SUPPLEMENTARY DATA

Table S1. Quantitative RT-PCR analysis: primer details

Gene	Sequence	Amplicon
Mki67	Fw: CCCACAAGCAATCTTCACAA Rv: GCTGCTGCTTCTCCTTCACT	214
Ccnb2	Fw: CTTCGAGACTCGGGGTTAGG Rv: CCATTCTCCAAGTGGGTCAT	189
Uhrf1	Fw: CGAACTATGGATGGGAAGGA Rv: CCGAGTCCCGTTCTTTTGTA	250
Ccl5	Fw: CCCTCACCATCATCCTCACT Rv: GAGCACTTGCTGCTGGTGTA	154
Itgb1	Fw: AACTGCACCAGCCCATTTAG Rv: ACATTCCTCCAGCCAATCAG	176
Dusp5	Fw: CGCGGGTCTACTTCCTTAAA Rv: CTGTAGGCGACGCTGAGAAC	154
Nr4a1	Fw: CTCTCCGAACCGTGACACTT Rv: TGGCGCTTTTCTGTACTGTG	191
18s	Fw: GTAACCCGTTGAACCCCATT Rv: CCATCCAATCGGTAGTAGCG	151
CD8	Fw: CCCACAAGCAATCTTCACAA Rv: GCTGCTGCTTCTCCTTCACT	214
CD4	Fw: CTTCGAGACTCGGGGTTAGG Rv: CCATTCTCCAAGTGGGTCAT	189
CD62L	Fw: CGAACTATGGATGGGAAGGA Rv: CCGAGTCCCGTTCTTTTGTA	250
CD44	Fw: CCCTCACCATCATCCTCACT Rv: GAGCACTTGCTGCTGGTGTA	154
Itgb1	Fw: AACTGCACCAGCCCATTTAG Rv: ACATTCCTCCAGCCAATCAG	176
Dusp5	Fw: CGCGGGTCTACTTCCTTAAA Rv: CTGTAGGCGACGCTGAGAAC	154
Nr4a1	Fw: CTCTCCGAACCGTGACACTT Rv: TGGCGCTTTTCTGTACTGTG	191
18s	Fw: GTAACCCGTTGAACCCCATT Rv: CCATCCAATCGGTAGTAGCG	151

Figure S1. Induction of CD69 on CD8hi versus CD8lo naive CD8 T cells

Frequencies of cells expressing CD69 or CD25 upon activation are shown on sort-purified CD8hi and CD8lo naïve CD8 T cells stimulated with anti-CD3 + anti-CD28 for 24 or 36 h as indicated. (n=3).

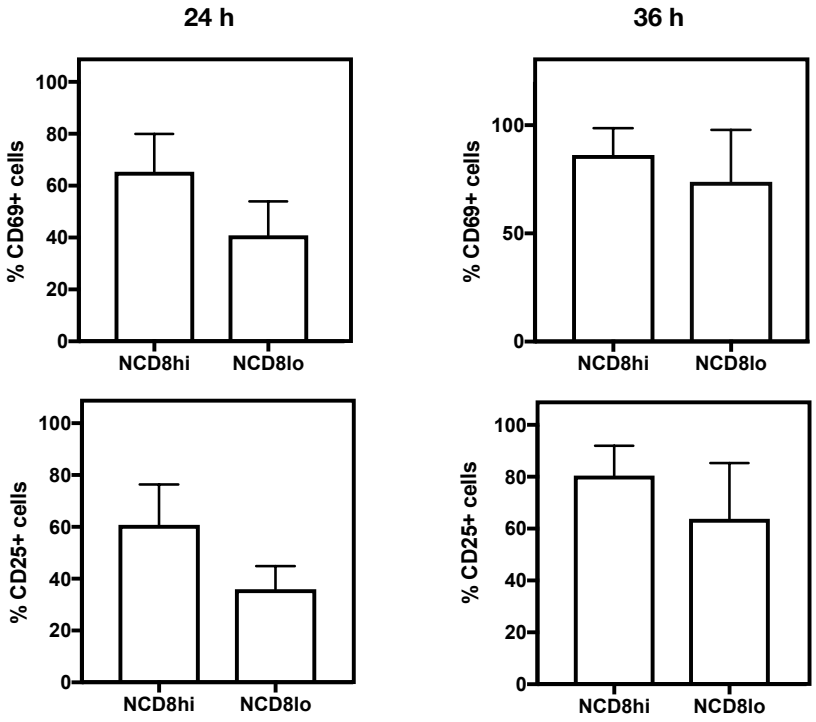

Figure S2. Cell-surface TCR levels on CD8hi versus CD8lo naive CD8 T cells of equal size

Top row: Naive (CD62Lhi CD44lo) CD8T cells were gated into CD8hi- and CD8lo-decile subsets, and their relative cell sizes (FSC; left panel) and cell-surface TCRß (right panel) levels were compared between naive CD8hi (red lines) and naive CD8lo (blue lines) cells of equal sizes (right panel).
Bottom row: Naive (CD62Lhi CD44lo) CD8T cells were first gated into CD8hi- and CD8lo-decile subsets, and then a cell size (FSC) gate was used to generate CD8hi and CD8lo subsets of equal sizes (left panel). The expression of TCRβ was then tested between naive CD8hi (red lines) and naive CD8lo (blue lines) cells of equal sizes (right panel).

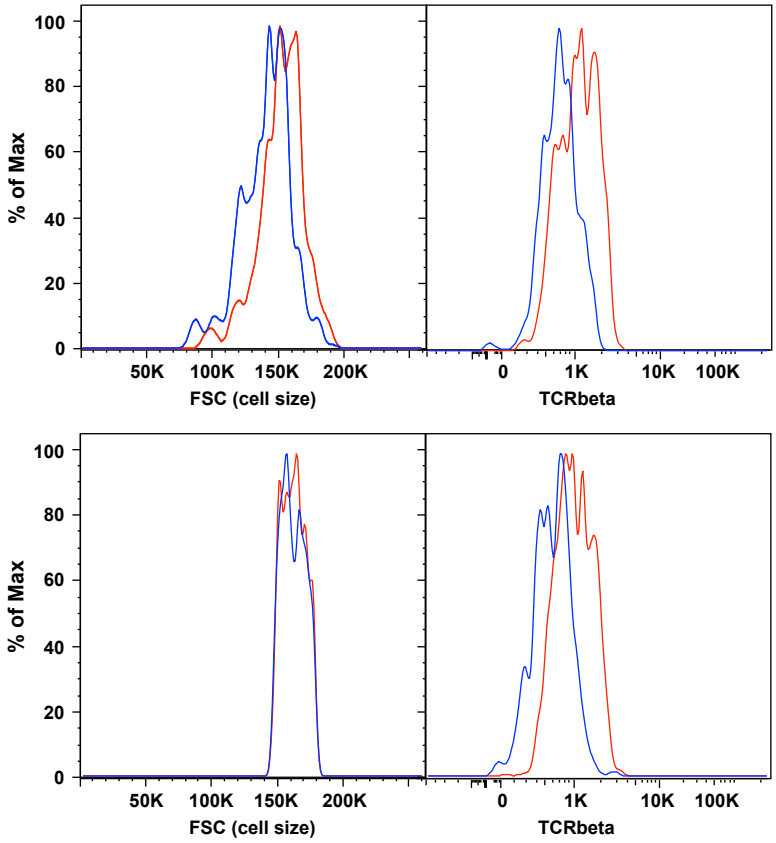

Figure S3. Compromised baseline signaling in CD8lo cells

Baseline levels of pZAP-70/pSyk and pERK on CD8hi and CD8lo naive CD8 T cells. Isotype controls are shown as gray histograms. Flow cytometry was done for pZAP70/pSyk and pERK in a two-step protocol using relevant unlabelled rabbit antibodies (Cell Signaling Technologies; dilutions as recommended by manufacturer), followed by fluorochrome-coupled secondary Fab'2 goat anti-rabbit IgG1 (0.5 µg/100µl).

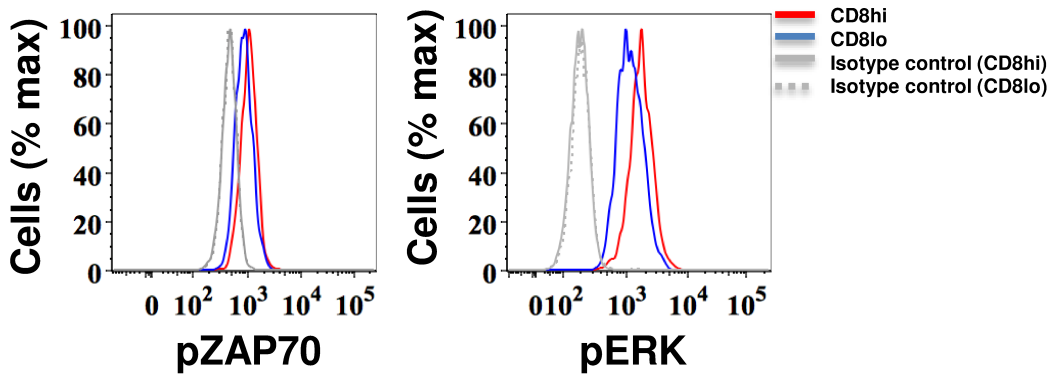

Figure S4. Comparison of mouse CD8hi and CD8lo RNA-Seq with human neonatal and adult CD8 T-cell expression array (GSE61570).

Comparison of RNA-Seq data on CD8hi and CD8lo naive CD8 T cells with a published dataset on human microarray analysis GSE61570 (Galindo-Albarran et al. 2016) on neonatal and adult CD8 T cells samples using Geo2R. (**A)** A Venn diagram showing significant overlap in genes upregulated in mouse CD8Hi cells and human neonatal CD8 cells ($p > 0.01$). (**B)** Expression heatmap of overlapping genes in mouse CD8hi and CD8lo cells based on RNA-Seq analysis. As expected, all overlapping genes are more highly expressed in CD8hi cells. (**C)** Gene Ontology analysis for over-represented biological processes. The genes that are more highly expressed in neonatal human CD8 cells and mouse CD8hi cells were subjected to Gene Ontology analysis. The Cell cycle term was found to be over-represented.