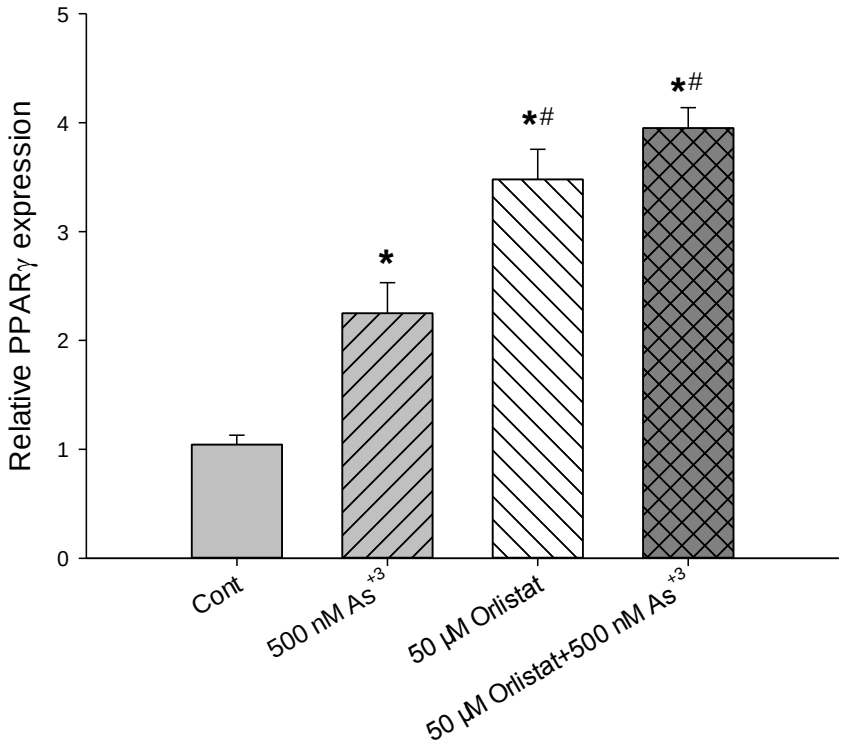

Figure S3. PPARγ mRNA level in THP-1 derived macrophages treated with As^{+3}, orlistat and their combinations. THP-1 derived macrophages were treated with 50 µM orlistat, 500 nM As^{+3}, and their combinations for 24 h, and the mRNA level PPARγ was measured by RT-qPCR. Relative expression of ACBA1 mRNA was analyzed with the Taqman assay (Hs01115513_m1, Cat No. 4331182, Thermo Fisher Scientific) on a QuantStudio 5 RT-PCR system (Thermo Fisher Scientific). *Significantly different compared to the control ($p<0.05$). #Significantly different compared to the 500 nM As^{+3} ($p<0.05$). Results are mean ± SD.

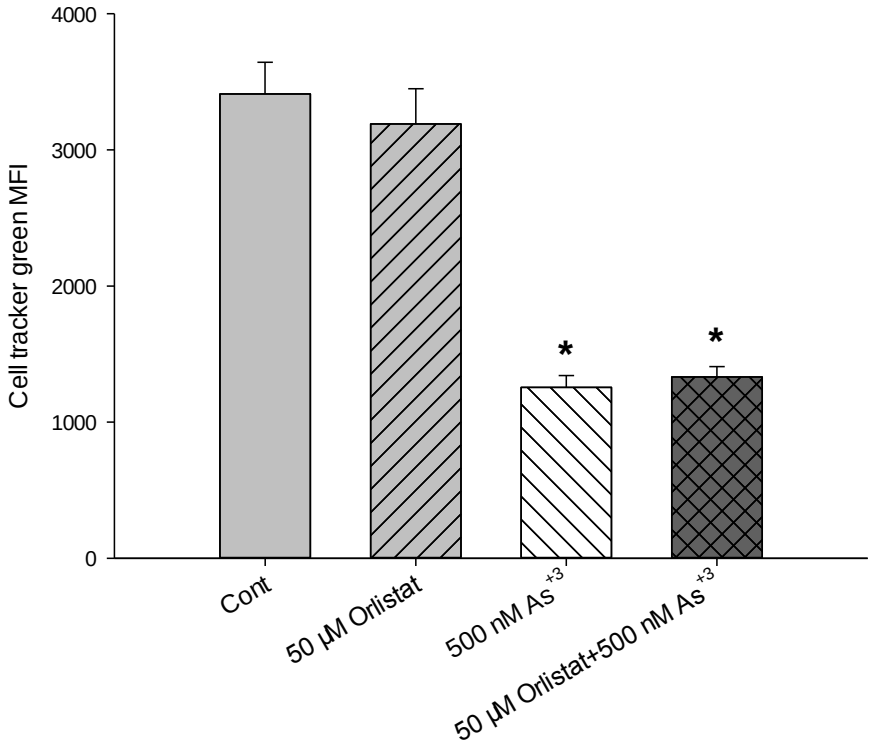

Figure S4. Phagocytosis activity of THP-1 derived macrophages with ABCA1 siRNA knockdown treated with As^{+3}, orlistat and their combinations. KD of ABCA1 with siRNA was performed in THP-1 derived macrophages and the cells were treated with 50 μM orlistat, 500 nM As^{+3} or 500 nM As^{+3} + 50 μM orlistat. Apoptotic CCRF-CEM cells were labeled with Cell Tracker Green and combined with the treated macrophages. Phagocytosis was analyzed by flow cytometry after 2 h of incubation. *Significantly different compared to the control ($p<0.05$). Results are mean ± SD.

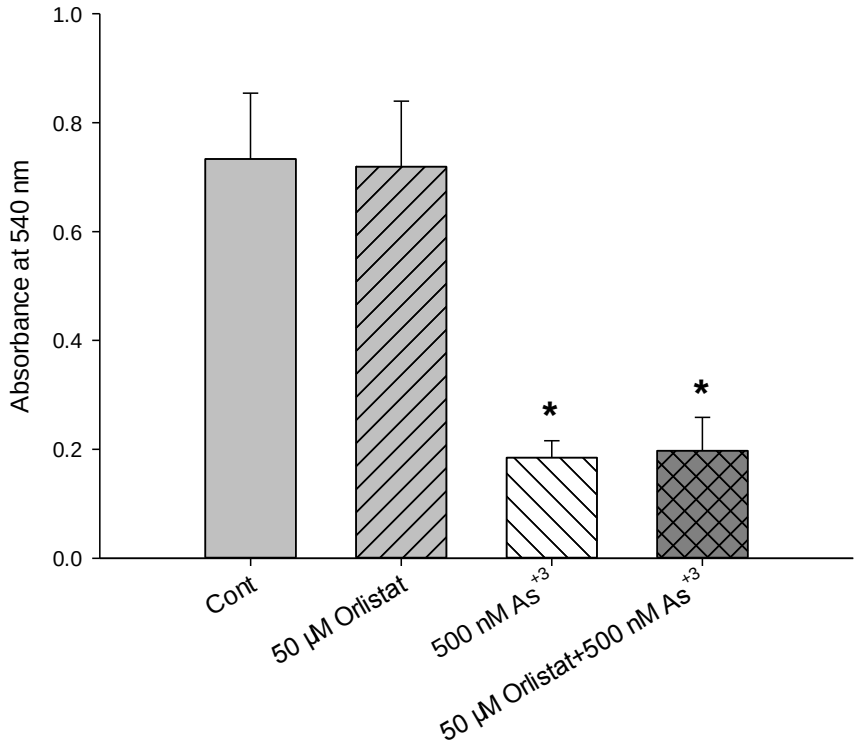

Figure S5. NO production of THP-1 derived macrophages with ABCA1 siRNA knockdown treated with As^{+3}, orlistat and their combinations. KD of ABCA1 with siRNA was performed in THP-1 derived macrophages and the cells were treated with 50 µM orlistat, 500 nM As^{+3} or 500 nM As^{+3} + 50 µM orlistat. NO in cell culture medium was analyzed by Griess Reagent on a Synergy Lx microplate reader (BioTek). *Significantly different compared to the control ($p<0.05$). Results are mean ± SD.