For RNA-seq analyses (bulk transcriptome), CD8 memory T cells were isolated ex vivo as described above and stimulated for 48 hours with CD3 and CD28 mAbs for a total culture time of 5 days in the presence of low and high NaCl concentrations. Total RNA was extracted from cells lysed in TRI reagent (Sigma-Aldrich) according to the manufacturer’s protocol. RNA was quantified using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific) and its quality was verified by an Agilent 2100 Bioanalyzer (Agilent) according to the manufacturer’s guidelines.

Library preparation for RNA-seq was performed using the TruSeq® Stranded Total RNA Sample Preparation Guide (Illumina) starting with 450 ng RNA as the input for each sample. Only 11 cycles were used for PCR amplification to minimize PCR bias. The barcoded libraries were sequenced on a HiSeq 2500 (Illumina) with paired-end, 100-bp reads. Approximately 6 Gb of sequencing reads were produced on average per sample. The reads were mapped to the reference transcriptome built from the human genome assembly hg38 (GRCh38) using STAR v2.6.1a 14. Transcripts were quantified with salmon v0.11.3 in the alignment-based mode 15. Downstream analyses were performed with the statistical framework R (ISBN 3-900051-07-0). Differentially expressed genes were identified using the R package DESeq2 16, considering the effect of samples originating from the same donor. Log fold change cutoff was set at 0 for determining differentially expressed genes using the Wald Test and the significance threshold was based on the false discovery rate (FDR) < 0.05. In total, there were 8120 upregulated, 8029 downregulated and 44474 dysregulated genes, out of which 1956 genes were significantly upregulated, and 1926 genes were significantly downregulated. Plots were produced with the R package ggplot2 17. For the principal component analysis (PCA), top 500 genes with highest variance were used. The clusterprofiler R package 18 was employed for overrepresentation analysis of GO terms within the ontology “Biological Process”, where the p value cutoff was set to 0.05 and the q value cutoff was set to 0.1. The enrichment plots show the top 20 entries for the significantly upregulated, downregulated and dysregulated genes respectively, which were ranked according to the shrunken fold change values calculated by DESeq2, as previously suggested 16. Access to our raw data is publicly available (GSE ….).