Supplementary figures

Figure S1

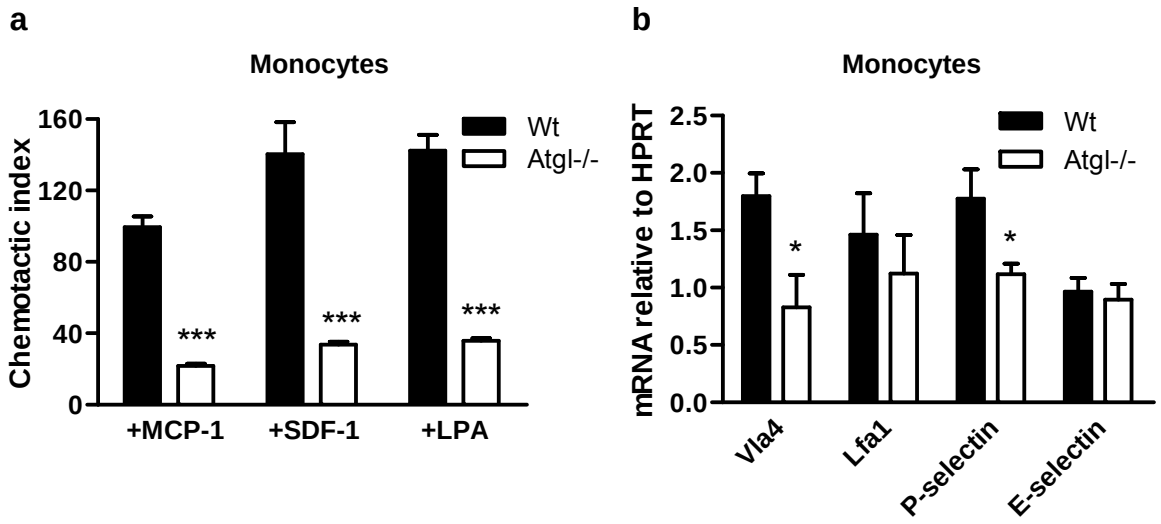

Fig. S1 Reduced migration of *Atgl-/-* monocytes (a) Monocytes from Wt and *Atgl-/-* mice were added to the upper chamber of transwell plates and were allowed to migrate through the membrane (pore size: 3 µm) into the lower chamber containing DMEM in the absence or presence of macrophage chemoattractant protein-1 (MCP-1; 50 ng/ml), stromal cell-derived factor-1 (SDF-1, 60 ng/ml) or lysophosphatidic acid (LPA, 9 ng/ml) at 37°C for 4 h. Migrated cells were counted by flow cytometry. Chemotactic indexes were calculated from the ratio of the cells that had migrated in the presence or absence of the respective chemoattractant. (b) Total RNA was isolated from Wt and *Atgl-/-* monocytes, reverse transcribed and mRNA expression of very late antigen 4 (Vla4), lymphocyte function-associated antigen 1 (Lfa1), P-selectin, E-selectin were determined by real-time PCR including normalization to hypoxanthine guanine phosphoribosyl transferase (HPRT). Data are expressed as mean values of 2 independent experiments ± SEM performed in triplicate repeats. * $p < 0.05$, *** $p \leq 0.001$.

Figure S2

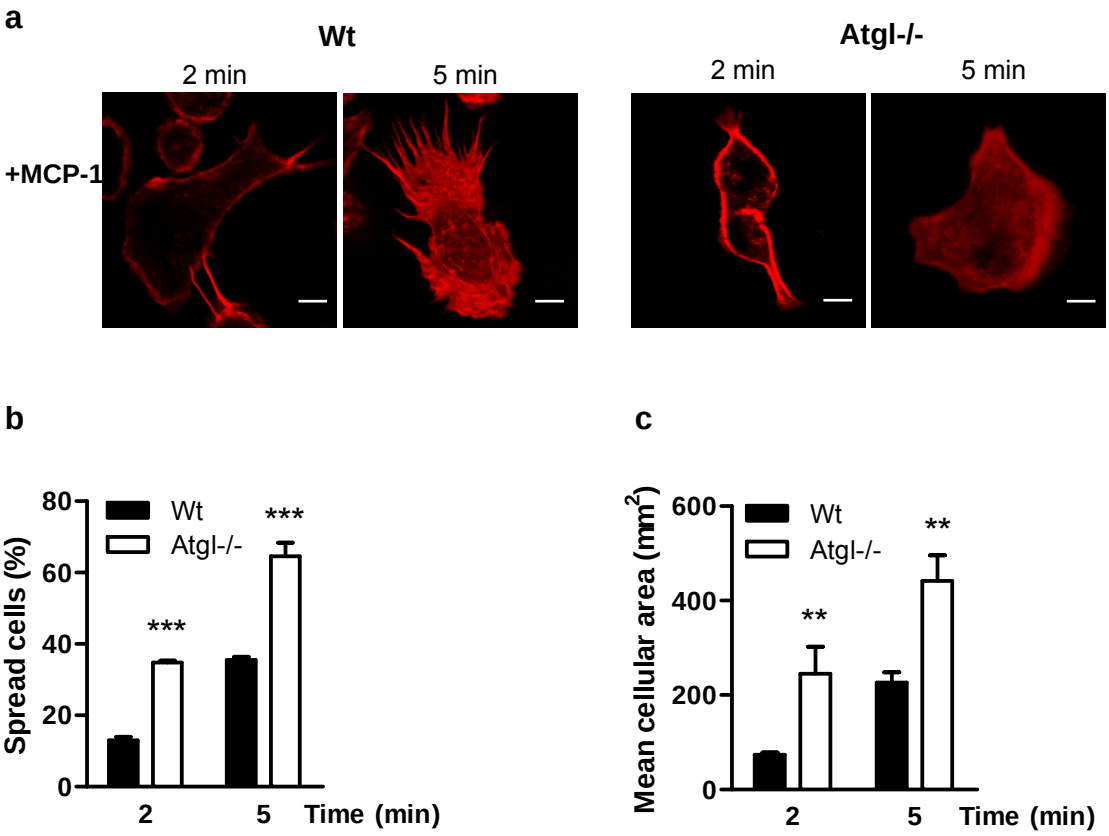

Fig. S2 Accelerated cell spreading in *Atgl-/-* macrophages Macrophages from Wt and *Atgl-/-* mice were plated on fibronectin-coated glass coverslips for 10 min in the presence of MCP-1 (50 ng/ml) for 2 and 5 min. Adherent cells were fixed and stained with phalloidin AlexaFluor-586. (a) Images were taken using a Leica AP5 AOBS confocal microscope. Scale bars: 5 µm. (b) Cell spreading was calculated by counting the percentage of spread cells. (c) Mean cellular area was measured using MetaMorph software. Four fields of cells (~ 100 cells per field) were counted for each condition. ** $p \leq 0.01$, *** $p \leq 0.001$.

Figure S3

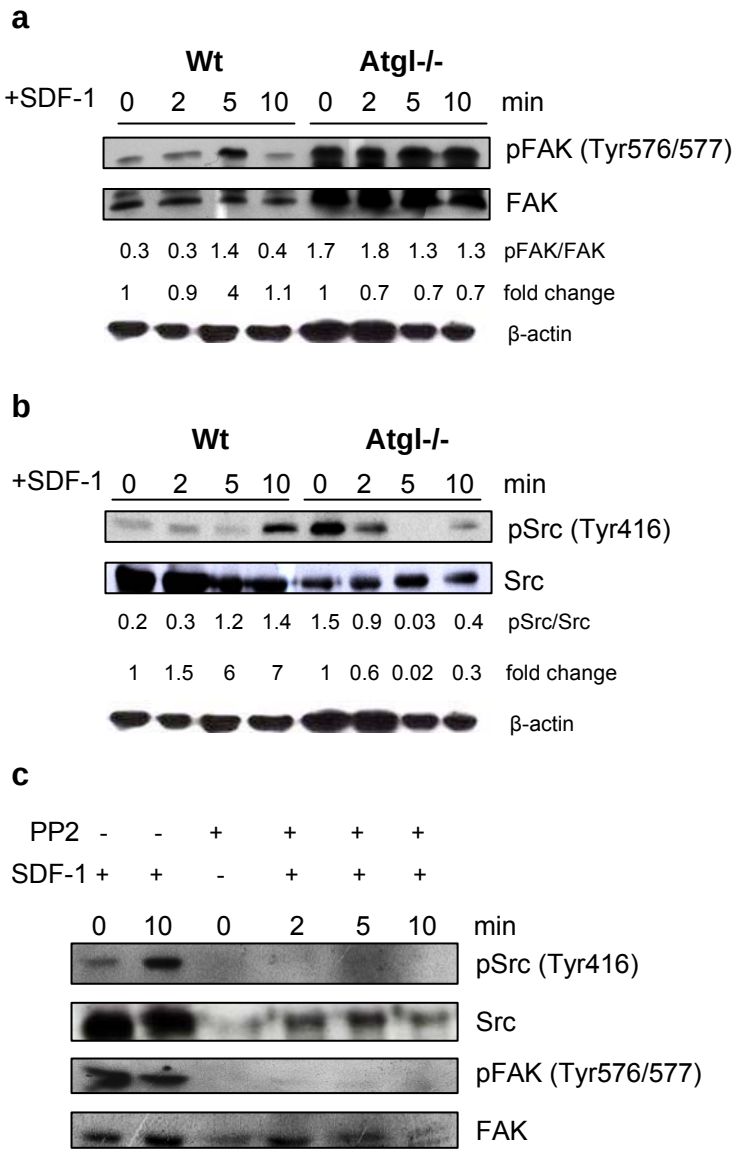

Fig. S3 Sustained FAK phosphorylation in a Src kinase-dependent manner in *Atgl-/-* macrophages Wt and *Atgl-/-* macrophages were incubated with SDF-1 (60 ng/ml) for 0, 2, 5 and 10 min. (a) Cell lysates were subjected to western blot analysis using antibodies specific for total and phosphorylated (p)FAK (Tyr576/577) and (b) total and pSrc (Tyr416). (c) *Atgl-/-* macrophages were preincubated with the Src kinase inhibitor 4-amino-5-(4-chlorophenyl)-7-(*t*-butyl)pyrazolo[3,4-d] pyrimidine (PP2, 10 μM) for 30 min and then exposed to SDF-1 (60 ng/ml) for 0, 2, 5 and 10 min. Western blot analysis was performed using the above mentioned antibodies. Ratios were calculated by dividing band intensities of phosphorylated to total protein expression. Fold changes were calculated relative to the ratios of unstimulated cells. Representative blots from 2 independent experiments are shown.

Figure S4

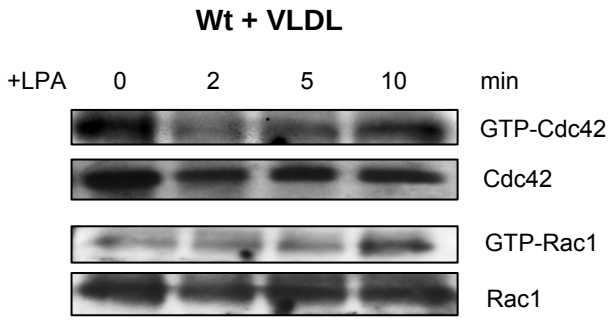

Fig. S4 Altered Cdc42 and Rac1 activation after stimulation of lipolysis Wt macrophages were serum-starved for 12 h and then loaded with VLDL (150 µg/ml) for 18 h. Therafter the cells were incubated in DMEM containing 9 ng/ml LPA for 0, 2, 5, and 10 min. Cell lysates were incubated with glutathione-sepharose beads complexed with GST-PBD fusion proteins to pull-down GTP-bound Rac1 and Cdc42. Precipitates were resolved by SDS-PAGE in parallel with whole cell lysates (total Cdc42 and Rac1). Protein expression of Cdc42 and GTP-Cdc42 as well as Rac1 and GTP-Rac1 was determined by western blot analysis using specific antibodies.