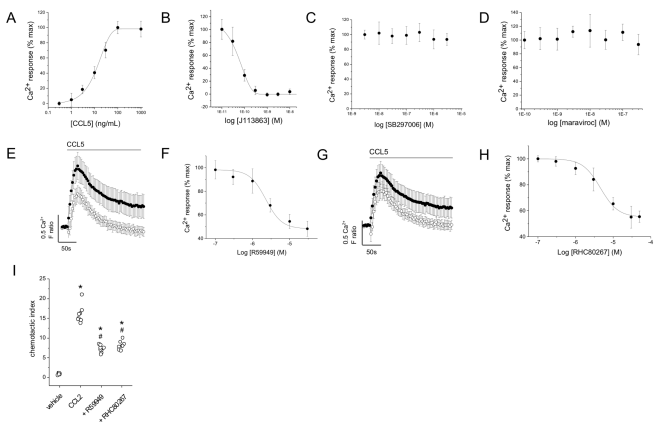
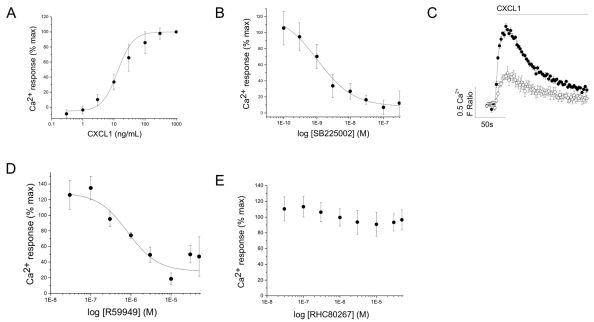
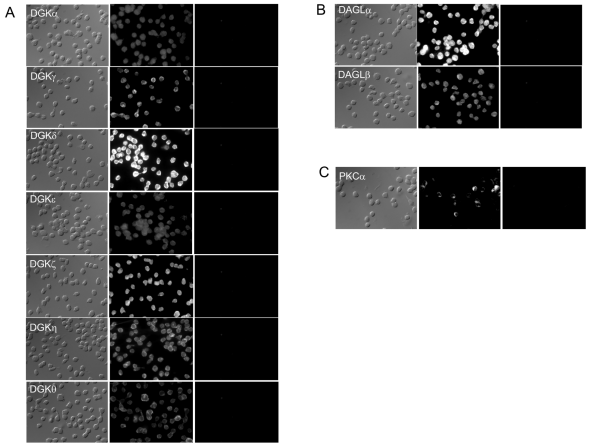


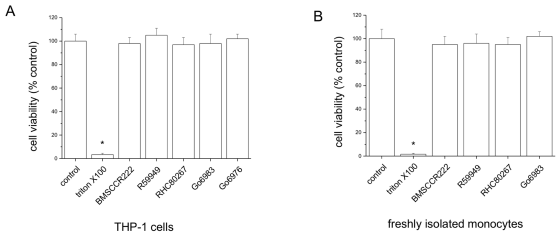
Supplemental Figure 1 Effect of DAG kinase and DAG lipase inhibition on CCL5-evoked response in CD14$^+$ human monocytes. (A) Concentration-response curve for CCL5-evoked intracellular Ca^{2+} responses (*N*=6). (B-D) Concentration-inhibition curve for selective CCR1 antagonist (B; J113863), CCR3 (C; SB297006) and CCR5 (maraviroc) (*N*=5). Responses evoked by 50ng/mL CCL5. (E) Average (*N*=6) intracellular Ca^{2+} responses evoked by CCL5 (50ng/mL) in the presence of R59949 (30μM; *open circles*) or vehicle control (*closed circles*). (F) Concentration-inhibition curve for R59949 against intracellular Ca^{2+} responses evoked by CCL5 (50ng/mL) (*N*=6). (G) Average (*N*=5) intracellular Ca^{2+} responses evoked by CCL5 (50ng/mL) in the presence of RHC80267 (30μM; *open circles*) or vehicle control (*closed circles*). (H) Concentration-inhibition curve for RHC80267 against intracellular Ca^{2+} responses evoked by CCL5 (50ng/mL) (*N*=5). (I) Effect of R59949 (30μM) and RHC80267 (30μM) on freshly isolated monocyte transmigration to CCL5 (5 ng/mL). * denotes $P<0.05$ versus vehicle and # denotes $P<0.05$ versus CCL5 alone. For intracellular Ca^{2+} measurements, F ratio is the Ca^{2+} response as measured by the Fura-2 emission intensity ratio when excited at 340 and 380 nm. Data in inhibition experiments is expressed as a percentage of the control response in the presence of vehicle alone.



Supplemental Figure 2 Effect of DAG kinase and DAG lipase inhibition on CXCL1-evoked response in CD14$^+$ human monocytes. (A) Concentration-response curve for CXCL1-evoked intracellular Ca^{2+} responses (*N*=6). (B) Concentration-inhibition curve for selective CXCR2 antagonist SB225002 (*N*=5). Response evoked by 100ng/mL CXCL1. (C) Average (*N*=6) intracellular Ca^{2+} responses evoked by CXCL1 (100ng/mL) in the presence of R59949 (30μM; *open circles*) or vehicle control (*closed circles*) (*N*=6). (D) Concentration-inhibition curve for R59949 against intracellular Ca^{2+} responses evoked by CXCL1 (100ng/mL) (*N*=5). (E) Concentration-inhibition curve for RHC80267 against intracellular Ca^{2+} responses evoked by CXCL1 (100ng/mL) (*N*=5).



Supplemental Figure 3 Immunocytochemical analysis of DAG kinase and DAG lipase isoform expression in THP-1 cells. Three image panels for each isoform investigated are shown and are bright field image (*lefthand panel*), fluorescence for experiments performed with primary and secondary Alexa-488 conjugated antibody (*central panel*), and negative control perform in the presence of secondary Alexa-488 conjugated antibody only (*righthand panel*). (A) DAG kinase (DGK) isforms α, γ, δ, ε, η, ζ and θ. (B) DAG lipase (DAGL) isoforms α and β. (C) Protein kinase C (PKC) isoform α.



Supplemental Figure 4 Compound cytotoxicity assay Cell viability as tested by lactate dehydrogenase (LDH) release. THP-1 cells (A) or freshly isolated human peripheral blood monocytes (B) were exposed to test compounds or positive control (0.1% Triton X100) for 30 mins perform measurement of LDH release (*N*=5). Compounds tested are BMSCCR222 (1μM), R59949 (30μM), RHC80267 (30μM), Go6983 (100nM) or Go6976 (100nM). Cell viability is controlled as percentage of viability in cells exposed to vehicle (*control*). * denotes $P<0.05$ versus vehicle.