SUPPLEMENTAL MATERIAL

Mende et al., http://www.jem.org/cgi/content/full/jem.20150308/DC1

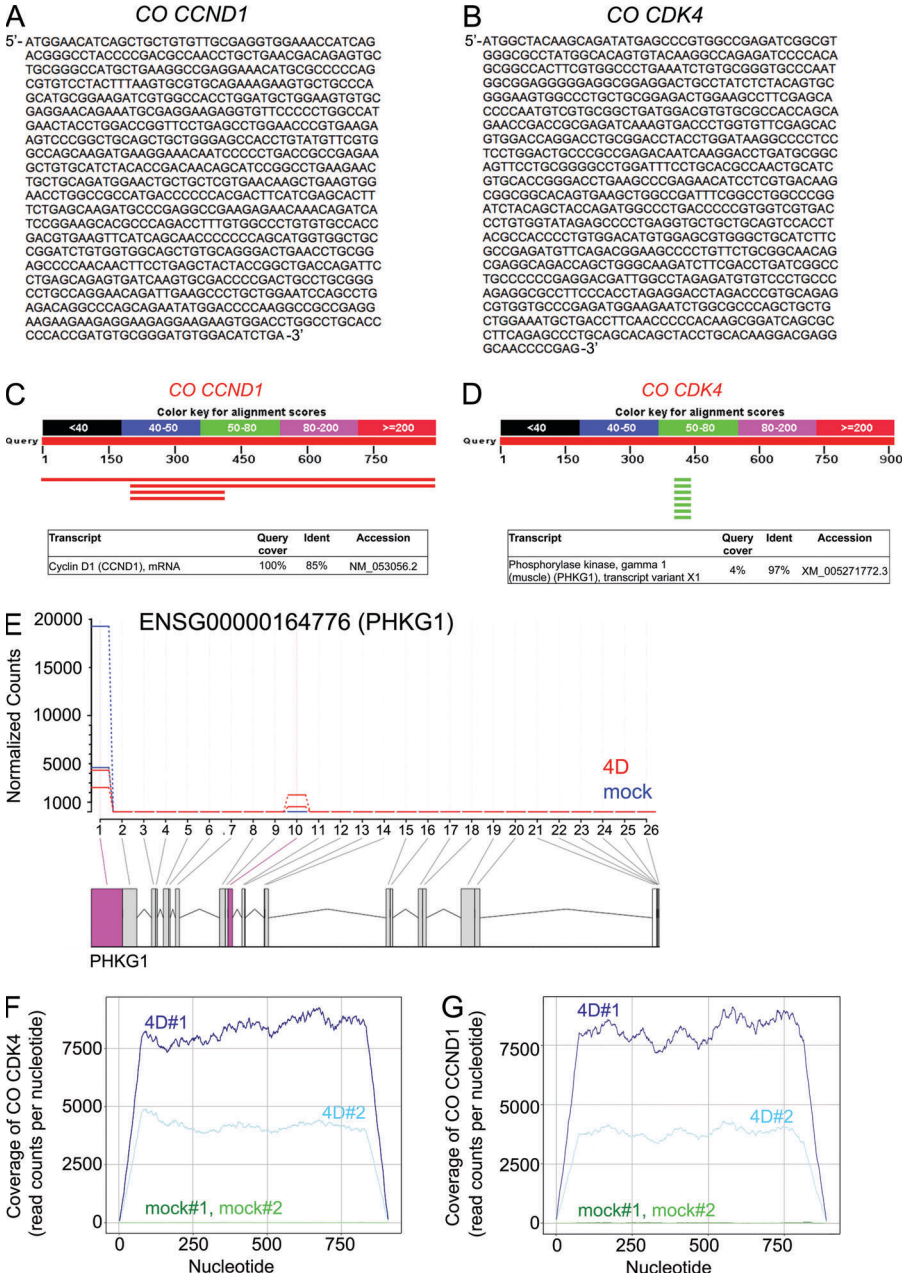

Figure S1. Sequence alignments for codon-optimized CCND1 and CDK4. (A and B) Codon-optimized (CO) sequences for the overexpression of CCND1 (A) and CDK4 (B). (C and D) In silico alignments. Plots show sequence alignments for CO CCND1 (C) and CO CDK4 (D) to the human genome using NCBI BLAST. (C) The entire codon-optimized sequence (query cover 100%) encoding for CCND1 aligns to NM_053056.2 (Homo sapiens cyclin D1, mRNA) and all transcript hits (red lines) align to the same sequence. (D) Only 4% (37 out of 909 nucleotides) of the codon-optimized sequence encoding for CDK4 aligns to NC_000007.14 (Homo sapiens phosphorylase kinase, γ1, PHKG1), suggesting a false-positive alignment for this sequence. All 7 BLAST hits (green lines) showed a similar distribution on the query sequence. (E) Alignments of sequencing read counts to *PHKG1*. Differential exon usage (DEXSeq; Anders et al., 2012) was used to show actual read counts in *PHKG1* and the neighborly areas. The graph shows exon bins (pink), which have significant differences (padj < 0.1) in exon usage between mock and 4D. The exon bin of interest is bin 10, which contains read counts in the predicted sequence that align with the codon-optimized CDK4 transcript (green line in B). Exon bin 1 is marked as significant as well. It overlaps with the 3′ UTR of *sulfatase modifying factor 2* (*SUMF2*) and therefore read changes originate from *SUMF2*, which is expressed in both conditions (mock and 4D, not depicted). (F and G) Plots show per nucleotide coverage of the codon optimized sequence encoding for CDK4 (F) and CCND1 (G). The entire sequence is covered by reads counts in 4D- but not in mock-transduced samples (#), verifying presence of corresponding transcripts.

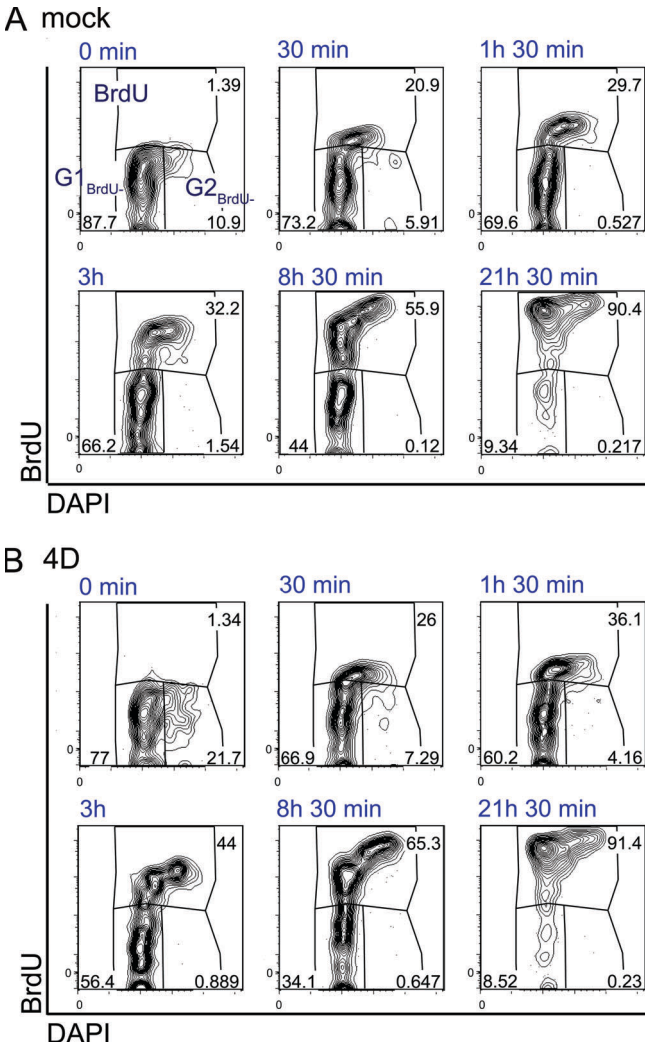

Figure S2. Gating strategy for the analysis of BrdU incorporation into 4D- or mock-transduced CD34⁺ CB cells. BrdU incorporation in cultivated mock- (A) or 4D-transduced (B) cells was measured over a time period of 28 h to determine cell cycle phase length. Dot plots are representative of different BrdU pulse times that were used to determine BrdU incorporation kinetics.

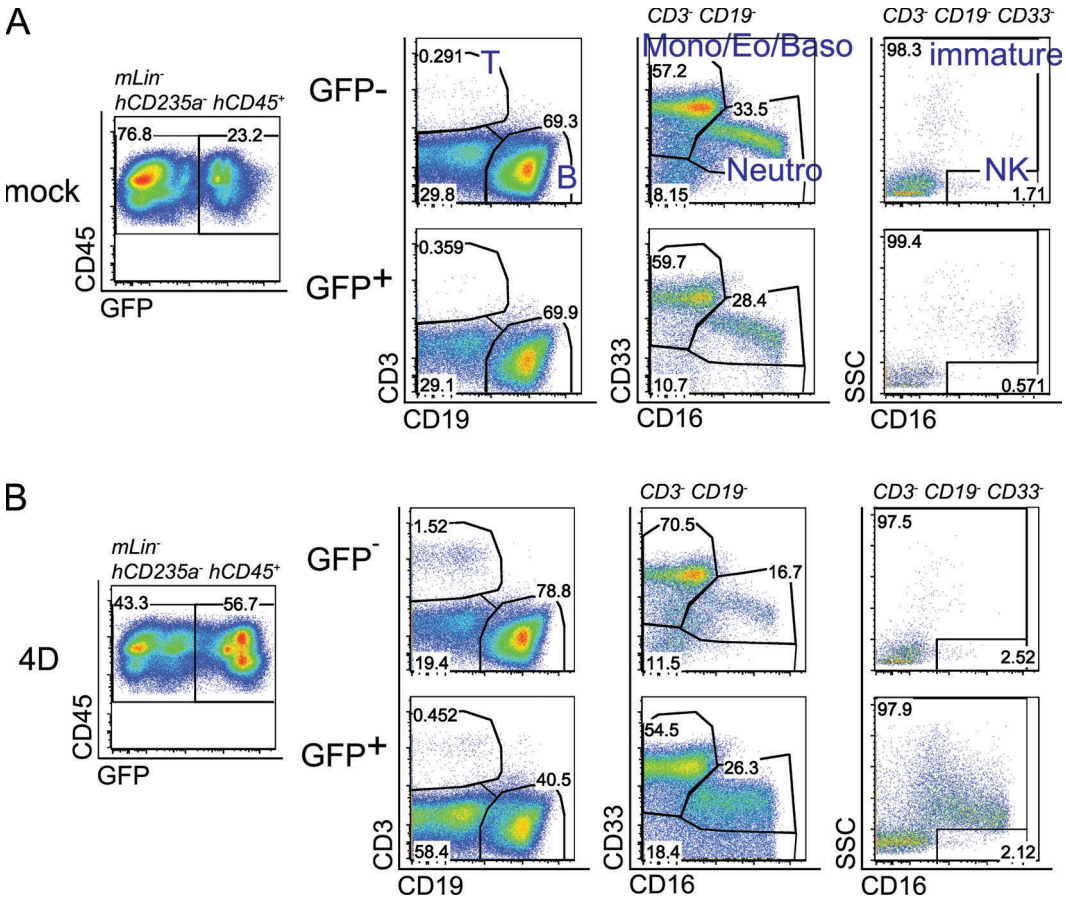

Figure S3. Gating strategy for the analysis of mature cell populations within GFP⁺ and GFP⁻ fractions of donor cells. Representative plots for mock- (A) or 4D-transduced (B) cells are shown.