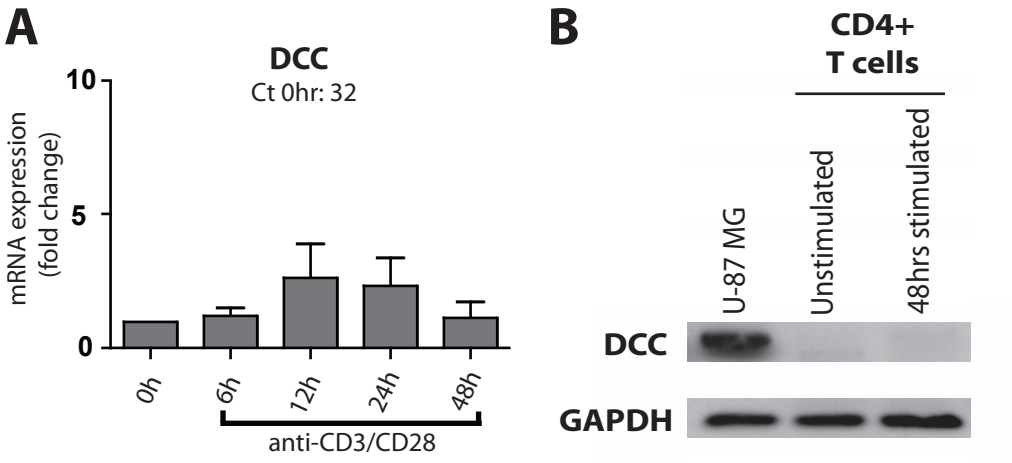

Figure S1. Expression of Netrin-1 and DCC on CD4+ T-lymphocytes: DCC (Panel A) expression was analyzed at the mRNA level by quantitative PCR on unactivated and on mitogen-activated human CD4+ T-lymphocytes. Illustrated is the mean fold change in mRNA expression ±SEM. Illustrated data is from n=3 independent experiments. (Panel B) Western blot analysis of DCC on positive control cells (U87MG) and on CD4+ T-cells.

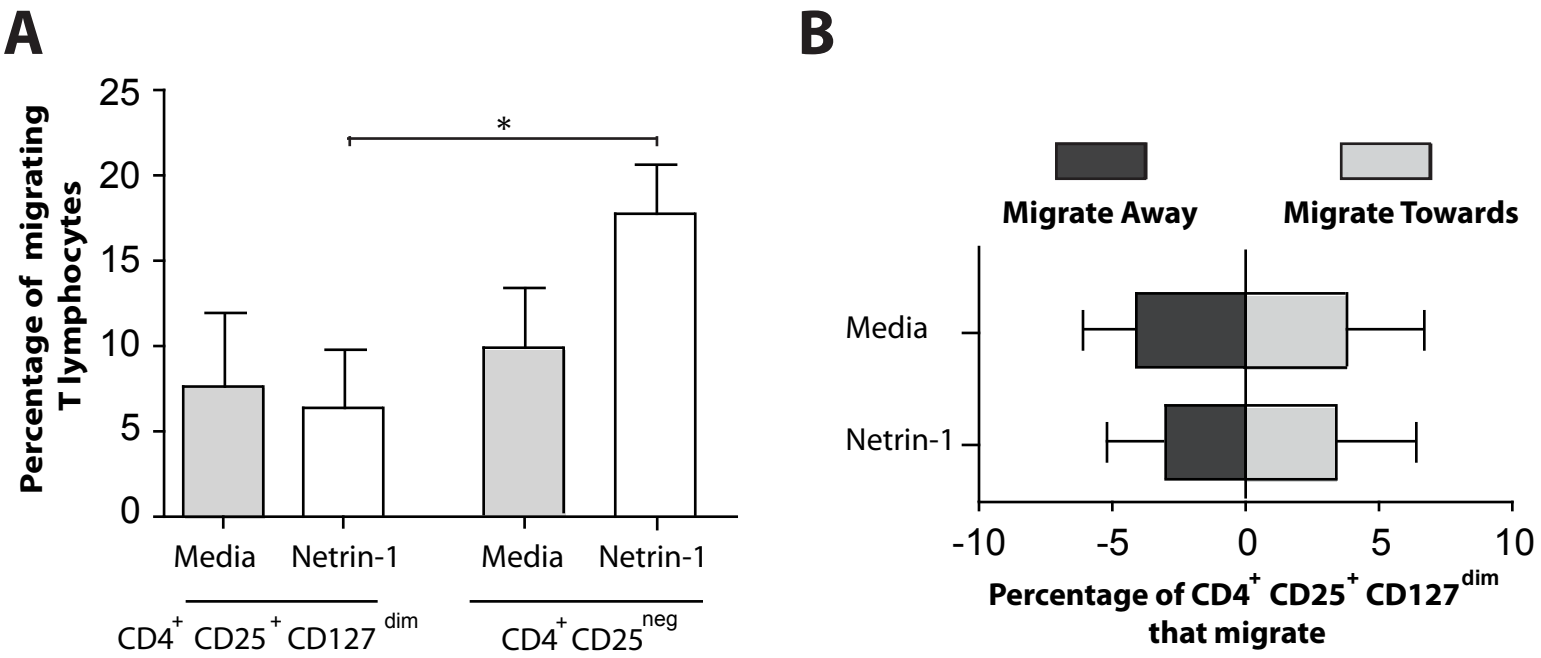

Figure S2. Minimal effect of Netrin-1 on the migration of T regulatory cells: Human CD4+ CD25neg and CD4+CD25+CD127dim cells and were isolated from PBMC and introduced into the microfluidic device and exposed to a Netrin-1 gradient (0.1µg/mL) over an 8-hr time period. A) The total percentage of migrating cells in media alone was compared to conditions in which cells were exposed to a Netrin-1 gradient. B) Directionality of migrating T regs in media alone and in the Netrin-1 gradient. As illustrated Tregulatory cells fail to respond to Netrin-1. Error bars represent mean±SEM. Illustrated data is from n≥3 independent experiments. * =p< 0.05.

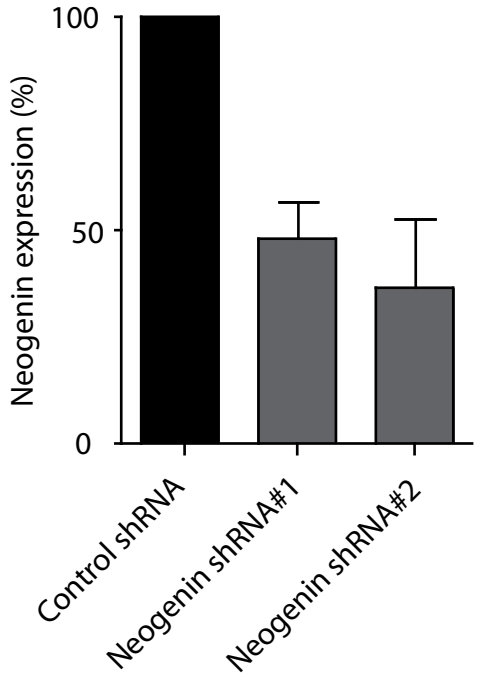

Figure S3. Knockdown efficiency of Neogenin: Human CD4+ T-lymphocytes were infected with two lentiviral Neogenin shRNA constructs as described in Methods, and were subsequently mitogen activated for 48hrs. Knockdown efficiency was determined by qPCR.

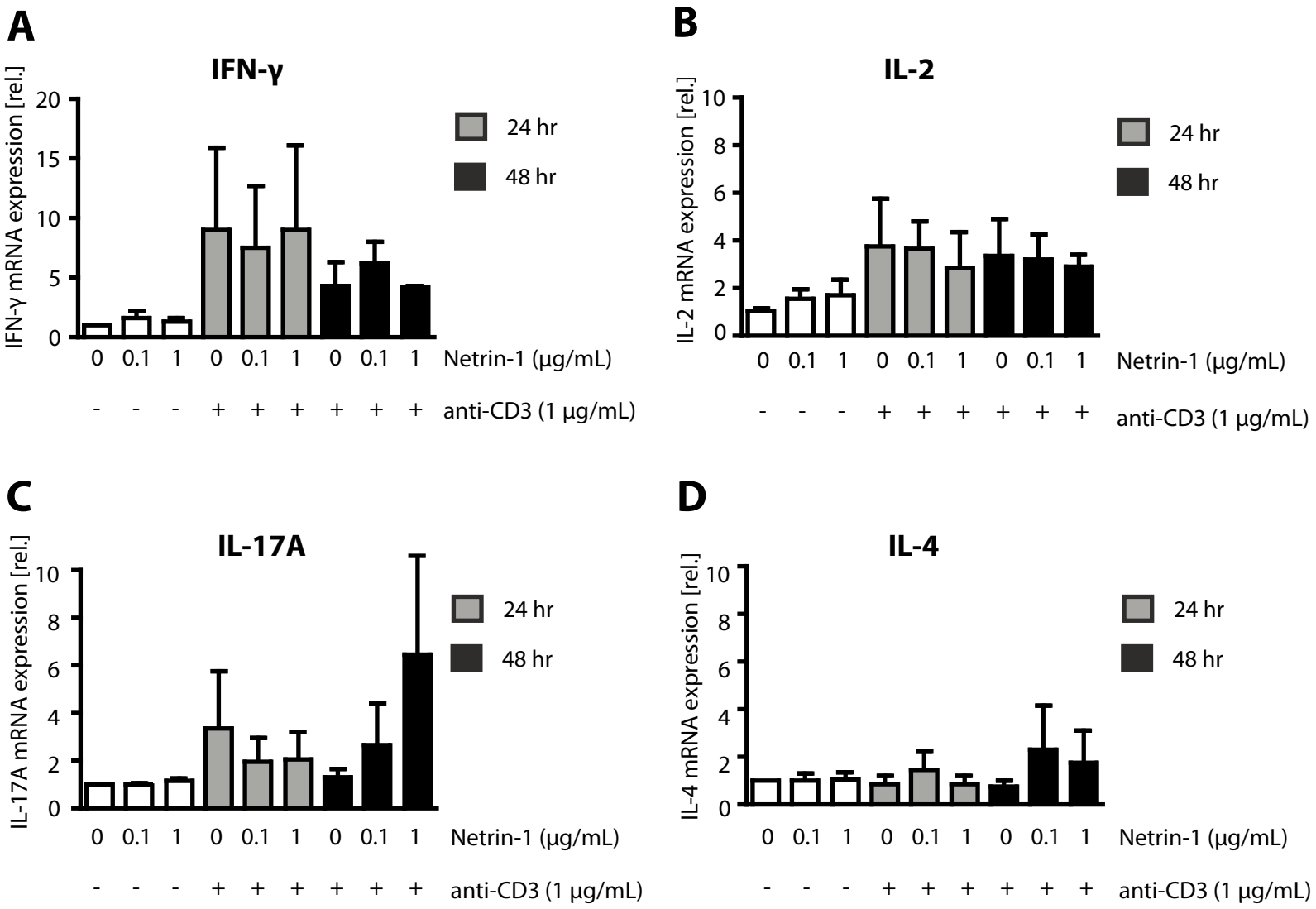

Figure S4. Netrin-1 does not regulate cytokine production in CD4+ T cells: CD4+CD25- T cells were activated with plate-bound anti-CD3 (1 µg/ml) for 24-48hrs in the absence or presence of increasing concentrations of Netrin-1 (0-1 µg/ml). Cytokine production was evaluated at the mRNA level by quantitative PCR. Illustrated is the representative expression of A) IFN-γ, B) IL-2, C) IL-17A and D) IL-4. Panels A-D show the mean fold change in mRNA expression ±SEM. Average of n=2 experiments are illustrated of a total of n≥3, all performed in duplicate.

Supplementary Movie 1 – CD4+ T cell migration in the presence of a Netrin-1 gradient: 48hrs mitogen activated CD4+ T cells were loaded in the microfluidic device and respond to a Netrin-1 gradient (0.1μg/mL) over an 8hr time period.

Supplementary Movie 2 – CD4+ T cell migration in media alone: 48hrs mitogen activated CD4+ T cells show spontaneous migratory behavior in the absence of a chemokine gradient over an 8hr time period.