Online Supplemental Material for

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Materials and Methods

RNA Amplification

In brief, first strand cDNA synthesis was performed in a 20-μl reaction volume containing 1–3 μg RNA, 200 ng (dT)-T7 primer [5′-GCATTAGCGGCCGCGAAATTAATACGACTCACTATAGGGAGA$(T)_{21}$V-3′], 100 mM DTT, 10 mM dNTP, 20 U RNase inhibitor, and 100 U Superscript II in first-strand buffer (Life Technologies), and incubation for 40 min at 42°C, 10 min at 50°C, and 10 min at 55°C, followed by heat inactivation for 15 min at 65°C. Second-strand synthesis was performed in 150 μl by adding 130 μl of an ice-cold premix containing 40 U DNA polymerase I, 2 U *E. coli* RNase H, 10 U *E. coli* DNA ligase, and 10 mM dNTP in second-strand buffer (Life Technologies) for 2 h at 15°C. After polishing with T4 DNA polymerase (Life Technologies) for 15 min at 15°C, inactivation for 10 min at 70°C, double-stranded cDNA was purified by extraction using phenol/choloroform/isoamylalcohol (Ambion) in gel tubes (Phase Lock; Eppendorf), followed by chromatography on a Bio-Spin Gel P6 column (Bio-Rad) and precipitated overnight using 1/25 volume 5 M NaCl (Ambion) and 2.5 volumes 95% ethanol. Samples used for genechip analyses are specified in Table S1.

cRNA Preparation and Hybridization

An in vitro transcription reaction for generation of biotin-labeled RNA target was performed using the BioArray HighYield RNA transcript labeling kit according to instructions in the Affymetrix technical manual (Enzo Diagnostics, distributed by Affymetrix, CA). The biotin-labeled target cRNA was then fragmented in fragmentation buffer (40 mM Tris-acetate (pH 8.1), 100 mM KOAc, 30 mM MgOAc) and used to prepare a hybridization mixture. This mixture, containing ~0.05 μg/μl fragmented cRNA, probe array controls and blocking reagents, was used for hybridization, as described in the Affymetrix technical manual (Affymetrix, San Jose, CA). The hybridization mixture was first hybridized to Test arrays to evaluate the quality of the cRNA. Satisfactory cRNA mixtures were subsequently hybridized to the Human Genome HGU95Av2 chips displaying probes for ~10,000 full-length genes for expression analysis. Chips were washed, stained with SA-PE, and scanned using an Affymetrix automated fluidics station and scanner, and the resultant image was captured as a data image file.

Supplemental Raw and Formatted Microarray Data

Data Set 1. Data Set 1 represents summary of data (average hybridization intensities, standard deviations, and presence calls) for all samples and all probesets. The file can be queried by probe Affymetrix Probe ID or by gene name at http://www.affymetrix.com/analysis/index.affx if one wants to search for a gene of interest. A GeneBank accession number can be entered for further information into http://www.ncbi.nlm.nih.gov./entrez/query.fcgi. Average hybridization intensities (Avg_int) and standard deviation (SD) for each NK cell subset are listed as well as the number of samples in which the gene was expressed (P-calls) out of nine dNK, five $CD56^{bright}$ pNK, and five $CD56^{dim}$ pNK samples.

Data Set 2. Microarray Suite analysis software (MAS 5.0) generated expression databased on Affymetrix algorithms and decision matrices. Data Set 2 contains signal intensities, detection calls Absent (A), Present (P), or Marginal (M), and detection p-values. Sample names correspond with Table SI.

Data Set 3. Filtered and normalized data. Signal intensity values were normalized by setting a trimmed mean to be the same for the 19 samples. The resulting data were used for T-test comparisons as described in the article's Materials and Methods section. The first column corresponds to row number in the 'pivotdata_19chips.txt' for the 5414 genes that passed the filter. Sample names correspond with Table SI.

The original " .CEL" files for the 19 chip hybridizations are available upon request. These were generated by Microarray Suite software (MAS 5.0) from image data files, and contain the intensities of each cell, i.e., the smallest divisions on the chip. A cell contains several thousand copies of a probe sequence. The .CEL files contain a captured image of the scanned GeneChip array and calculations of the raw intensities for probe sets.

Gene Classification

Genes were classified based on information provided by Affymetrix (http://www.affymetrix.com/analysis/index.affx (Liu, G., A. E. Loraine, et al. 2003. NetAffx: Affymetrix probesets and annotations. *Nucleic Acids. Res.* 31: 82–86), and on additional information available in the Gene Ontology Consortium functional annotations (http://www.geneontology.org), Locuslink (http://www.ncbi.nlm.nih.gov/LocusLink/), OMIM (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=OMIM), and PubMed (http://www.ncbi.nlm.nih.gov./entrez/query.fcgi) databases.