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Supplementary Materials for

LINE1 modulate human T cell function by regulating protein synthesis during the life span

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The PDF file includes:

Other Supplementary Material for this manuscript includes the following:

Supplementary figures and figure legends

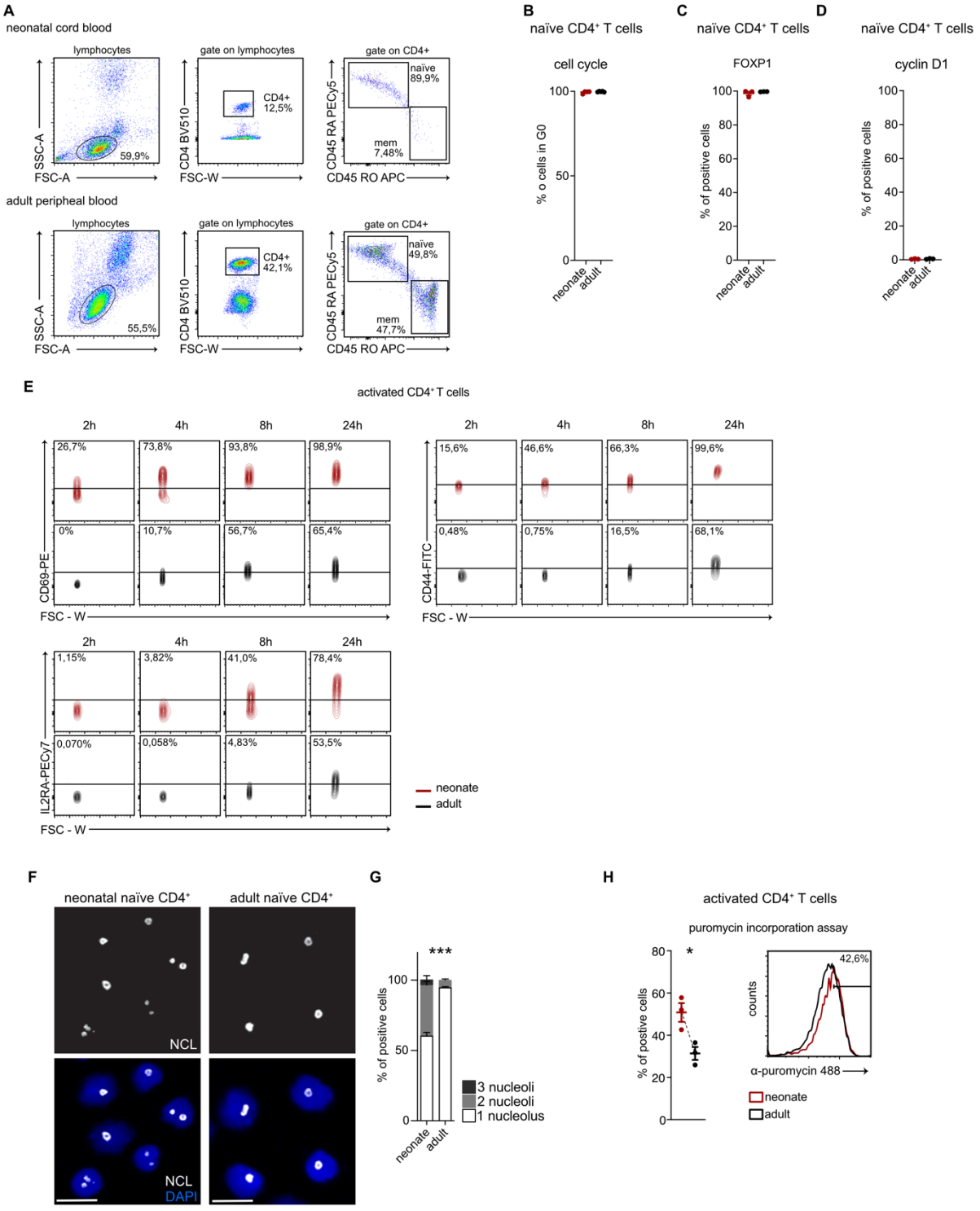

Fig. S1. Neonatal naïve CD4$^+$ T cells display a distinctive quiescent phenotype primed for activation.

(**A**) Representative sorting strategy of naïve CD4$^+$ T cells derived from umbilical cord blood (up) and adult peripheral blood (bottom). (**B**) Percentage of Ki-67 positive neonatal or adult naïve CD4$^+$ T cells (n = 4). (**C**) Percentage of FOXP1 positive neonatal or adult naïve CD4$^+$ T cells (n = 3). (**D**) Percentage of CyclinD1

positive neonatal or adult naïve $CD4^+$ T cells ($n = 3$). (**E**) Representative counter plot of CD69, CD44, IL2RA positive naïve $CD4^+$ T cells isolated from neonates or adults and activated for 2, 4, 8 or 24 h. (**F**) Confocal fluorescence microscopy images of Nucleolin immunofluorescence staining (grey) in naïve $CD4^+$ T cells isolated from neonates or adults. Original magnification 63x, scale bar 5µm. (**G**) Quantification of nucleoli number displayed by neonatal or adult naïve $CD4^+$ T cells ($n = 3$). *** $P < 0.001$, $F=322.1$, Two-way ANOVA. (**H**) Percentage of puromycin-positive neonatal or adult naïve $CD4^+$ T cells activated for 24 h ($n = 3$). * $P = 0.0226$, unpaired Two-tailed *t*-test. All data represent mean ± s.e.m, *n* refers to individuals.

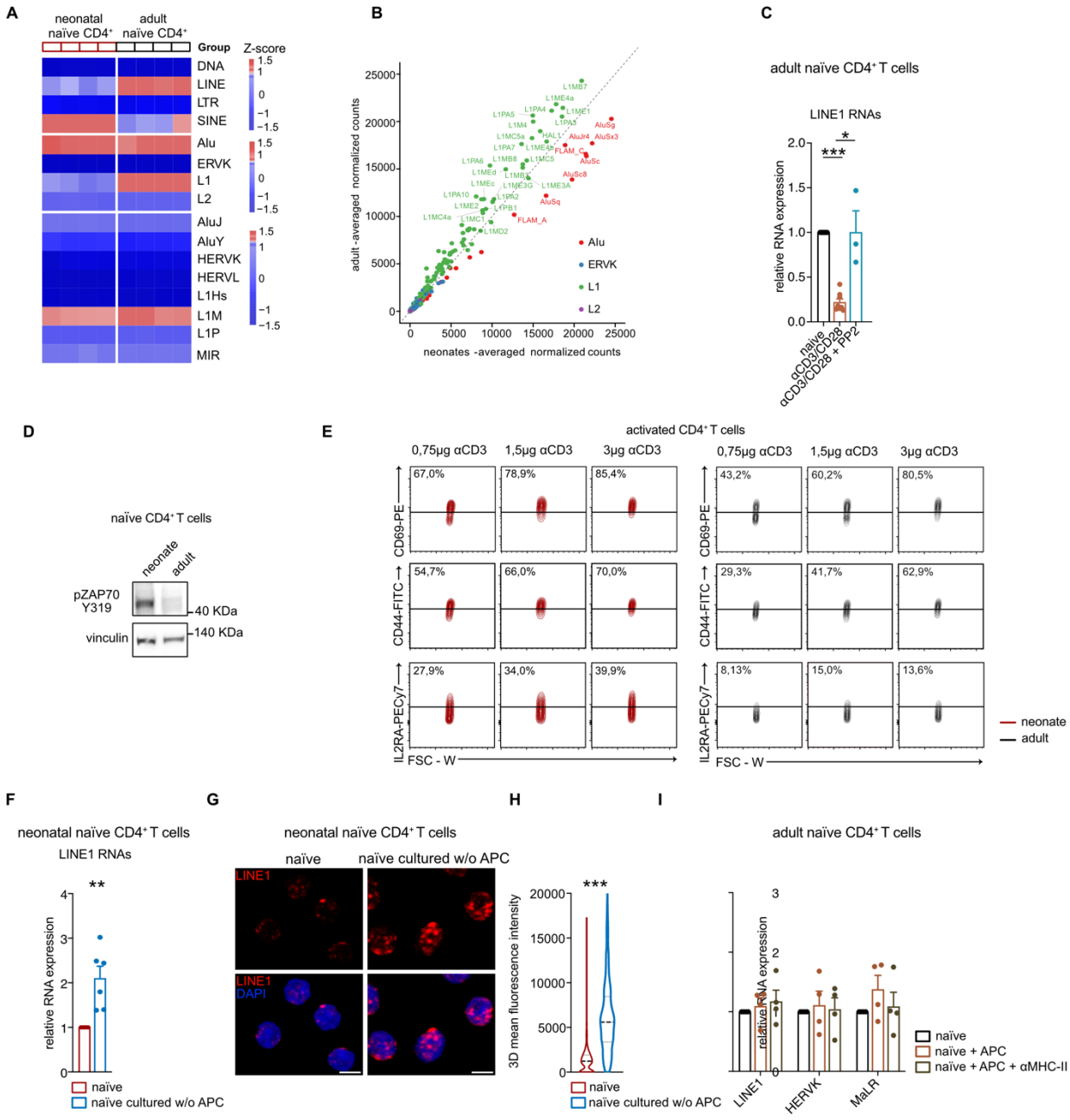

Fig. S2. Tonic TCR stimulation regulates LINE1 expression in neonatal naïve CD4$^+$ T cells.

(**A**) Heatmap of TE expression at class, family and subfamilies in neonatal or adult naïve CD4$^+$ T cell RNA-seq (n = 4). Z-score was calculated on DESeq2 normalized TE counts (see methods). (**B**) Scatter plot of averaged DESeq2 normalized counts of TE subfamilies in neonatal or adult naïve CD4$^+$ T cell RNA-seq (n = 4). (**C**) LINE1 expression by RT-qPCR in adult naïve CD4$^+$ T cells or naïve CD4$^+$ T cells activated for 8 h with anti-CD3/anti-CD28 beads with or without Lck inhibitor (PP2) (anti-CD3/anti-CD28 n = 8; anti-CD3/anti-CD28 + PP2 n = 3). *** P < 0.001; * P = 0.04, paired Two-tailed t-test. (**D**) Western blot analysis of phospho-ZAP70 (Tyr319) in neonatal or adult naïve CD4$^+$ T cells. Data were normalized on Vinculin. (**E**)

Representative counter plot of CD69, CD44, IL2RA positive naïve $CD4^+$ T cells isolated from neonates or adults and activated for 16 h with different anti-CD3 concentrations. (**F**) LINE1 expression by RT-qPCR in neonatal naïve $CD4^+$ T cells either freshly isolated or cultured without APC ($n = 6$). ** P = 0.010, paired Two-tailed *t*-test. (**G**) Confocal fluorescence microscopy images of LINE1 RNA FISH (red) in neonatal naïve $CD4^+$ T cells either freshly isolated or cultured without APC, original magnification 63x, scale bar 5µm and **(H)** relative quantification (at least 200 nuclei, $n = 2$). *** P < 0.001, unpaired Two-tailed *t*-test. (**I**) LINE1, HERVK and MaLR expression by RT-qPCR in adult naïve $CD4^+$ T cells either freshly processed or cultured in complete medium with autologous APC, with or without α-MHC-II ($n = 4$). All data represent mean ± s.e.m, *n* refers to individuals.

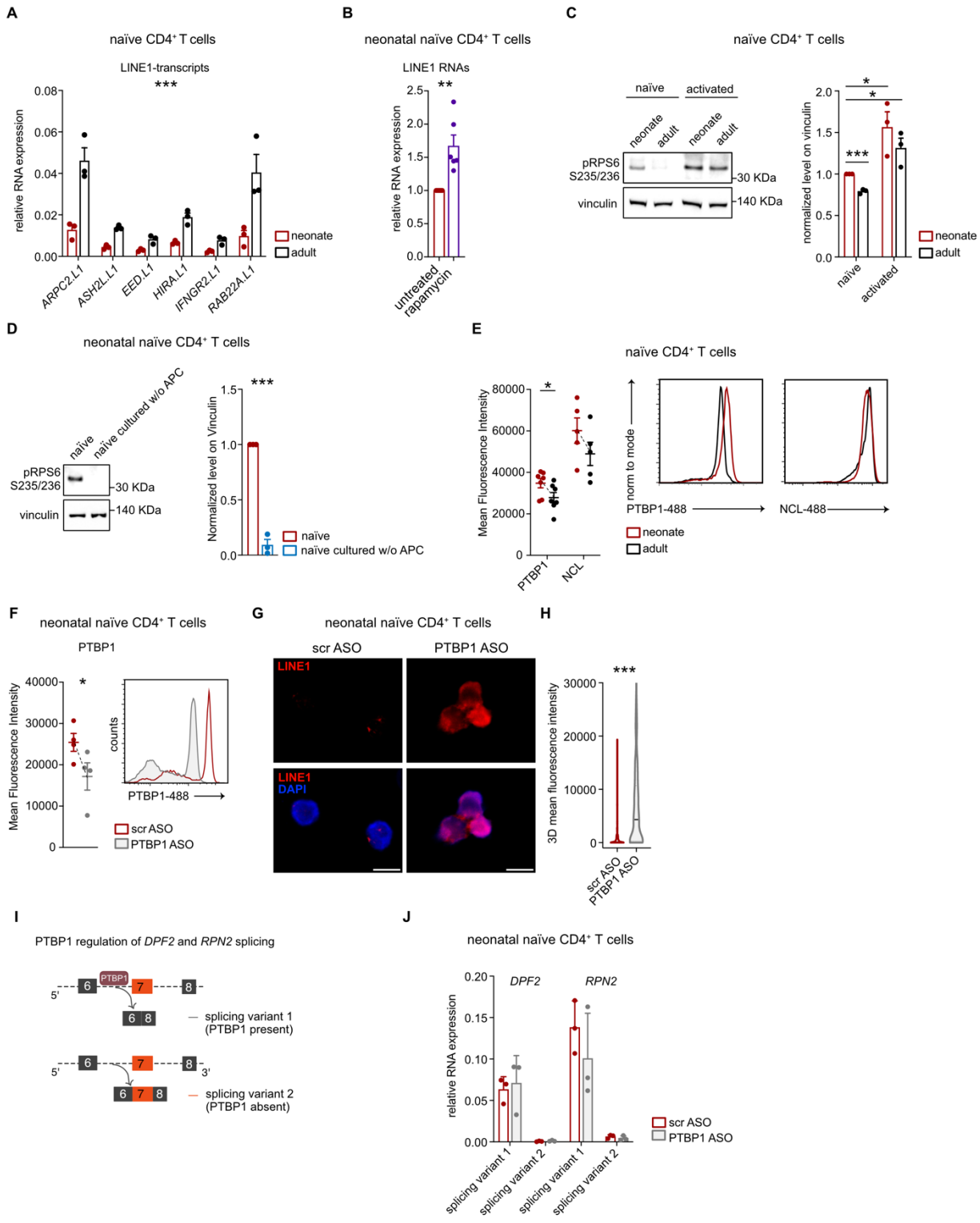

Fig. S3. PTBP1 suppresses the splicing of intronic LINE1 sequences in neonatal naïve CD4⁺ T cells.

(**A**) LINE1-transcripts expression by RT-qPCR in neonatal or adult naïve CD4⁺ T cells ($n = 3$). *** $P < 0.001$, $F = 54.4$, Two-way ANOVA. (**B**) LINE1 expression by RT-qPCR in neonatal naïve CD4⁺ T cells treated with or without rapamycin ($n = 6$). ** $P = 0.009$, paired Two-tailed *t*-test. (**C**) Western blot analysis and quantification of phospho-RPS6 (Ser235/236) in neonatal or adult naïve CD4⁺ T cells stimulated (10 min) or

not with anti-CD3-anti-CD28 (*n* = 3). Data were normalized on Vinculin. Naïve neonatal vs adult: *** P = 0.0002; neonatal activated vs neonatal naive: * P = 0.039, unpaired Two-tailed *t*-test.; neonatal naïve vs adult activated: * P = 0.0304, unpaired One-tailed *t*-test. (**D**) Western blot analysis and quantification of phosphor-RPS6 (Ser235/236) in neonatal naïve $CD4^+$ T cells freshly isolated or cultured without APC (*n* = 3). Data were normalized on vinculin. *** P < 0.001, unpaired Two-tailed *t*-test. (**E**) PTBP1 and NCL MFI in neonatal or adult naïve $CD4^+$ T cells (PTBP1 *n* = 7; NCL *n* =5). * P = 0.03, unpaired One-tailed *t*-test. (**F**) PTBP1 MFI in neonatal naïve $CD4^+$ T cells treated with PTBP1 or scr ASOs (*n* = 4). * P = 0.034, paired Two-tailed *t*-test. (**G**) Confocal fluorescence microscopy images of LINE1 RNA FISH in neonatal naïve $CD4^+$ T cells treated with PTBP1 or scr ASOs. Original magnification 63x, scale bar 5µm, and (**H**) relative quantification (at least 200 nuclei, *n* = 2). *** P < 0.001, paired Two-tailed *t*-test. (**I**) Scheme of RT-qPCR for alternative splicing variants regulated by PTBP1 (*23*). (**J**) *DPF2* and *RPN2* alternative spicing variant expression by RT-qPCR in neonatal naïve $CD4^+$ T cells treated with PTBP1 or scr ASOs (*n* = 3). All data represent mean ± s.e.m, *n* refers to individuals.

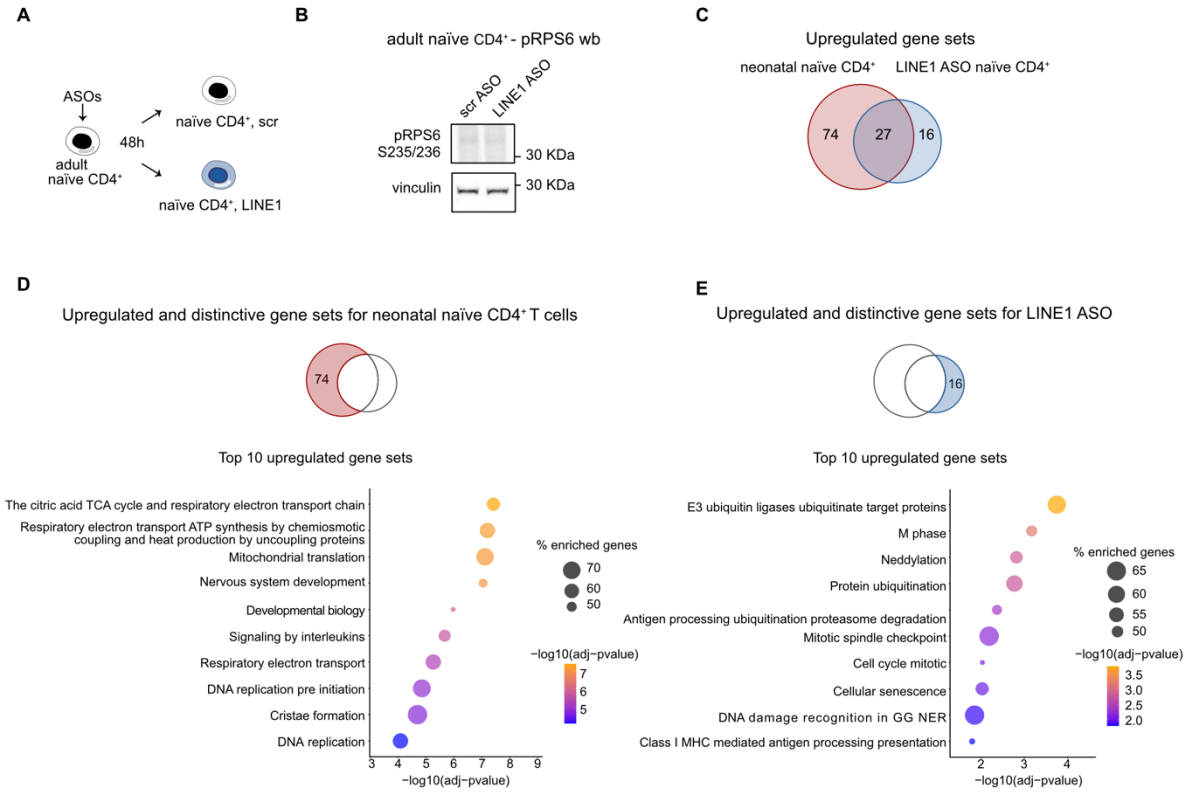

Fig. S4. Gene sets related to protein translation and cell cycle progression are distinctively upregulated in neonatal naïve CD4$^+$ T or in adult naïve CD4$^+$ T cells depleted of LINE1.

(**A**) Schematic representation of the strategy for LINE1 knockdown in adult naïve CD4$^+$ T cells with ASOs. (**B**) Western blot analysis of phospho-RPS6 (Ser235/236) in adult naïve CD4$^+$ T cells treated with LINE1 or scr ASOs (n = 2). Data were normalized on Vinculin. (**C**) Venn diagram showing the intersection between the gene sets upregulated in the comparison neonatal vs adult naïve CD4$^+$ T cells and those upregulated in the comparison adult naïve CD4$^+$ T cells LINE1 vs scr ASOs. (**D**) GSEA of neonatal vs adult naïve CD4$^+$ T cells (n = 4). Bubble plot showing the top 10 enriched gene sets among the 74 gene sets that are upregulated and specific for neonates from "Curated Reactome" database (MSigDb), P adjusted ≤ 0.05. (**E**) GSEA of adult naïve CD4$^+$ T cells treated with LINE1 vs scr ASOs (n = 4). Bubble plot showing the top 10 enriched gene sets among the 16 gene sets that are upregulated and specific for LINE1 ASOs condition from "Curated Reactome" database, P adjusted ≤ 0.05.

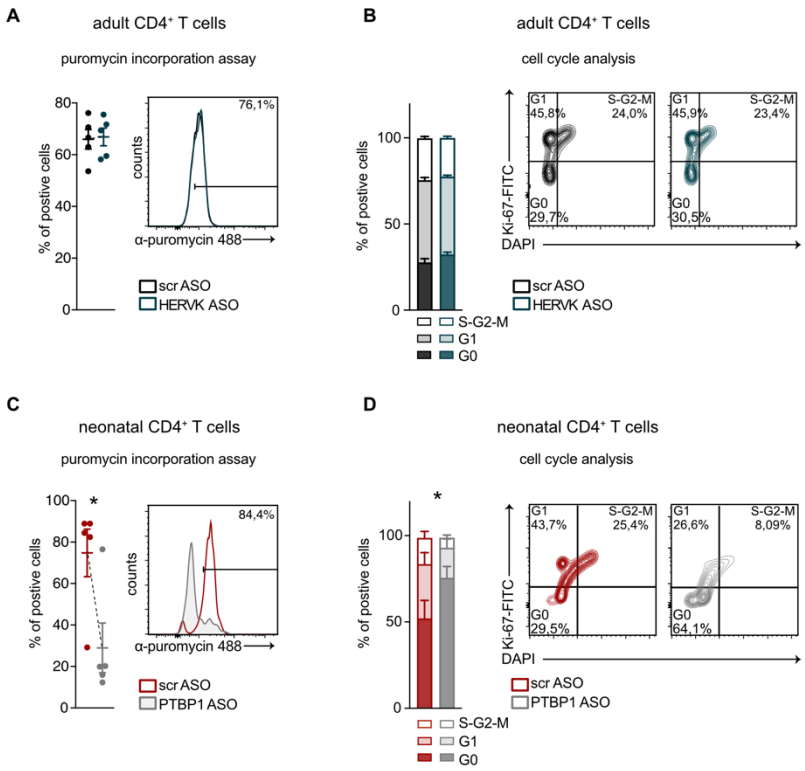

Fig. S5. LINE1 expression controls protein synthesis and cell cycle progression.

(**A**) Percentage of puromycin-positive adult naïve CD4$^+$ T cells treated with HERVK or scr ASOs and activated for 24 hours (n = 5). (**B**) Cell-cycle analyses in adult CD4$^+$ T cells treated with HERVK or scr ASOs and activated for 36 hours (n = 5). (**C**) Percentage of puromycin-positive neonatal naïve CD4$^+$ T cells treated with PTBP1 or scr ASOs and activated for 24 hours (n = 5). * P=0.04, paired Two-tailed t-test. (**D**) Cell-cycle analyses in neonatal CD4$^+$ T cells treated with PTBP1 or scr ASOs and activated for 36 hours (n = 5). * P = 0.0145, F = 5.076, Two-way ANOVA. All data represent mean ± s.e.m, n refers to individuals.

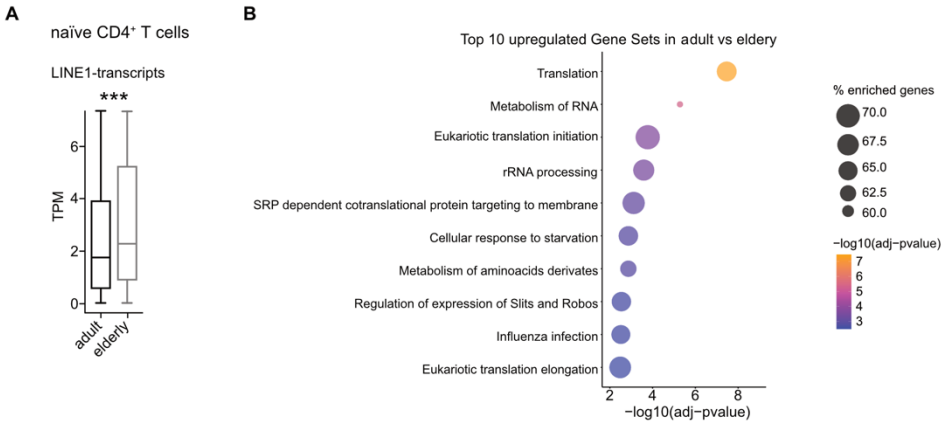

Fig. S6. Elderly naive CD4$^+$ T cells are characterized by increase in LINE1 expression and decrease in protein synthesis.

(**A**) Expression levels of the 461 LINE1-transcripts identified in (*15*), using RNA-seq datasets from adult and elderly naïve CD4$^+$ T cells (n = 4). *** P = 1.865e−11, unpaired Two-tailed Wilcoxon rank sum test. (**B**) GSEA of adult vs elderly naïve CD4$^+$ T cells (n = 4). Bubble plot showing the top 10 enriched gene sets among the 17 gene sets that are upregulated and specific for adult condition from "Curated Reactome" database, P adjusted ≤ 0.05.

Table S1. LINE1-transcript expression quantified in quiescent neonatal and adult naive $CD4^+$ T cells RNA-seq datasets.

Table S2. GSEA performed in i) neonatal versus adult naïve $CD4^+$ T cells ii) adult naïve $CD4^+$ T cells where LINE1 were downregulated using LINE1 ASOs versus control cells (scr ASOs) iii) common upregulated genesets from the two comparisons.

Table S3. Sample list of infants, toddlers, children, teenagers and elderly enrolled in the study.

Table S4. LINE1-transcript expression quantified in quiescent adult or elderly naive $CD4^+$ T cells RNA-seq datasets.

Table S5. GSEA performed in i) adult vs elderly naïve $CD4^+$ T cells.

Table S6. List of primer sequences used in qRT-PCR analysis.