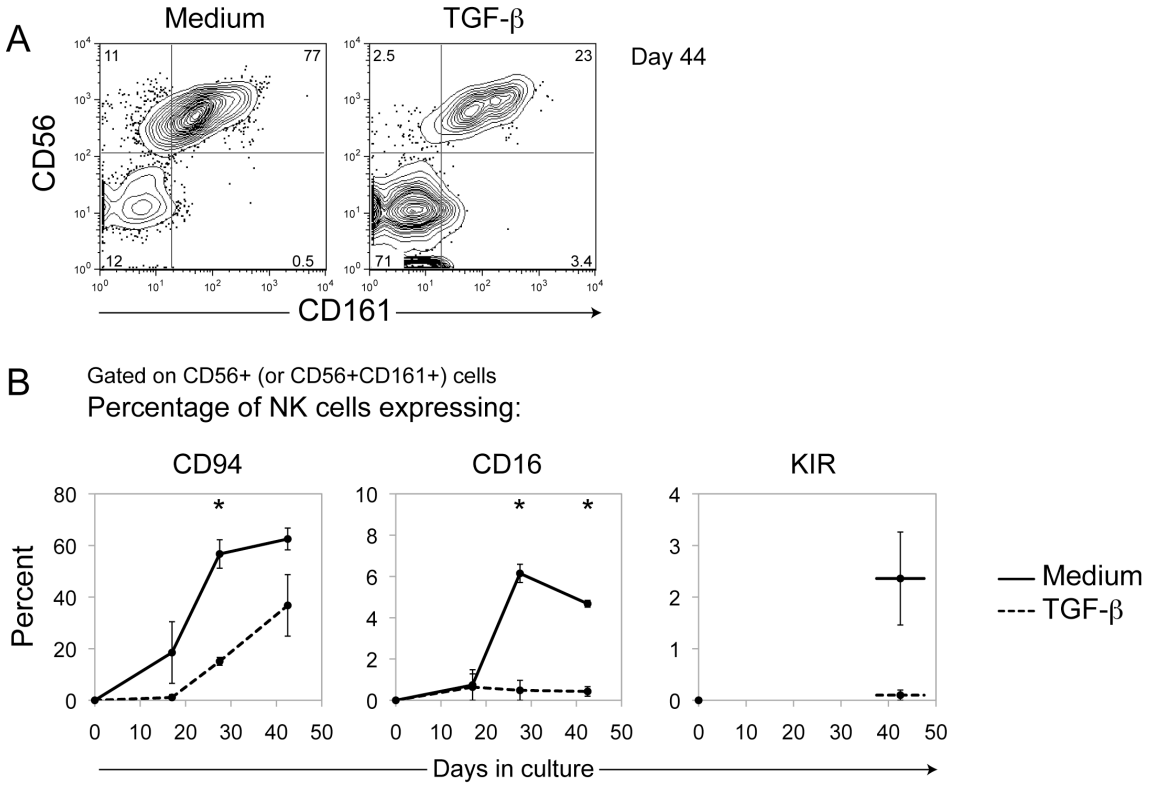
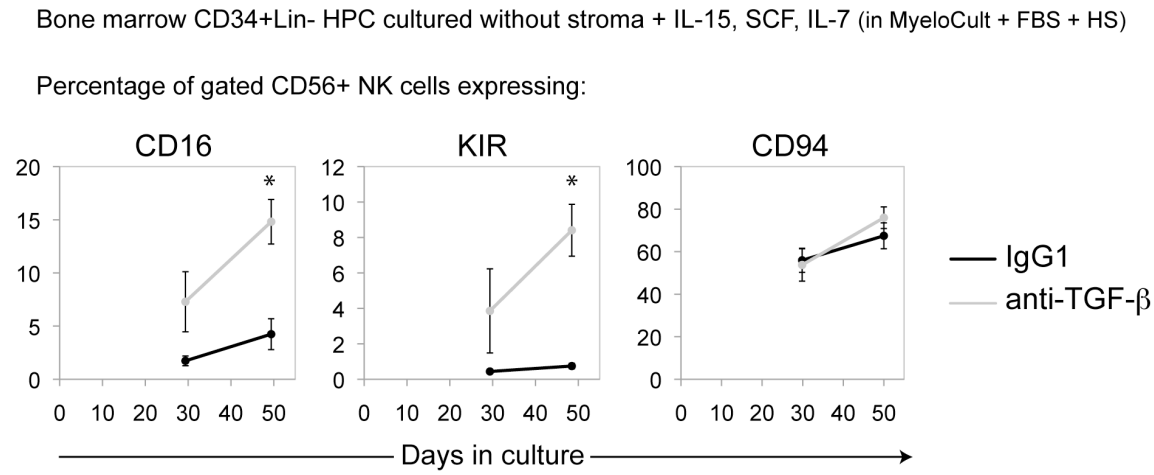
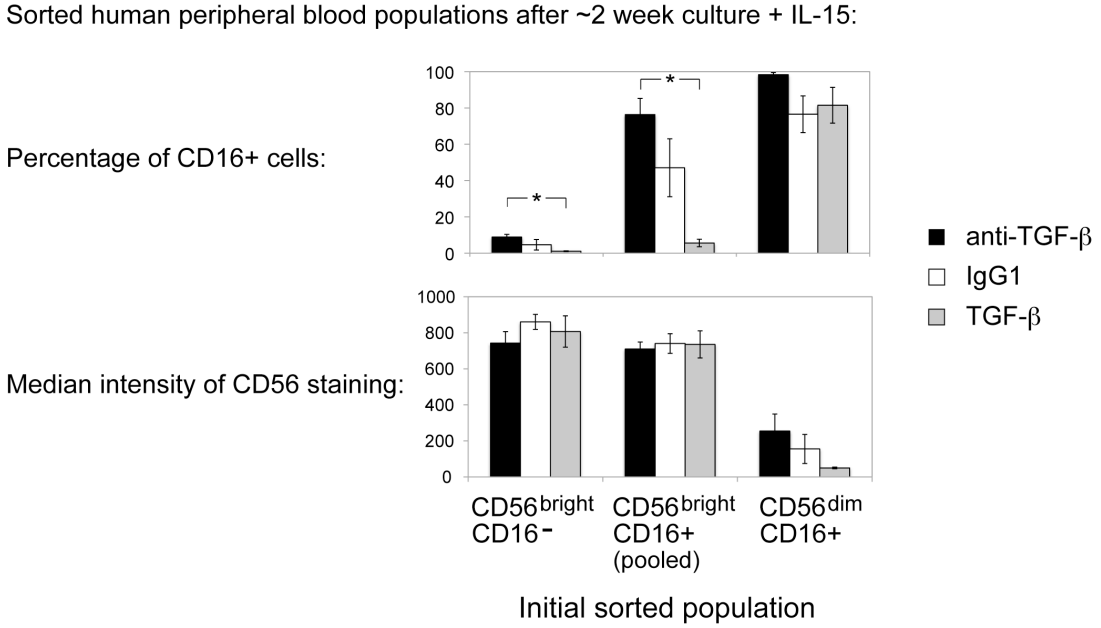
Bone marrow CD34^{+}CD38$^{low/-}$ HPC cultured with OP9 stromal cells + IL-15, SCF, Flt3L (in MEMα + FBS)



Supporting Information Figure 1. TGF-β1 delays or inhibits acquisition of CD94, CD16 and KIR on NK cells developing from bone marrow CD34^{+}CD38$^{low/-}$ HPC on OP9 stromal cells. Further characterization of experiments performed as described in Figure 1a. (A) Staining of CD56 versus CD161 (NKR-P1A) (gated by FSC/SSC/PI). (B) Averages of three experiments from two donors. Percentages of cells expressing CD94, CD16, or KIR were measured on gated CD56^{+} (or CD56^{+}CD161^{+}) NK cells. KIR were detected with pooled mAb HP-3E4, CH-L, and DX9. Data show means of measurements taken at approximately the same duration from different experiments ±SEM; * indicates $p<0.05$ in two tailed Student's t-test.



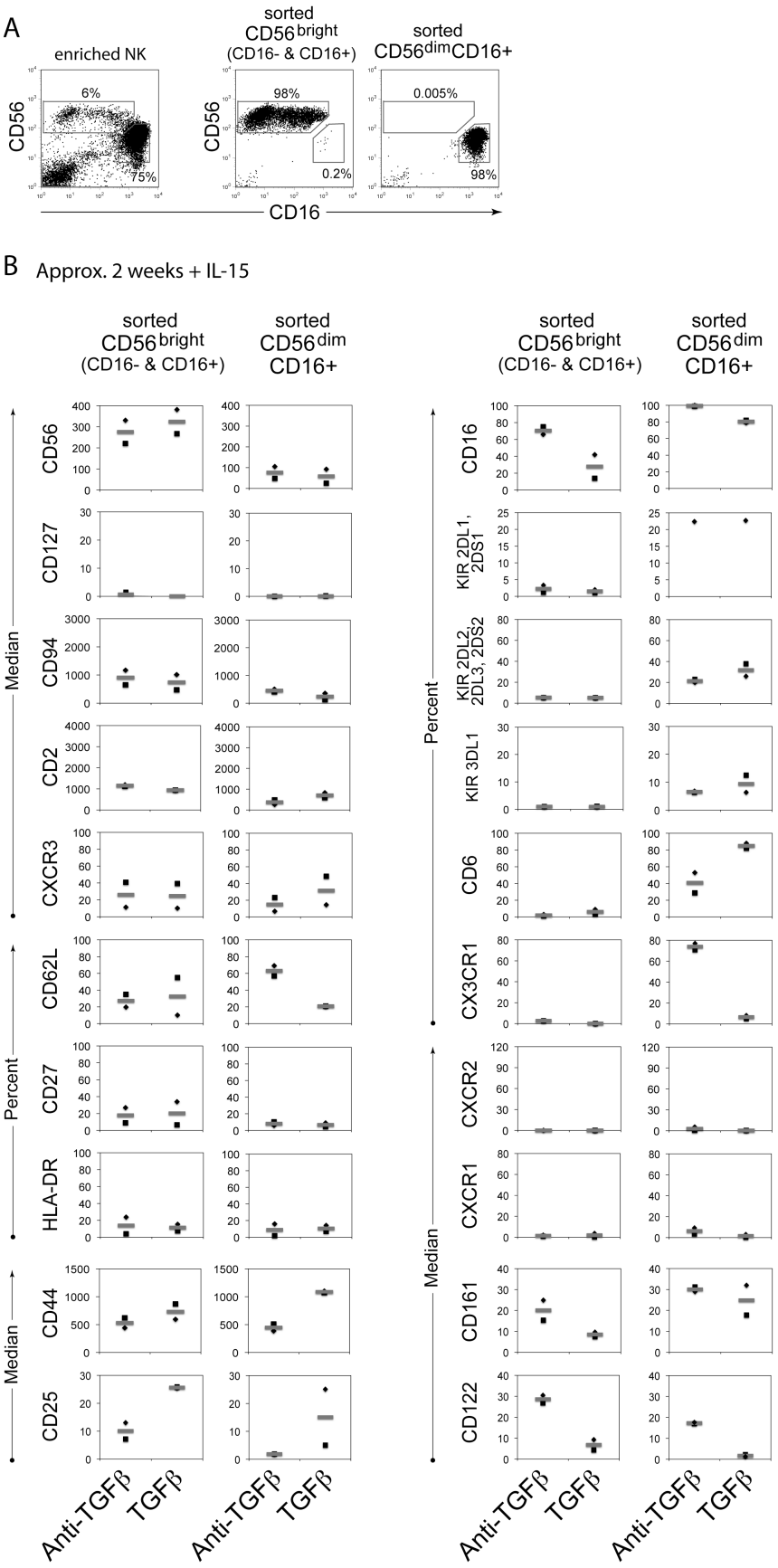
Supporting Information Figure 2. TGF-β affects the percentage of CD16$^+$ and KIR$^+$ NK cells developing from bone marrow CD34$^+$Lin$^-$ HPC *in vitro* (without stromal cells). Averages of 7-10 experiments performed as described in Figure 1c. Percentages of cells expressing CD16, KIR, or CD94 were measured on gated CD56$^+$ NK cells. KIR were detected with pooled mAb HP-3E4, CH-L, and DX9. Data show means of measurements taken at approximately the same duration from different experiments ±SEM; * indicates $p<0.05$ in two tailed Student's t-test. Effects on acquisition of CD94 may require higher TGF-β concentrations (as Supporting Information Figure 1).



Supporting Information Figure 3. TGF-β inhibits and down-regulates CD16 expression on CD56^{bright} NK cells from human peripheral blood. In addition, TGF-β appeared to down-regulate CD56 expression on sorted CD56^{dim}CD16^{+} NK cells, while TGF-β addition or neutralization had less effect on CD56 levels on sorted CD56^{bright} NK cells. This may indicate that altered TGF-β signaling may contribute to the "CD56^{dim}" phenotype following commitment to the CD56^{dim}CD16^{+} stage. Averages of three experiments performed as Figure 3a-c. Briefly, human blood NK populations were sorted as indicated, then cultured with IL-15 (20ng/ml) plus IgG1, or anti-TGF-β 1D11 mAb (10μg/ml), or added TGF-β1 (2ng/ml). After approximately two weeks, cells were analyzed by flow cytometry. Results from sorted CD56^{bright}CD16^{low} and CD56^{bright}CD16^{high} populations were pooled for analysis and labeled CD56^{bright}CD16^{+}. Error bars represent SEM; * indicates $p<0.05$ in two tailed Student's t-test.

Supporting Information Figure 4 (next page). TGF-β1 affects expression of several cell surface molecules on NK cells. (A) NK cells enriched from human peripheral blood were sorted into CD56^{bright} and CD56^{dim}CD16^{+} subsets as indicated. (Sort purity is also shown). (B) Phenotype of cells after culture for approximately 14 days with IL-15 (20ng/ml) plus anti-TGF-β 1D11 mAb (10μg/ml) or added TGF-β1 (2ng/ml). Median fluorescence intensity (minus median fluorescence of IgG control) or percentage of cells staining positively is depicted for each mAb. Two independent experiments are shown (along with the mean).

Supporting Information Figure 4



Supporting Information: TGF-β affects development and differentiation of human natural killer cell subsets
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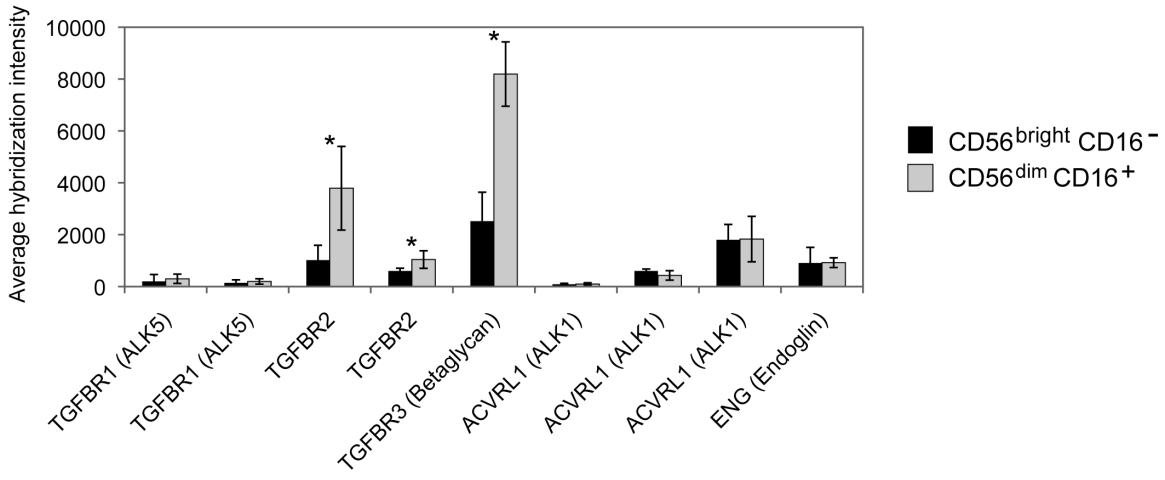

Supporting Information Figure 5. TGF-β receptor expression on human NK cell subsets. Microarray data, derived from reference [4] online supplementary file #2, comparing sorted $CD56^{bright}CD16^{-}$ and $CD56^{dim}CD16^{+}$ NK cell subsets. Shown are average hybridization intensity and SD for several probe-sets recognizing TGF-β receptors. * indicates $p<0.05$ in a two-tailed Student's t test. A second unpublished microarray dataset (Affymetrix human genome U133 Set) comparing $CD56^{bright}CD16^{-}$ and $CD56^{dim}CD16^{+}$ NK cells confirms the trends observed for TGFBR2 and TGFBR3 expression and suggests that $CD56^{dim}CD16^{+}$ cells may also express higher levels of TGFBR1 transcripts compared with $CD56^{bright}CD16^{-}$ NK cells (not shown).