Sorting and reculture of cells generated on OP9-DL1 stromal cells

CD34^+Lin^- CB cells were cultured on OP9-DL1 in presence of Flt-3L, SCF, IL-7 and IL-15 as described. After 20 days of culture the cells were stained for CD56 and CD45 and sorted into CD56^-, CD56^{dim} and CD56^{high} subsets with a FACSVantage Cell Sorter. The different subsets were recultured on OP9-DL1 with the same cytokines for different time point as indicated on Figure S1.

It is clear that the CD56^- become progressively CD56^{dim} and CD56^{high} in function of length of culture time, the CD56^{dim} subset becomes CD56^{high} and the CD56^{high} subset remains CD56^{high}.

Gene expression analysis

Sorted CD56^+ NK cells from OP9-control and OP9-DL1 cocultures were resuspended in RLT buffer (Qiagen) and stored at -70°C prior to RNA extraction. RNA was isolated using RNeasy (Qiagen) and converted into cDNA using Superscript RT II with random hexamers (Invitrogen). Real-time PCR reactions were performed using qPCR Core kit for SYBR® Green I (Eurogentec) on a 7300 Real-time PCR system (Applied Biosystems). Primer sequences are: CD122fw: AGACCCTCTGAATTCTTTTCC. CD122rev: CAGGGCTGAAGGACGATGAG. CD127fw: TGGCTGGGAATGTCAGTGC; CD127rev: CATTCTTGCCACTCTCCCTGC. Relative expression levels were calculated for each gene using the ΔΔCt method using β-actin for normalization.