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Supplemental Information

$CD8^+$ T Cells from Human Neonates Are Biased toward an Innate Immune Response

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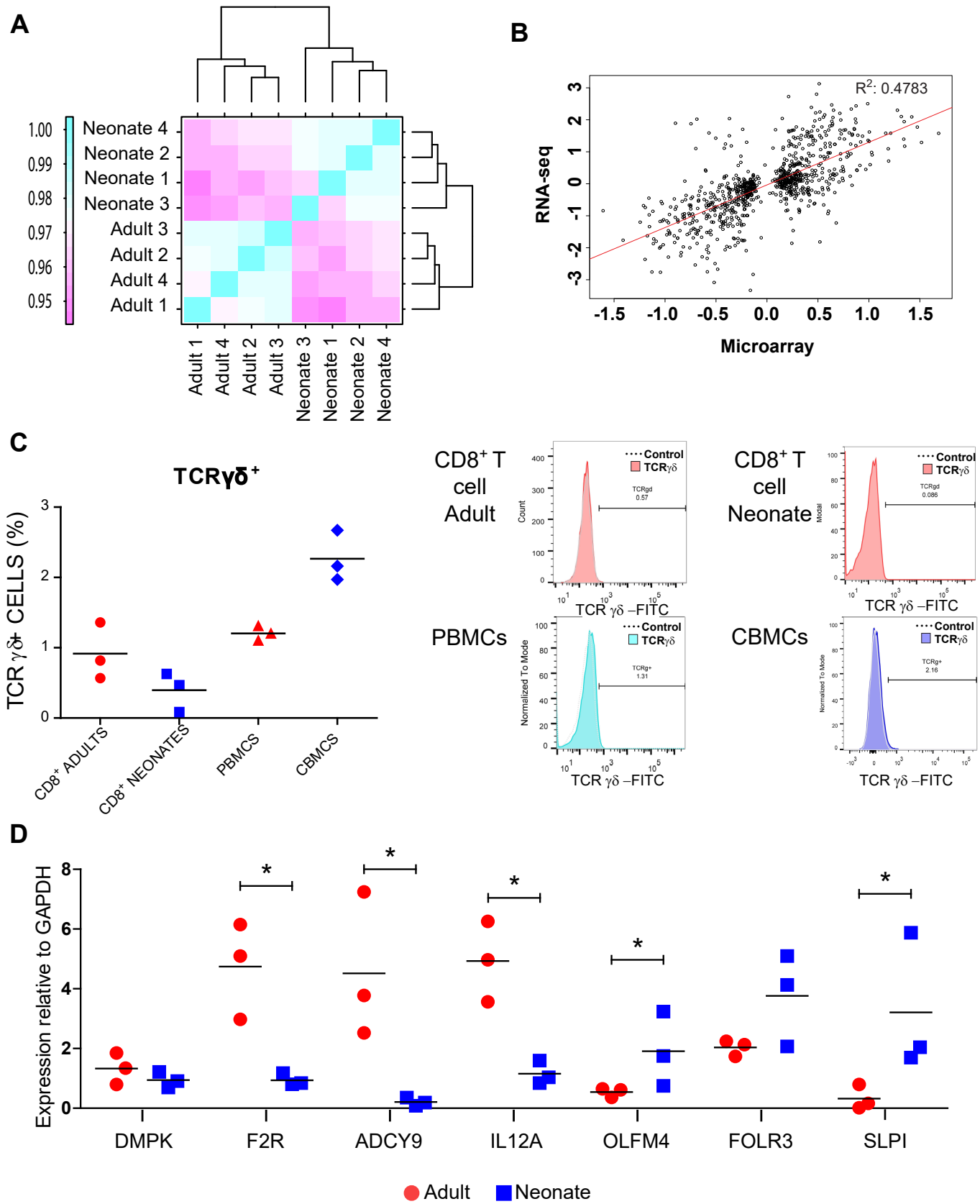
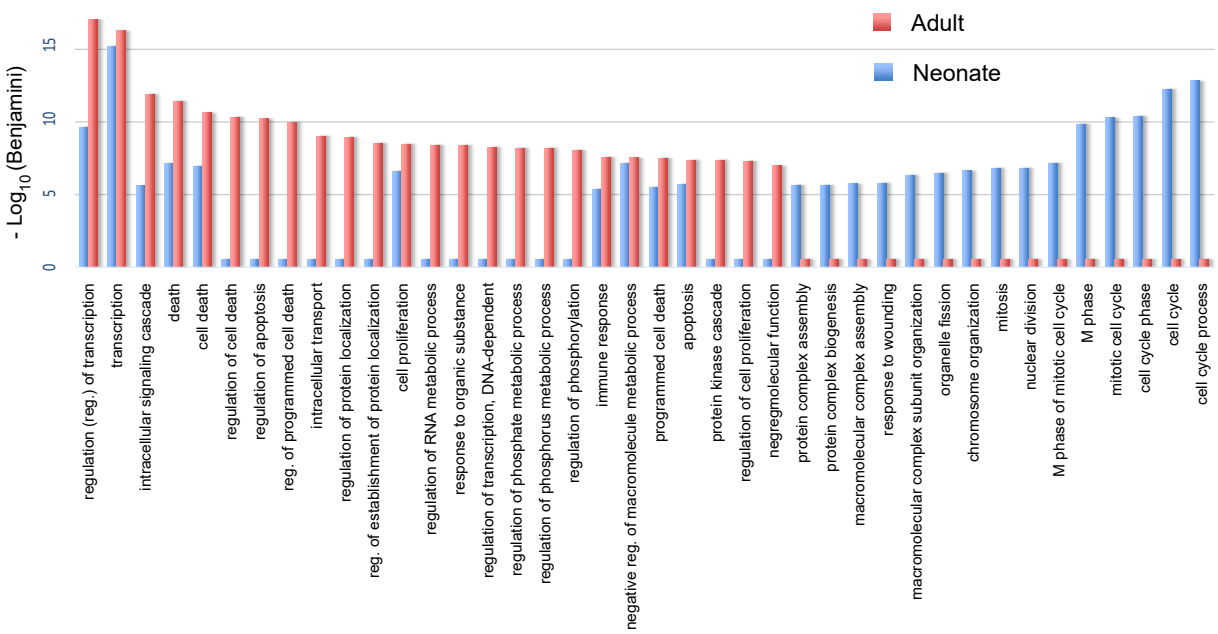
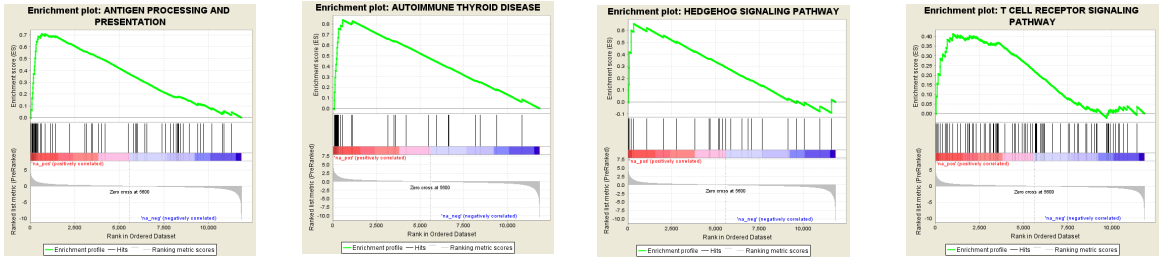


Figure S1. Unsupervised clustering and gene expression validation, related to Figure 1. A) Pearson correlation and unsupervised hierarchical clustering of naïve neonate and adult CD8$^+$ T cells. B) Scatter plot shows the correlation between microarray and RNAseq data of significative genes, each axis are the fold change Adult/Neonate in $\log_2$ scale. C) Flow cytometry showing the percentage of TCR$\gamma\delta^+$ cells in each population (by triplicate) and one representative flow cytometry histogram for each group (right panel). D) RT-qPCR showing the transcription levels of randomly chosen genes, used to validate the mirroarray data. Four genes were overexpressed in adult cells and tree in the neonate population. Statistical significance was assessed by Mann-Whitney U test. *$P < 0.05$.

A

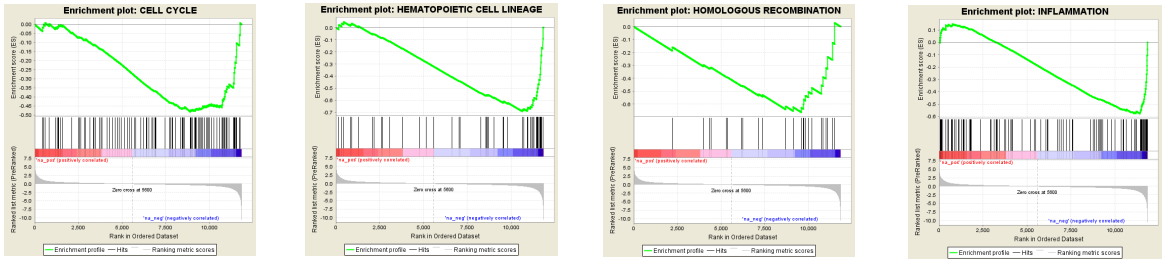


B **Adult**



NAME	SIZE	ES	NES	NOM p-val	FDR q-val	FWER p-val
ANTIGEN PROCESSING AND PRESENTATION	60	0.708	2.263	0	0	0
AUTOIMMUNE THYROID DISEASE	30	0.837	2.368	0	0	0
HEDGEHOG SIGNALING PATHWAY	27	0.655	1.801	0	0.016	0.137
T CELL RECEPTOR SIGNALING PATHWAY	88	0.412	1.451	0.019	0.195	0.981

C **Neonate**



NAME	SIZE	ES	NES	NOM p-val	FDR q-val	FWER p-val
HEMATOPOIETIC CELL LINEAGE	48	-0.6894538	-1.9194214	0	0.009833152	0.011
HOMOLOGOUS RECOMBINATION	24	-0.6635029	-1.5962675	0.014423077	0.3153934	0.837
CELL CYCLE	105	-0.4831129	-1.4900504	0.006702413	0.3254052	0.991
INFLAMMATION	109	-0.57814723	-1.7931628	0	0.05568169	0.117

Figure S2. GO terms enrichment and GSEA analysis of neonatal and adult cells, related to Figure 2. A) Differential GO Terms enriched in neonatal or adult CD8$^+$ T cells. Differentially expressed genes were analysed with DAVID software to associate genes to GO terms. The top 25 more significant GO terms are represented for each case. Significance of enrichment in each pathway is shown in bars. B) and C) Gene Set Enrichment Analysis of neonate or adult CD8$^+$ T cell samples, selected significantly enriched pathways are shown.

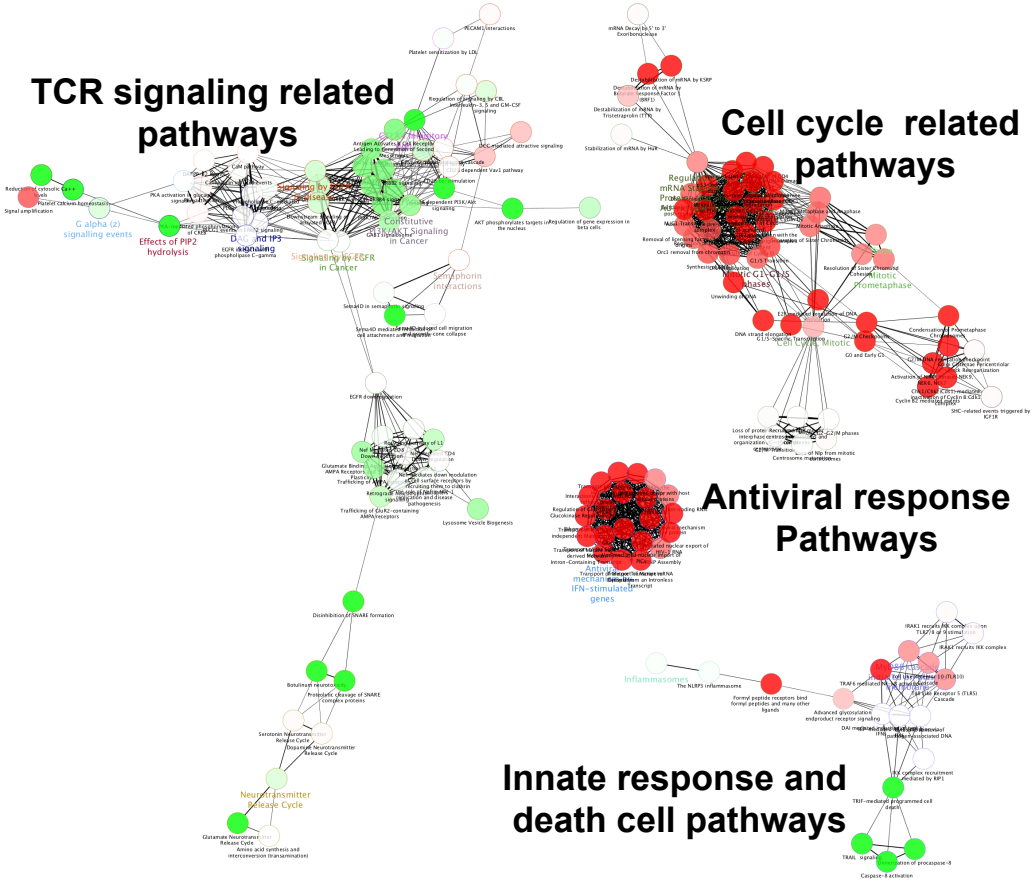


Figure S3. Reactome analysis, related to Figures 3, and 4. Major networks of related pathways are shown, each circle represents a reactome pathway. Neonate cells enriched pathways are represented in red, and pathways enriched in adult cells are shown in green.

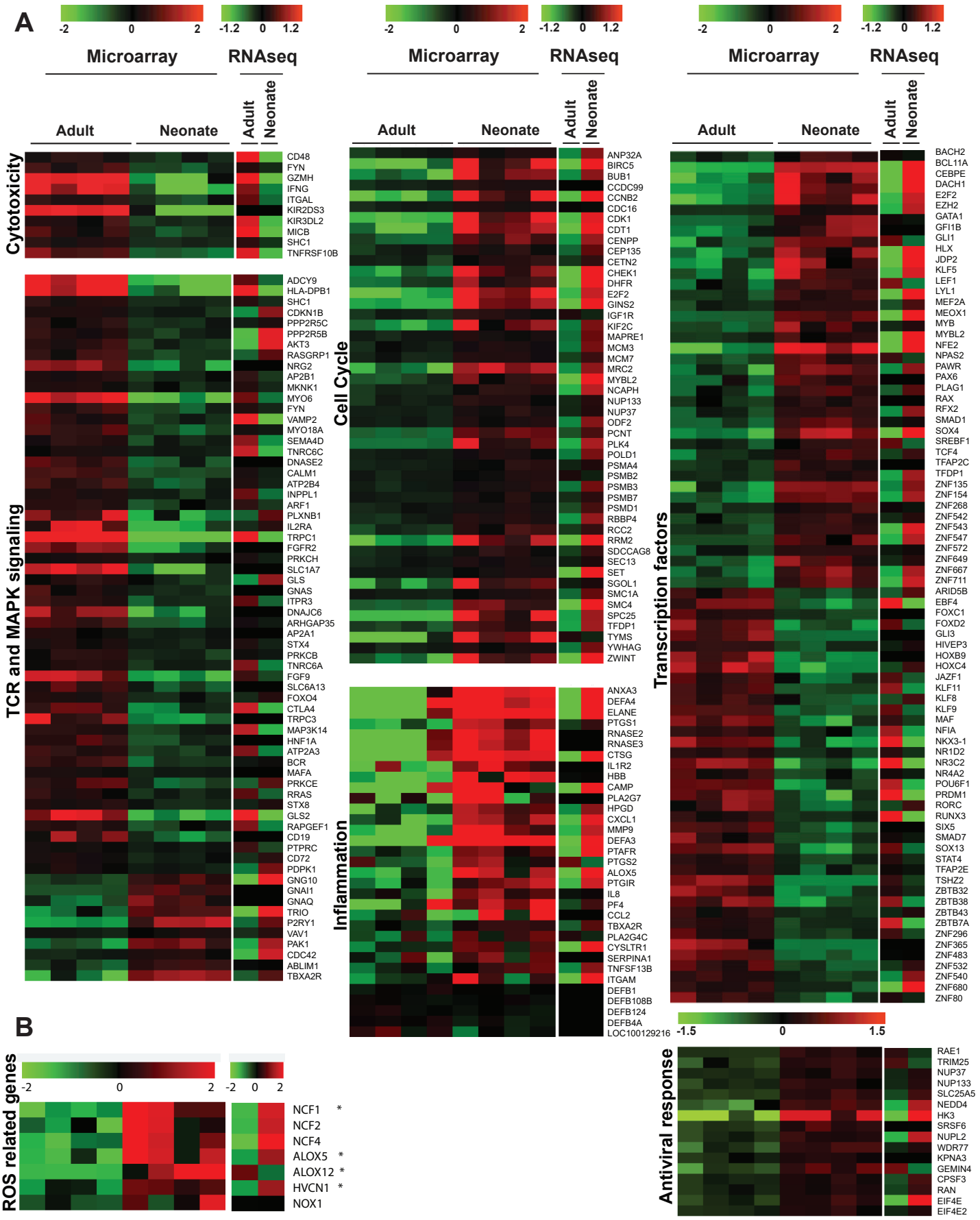


Figure S4. Comparison between the transcriptomic signatures and the RNA-seq data, related to Figures 3, 4, and 5. A) The heatmaps show the expression level of the different gene sets described in the figures 1, 3, 4 and 5 genes quantified by microarray or RNA-seq analyses. B) Heatmap shows the genes likely to be associated with reactive oxygen species (*significant in LIMMA analysis of transcriptome).

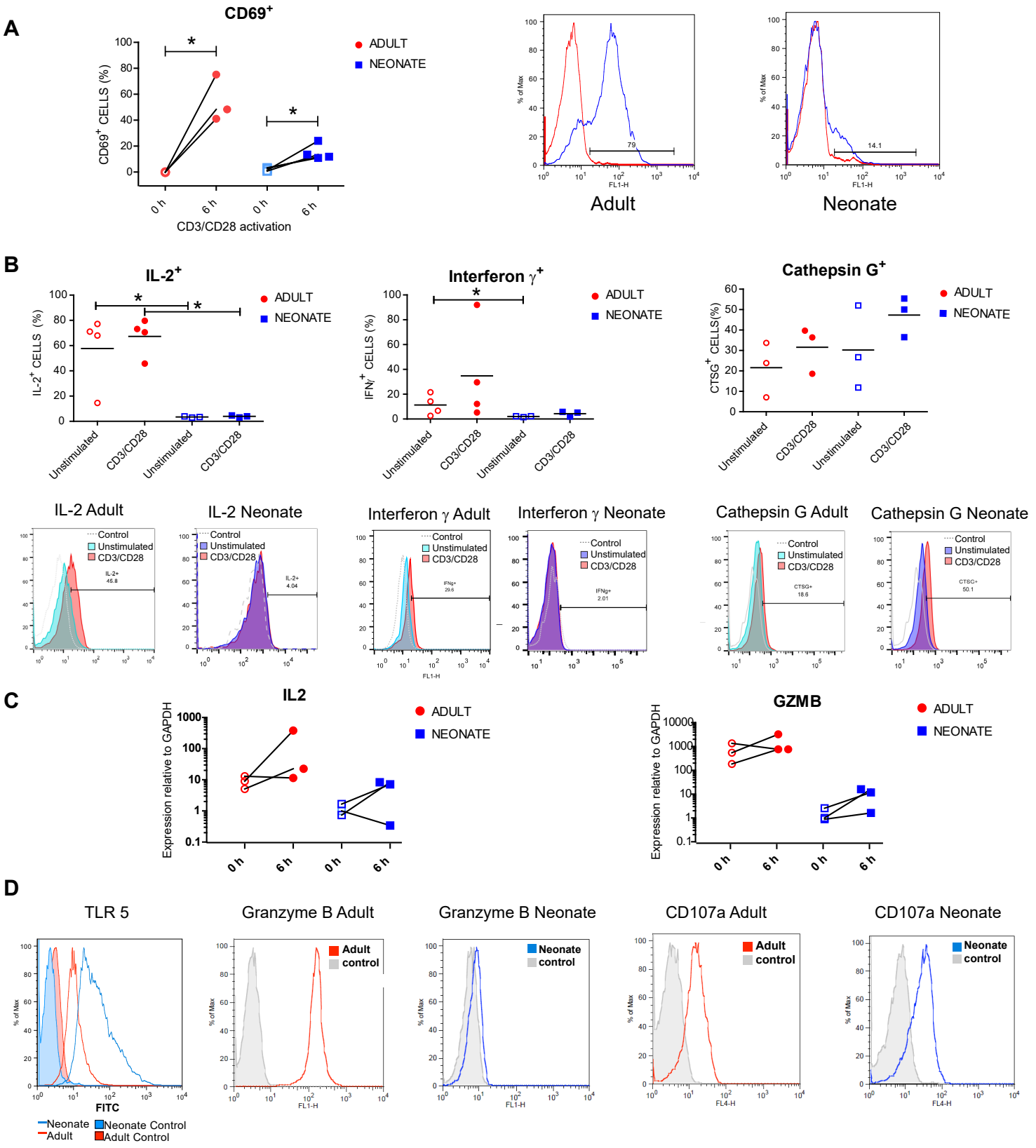


Figure S5. Activation profile and flow cytometry validation, related to Figures 1, 3, and 4. Flow cytometry evaluations of the percentage of positive cells before and after 6 hours stimulation with CD3/ CD28 crosslinking: A) Naïve $CD8^+$ T cells that were $CD69^+$ (right panel shows the representative histograms), B) Interleukin 2, Interferon gamma, and Cathepsin G (one representative histogram in lower panel). C) RT-qPCR shows the transcription levels relative to control gene expression before and after 6 hours of CD3/CD28 crosslinking for adult and neonate CD8-T cells. D) Histograms shows one representative cytometry analysis of at least three evaluations of independent samples from neonate or adult $CD8^+$ T cells: TLR5, Granzyme B and CD107a (controls corresponds to methanol permeabilized cells). Statistical significance was assessed by Mann-Whitney U test. *$P < 0.05$.

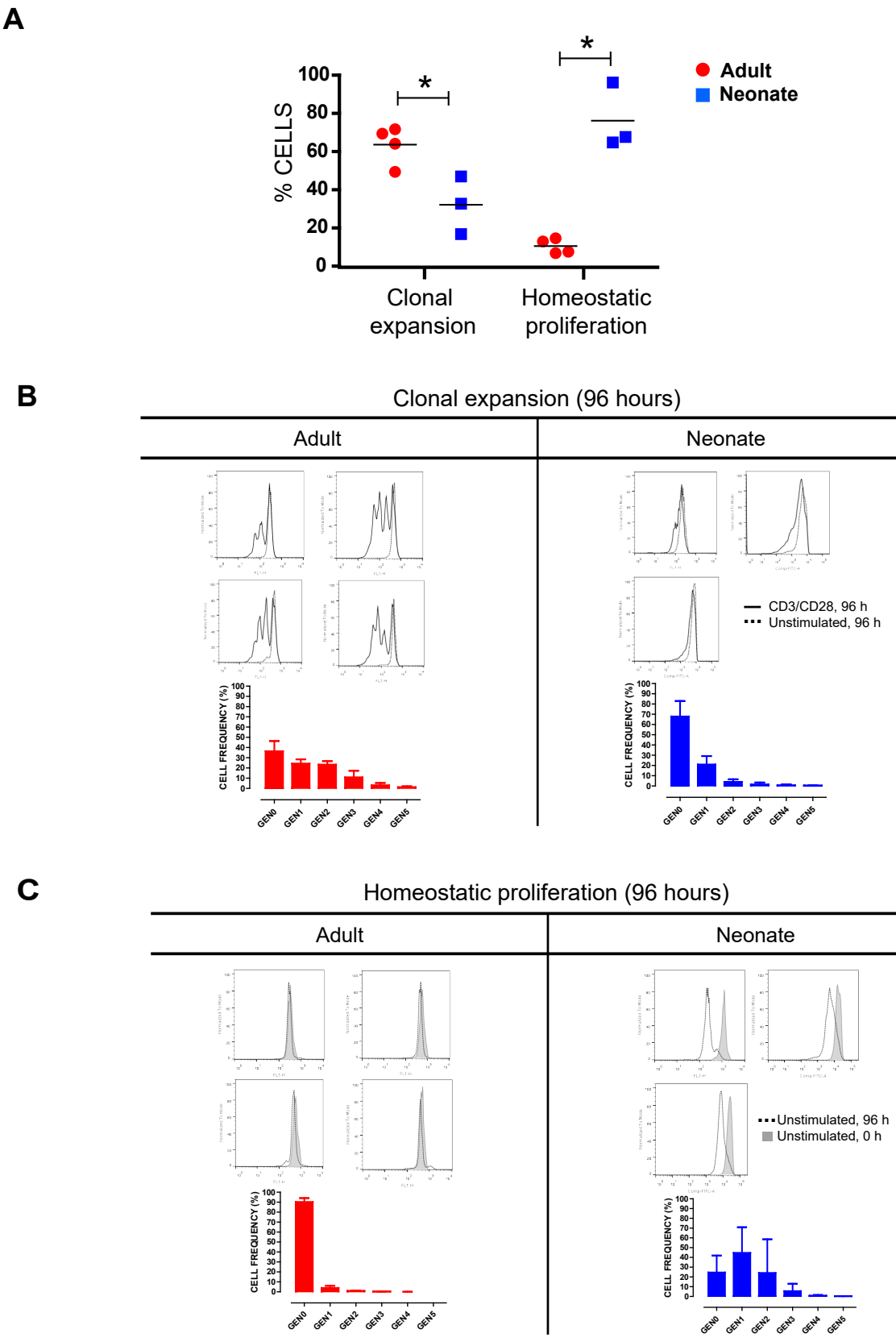


Figure S6. Proliferation analysis, related to Figure 4. A) The porcentage of cells that have divided after 96 hours of culture due to clonal expansion (CD3/CD28 activation) or homeostatic proliferation (unstimulated) of four adults and tree neonates are shown. B) Clonal expansion of the $CD8^+$ T cell population gate of PBMCs or CBMCs after 96 hours stimulation with CD3/CD28 crosslinking. C) Homeostatic proliferation analysis corresponding to 0 hours or 96 hours of culture without any stimulus; in the $CD8^+$ T cell population gate of PBMCs or CBMCs. Upper panels correspond to flow cytometry histograms of each sample; lower panel shows the percentage of cell populations in one range of CFSE dilution fixed from 1 to 5 cell divisions.

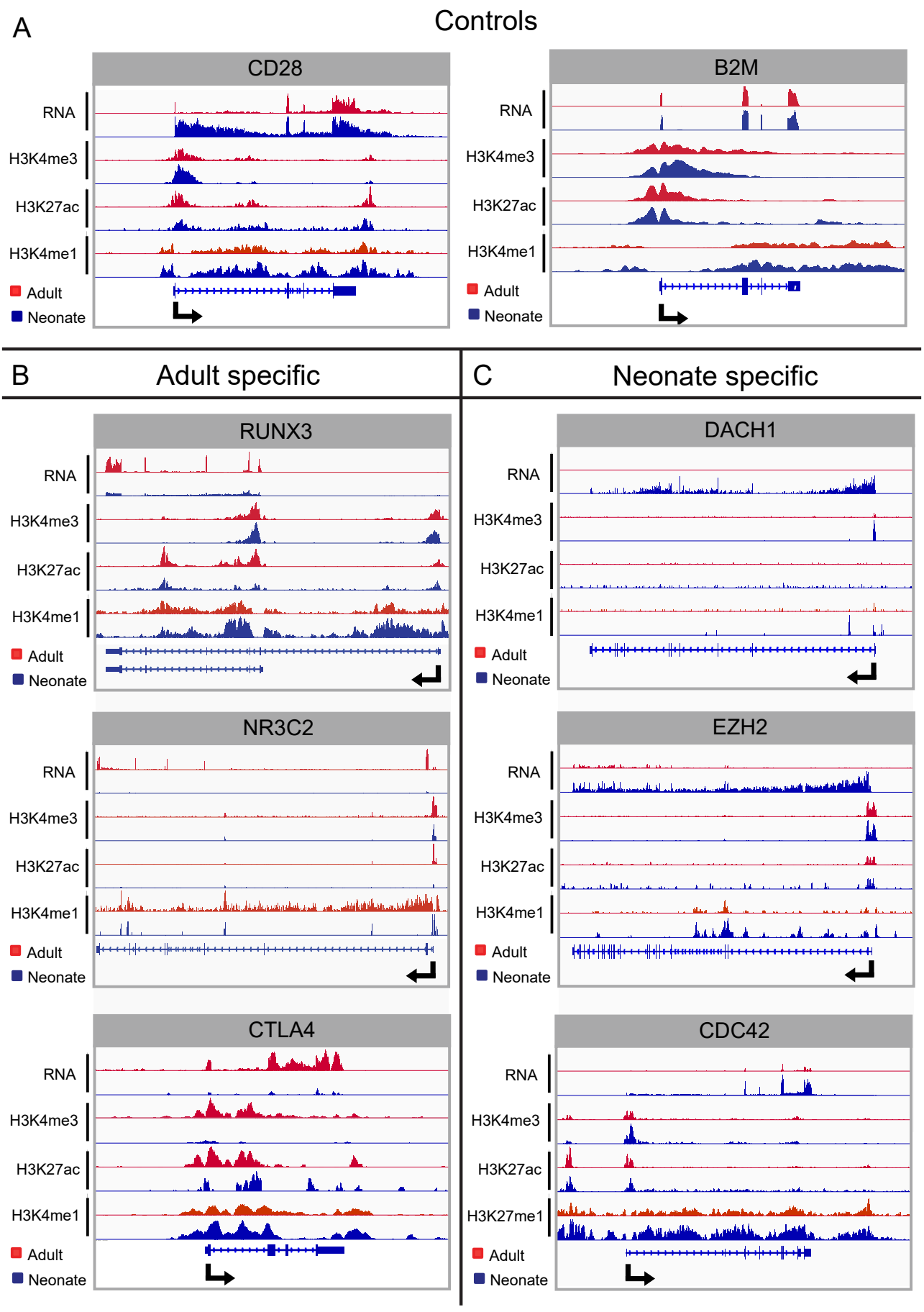


Figure S7. Additional examples of epigenetic marks and RNA-seq profiles, related to Figure 6. A) Two control genes showing equivalent levels of RNA-seq and ChIP-seq signal between adult and neonate samples. B-C) Three examples of genes up-regulated in adult CD8$^+$ T cells (B) or up-regulated in neonate CD8$^+$ T cells (C). Scales were adjusted with respect to control genes.

Table S1. Files for Epigenetic analysis, related to experimental procedures (excel spreadsheet).

DataSet 1. Transcriptome data from 4 adults and 4 neonates and the LIMMA analysis, related to experimental procedures (excel spreadsheet).

DataSet 2. Differential chromatin states between adult and neonate $CD8^+$ T cells, related to experimental procedures (excel spreadsheet).