Supplementary Figure 4. Effect of PRAS40 silencing and chemokines on insulin-action. Human primary skeletal muscle cells were transfected with either a non-target (NT) or PRAS40 siRNA on day 3 of differentiation. At day 6 of differentiation, cells were serum-starved or incubated for 24 h with 2 μg/ml chemerin, or 2 ng/ml monocyte chemoattractant protein 1 (MCP-1). Then cells were kept untreated (+; open bars) or exposed for 10 min to 100 nmol/l insulin (+; filled bars). Cell lysates were analyzed by Western blotting for phosphorylation of Akt-Ser473, or by magnetic bead based assays for phosphorylation of p70S6 kinase-Thr412 (b). Membranes were reprobed with antibodies for β-actin to verify equal loading. Data are presented as representative blots and a bar graph showing the mean ± standard error of the mean of at least 5 independent experiments using cells from different donors. Values obtained for insulin-stimulated NT-siRNA transfected cells were considered as control and set at 100%. The effects of PRAS40 silencing and chemokines were analyzed using a two-way ANOVA followed by post-hoc Bonferroni testing for multiple comparisons. ***, p<0.001, **, p<0.01, *, p<0.05 versus cells transfected with NT-siRNA; †, indicates p<0.05 for the effect of insulin- (+) versus untreated-cells (-).