Figure S1

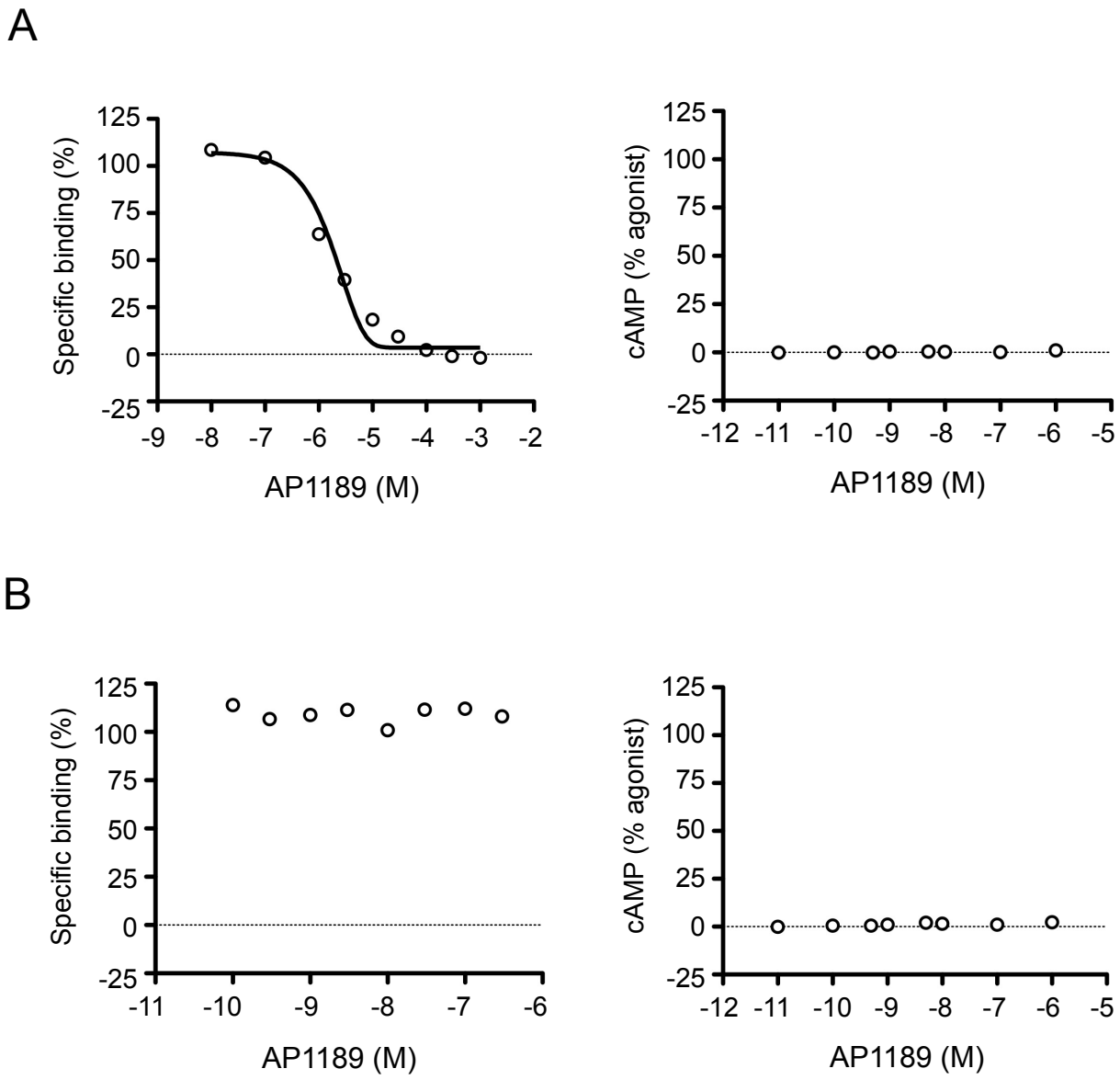


**Figure S1. Binding and activation of $MC_1$ and $MC_3$.** Murine B16-F10 cells expressing $MC_1$ (A) and CHO cells stably transfected with human $MC_3$ (B) were used to study AP1189 binding properties to these receptors and cAMP activity by radioligand binding assay using [125I]-NDP-αMSH. For cAMP measurements, cells were stimulated for 15min with AP1189 and cAMP measured using the cAMP EIA kit from Cayman Chemical.

Figure S2

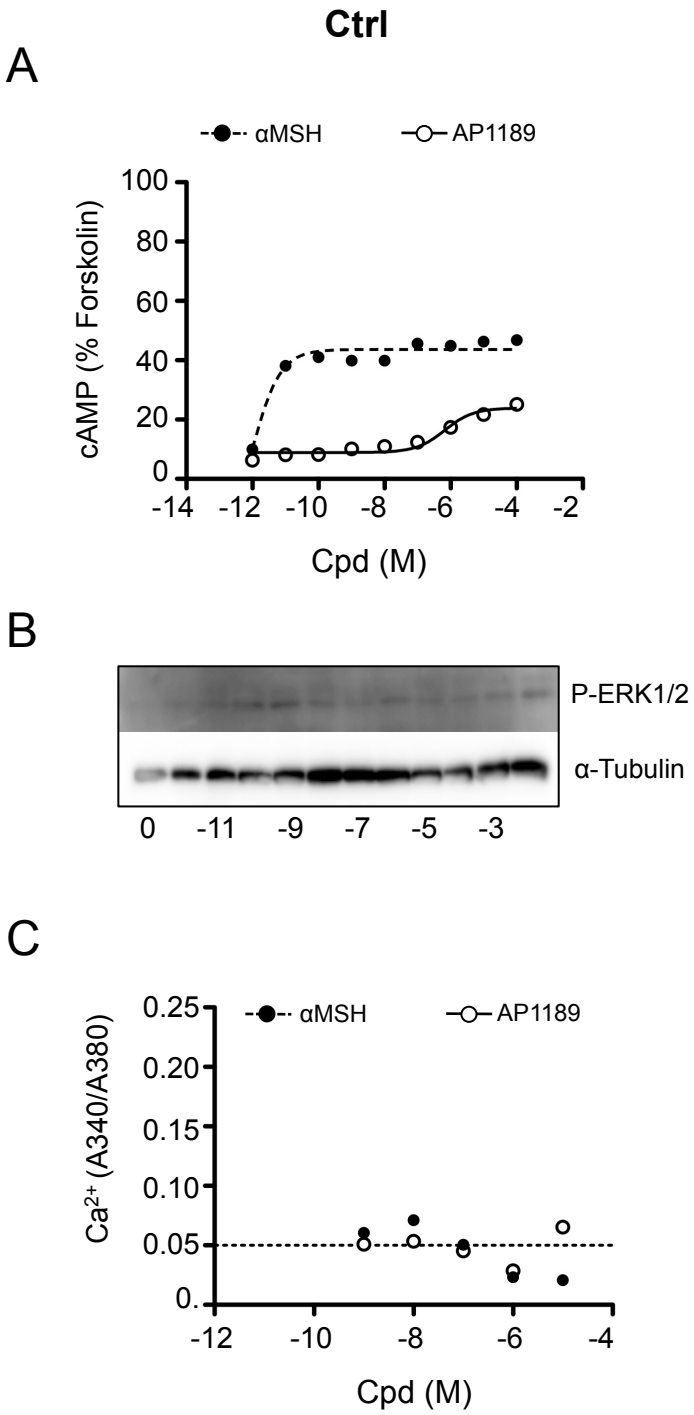


**Figure S2. cAMP, ERK1/2 phosphorylation and $Ca^{2+}$ flux in control cells.** HEK293A cells were transiently transfected with empty vector pCMV6 and used 24h after transfection. (A) Cells were stimulated with AP1189 and αMSH for 15min. cAMP was measured by EIA. Forskolin (3μM) was used as positive control (B) Cells were stimulated with AP1189 for 8min and with αMSH for 5min. ERK1/2 phosphorylation was analyzed by western blot using α-Tubulin as loading control. (C) Intracellular Ca2+ mobilization was measured using Fura-2 AM labelled cells in the NOVOstar microplateplate reader. Ionomycin (1μM) was used as positive control. The ratio of fluorescence emission at 510 nm after sequential excitation at 340 and 380 nm was recorded.

Figure S3

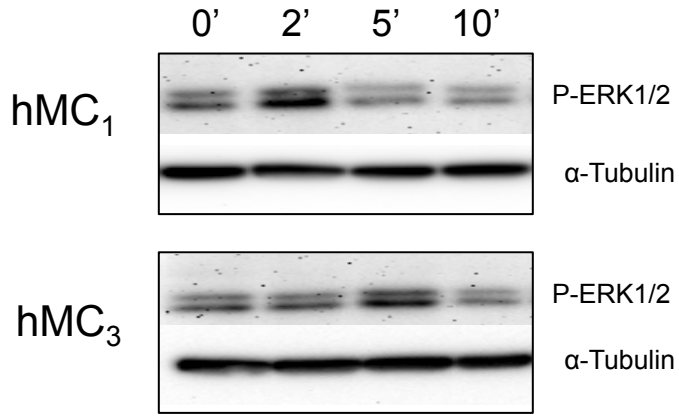


**Figure S3. ERK1/2 phosphorylation by human $MC_1$ and $MC_3$.** HEK293A cells were transiently transfected with the human $MC_1$ or $MC_3$ and used 24h after transfection. A time course study was performed in which cells were stimulated for 2, 5 or 10min with 1nM AP1189. α-Tubulin was used as loading control.

Figure S4

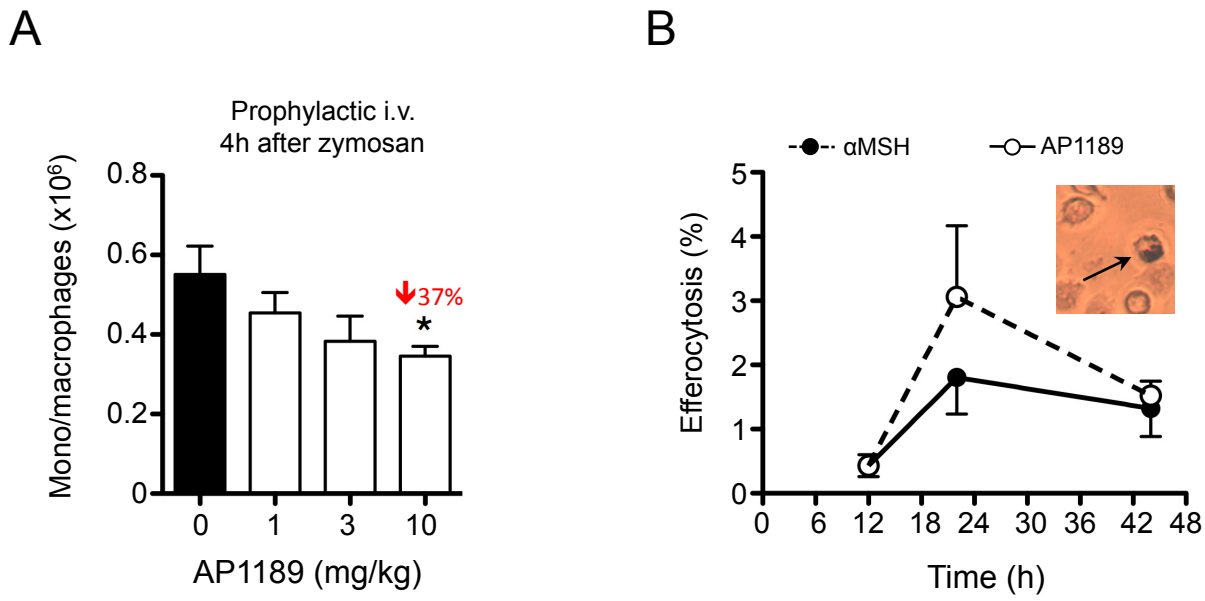


**Figure S4. Effect of AP1189 on monocytes in the zymosan-induced peritonitis model.** Acute peritonitis was induced with one single injection of 1mg zymosan and leukocyte infiltration was analyzed by flow cytometry. (A) Prophylactic experimental design: AP1189 at the doses indicated was administered i.v. 30min before zymosan injection and cells analysed at the 4h time-point. (B) AP1189 (1mg/kg) or vehicle were injected i.p. at 12 h post-zymosan, and peritoneal cells collected at the 22h or 44h time-point. Cells were cultured in vitro for 1h in fully supplemented RPMI media, then washed to remove non-adherent cells and fixed. The myeloperoxidase assay was then performed and efferocytosis analysed by cell counting. The proportion of phagocytic cells (see arrow in image insert) was then calculated.