SUPPLEMENTAL FIGURES
Nicolet, *et al*. 2021

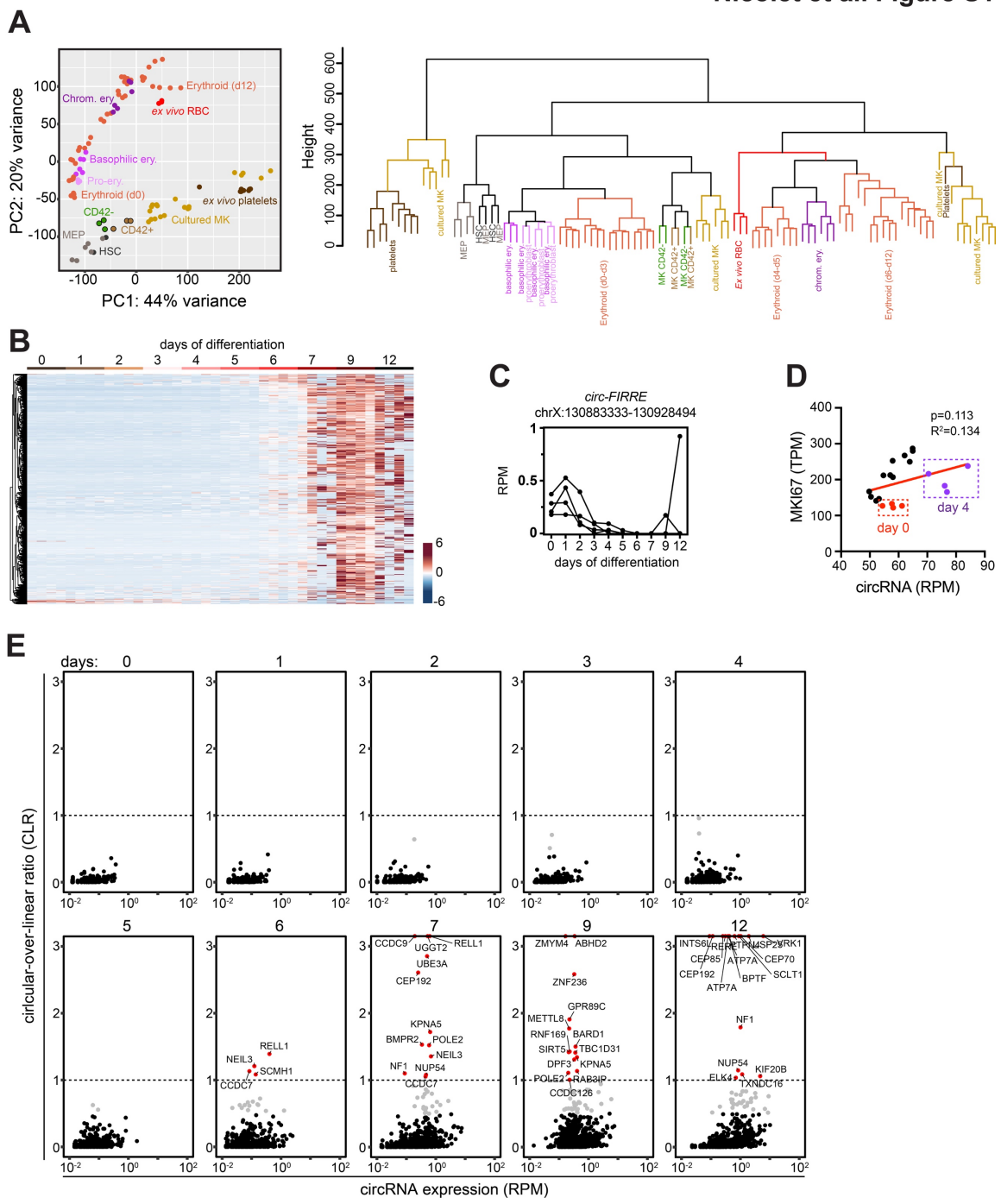

Figure S1: expression of high confidence circRNA and their relation to mRNA in erythropoiesis.

(**A**) Principal component analysis (PCA) (left panel) and clustering analysis (hclust; "complete" method; right panel) of mRNA expression of erythroid differentiation used in Figure 1 and megakaryoid differentiation used in Figure 3 (see Table S1). (**B**) Heatmap of high confidence circRNA expression during erythropoiesis (RPM; n=950). Color scale represents Z-score. (**C**) *Circ-FIRRE* expression level at indicated days of erythroid differentiation. (**D**) Plot showing the relation between MKI67 (KI-67) expression and overall circRNA expression (RPM; low-confidence circRNA) in nucleated cells. Red line indicated the linear regression model. (**E**) Circular-over-linear ratio (CLR) was calculated for all high confidence circRNA. CLR was plotted against the circRNA expression (RPM: Reads per million mapped reads) at the indicated day of differentiation.

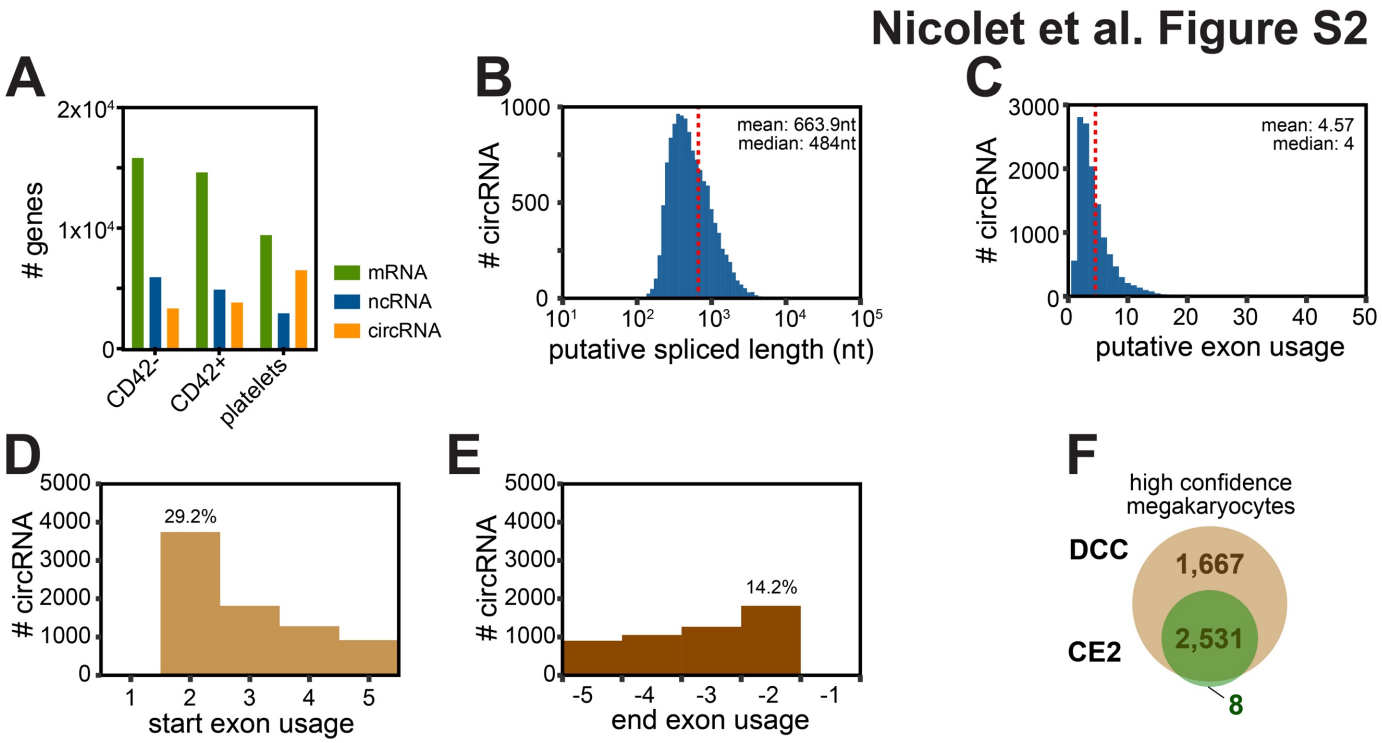

Figure S2: Characteristics of circRNA expression in megakaryocytes

(**A**) Number of genes encoding mRNA, ncRNA and circRNA detected during megakaryocyte maturation and compared to platelets (cut-off for mRNA and ncRNA >0.1 TPM; low confidence circRNA). (**B**-**C**) Characterization of low confidence circRNA putative exon usage (**B**) and putative spliced length (**C**), using splicing annotations from the linear isoforms. (**D**-**E**). Characterization of start (**D**) and end (**E**) exon usage of 'low confidence' circRNAs, based on the circRNA junction positions and canonical mRNA splicing annotations. Percentage indicated the fraction of circRNA in the bar. (**F**) CircRNA detection of high confidence circRNAs.

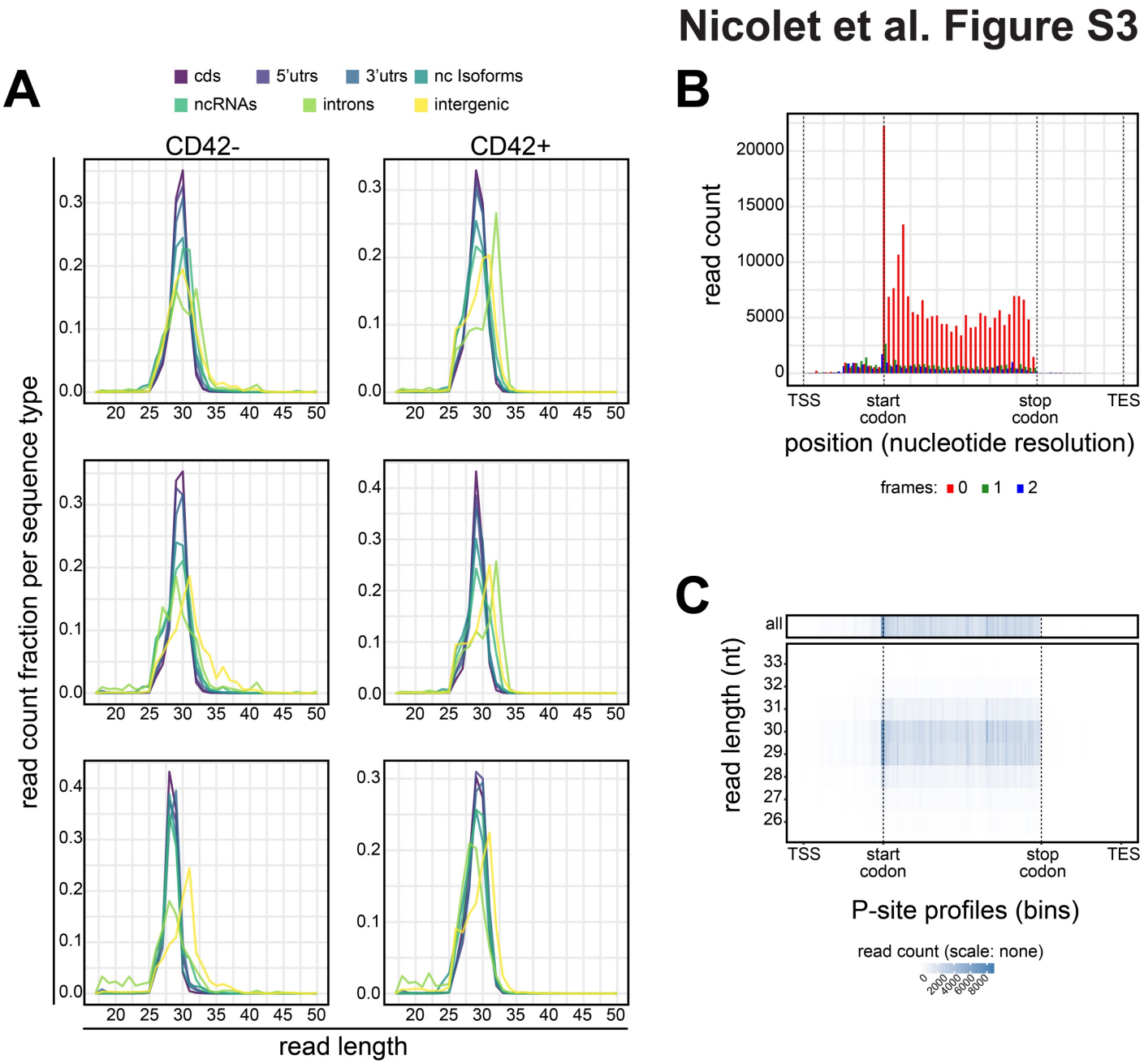

Figure S3: Quality control of ribo-seq data in megakaryocytes

Ribo-seQC (Calviello et al., 2019) was used to assess the quality of ribo-seq reads of megakaryocytes. (**A**) Diagram depicting the mapped read length distribution of all 6 megakaryocyte ribo-seq samples within the coding sequence (CDS), 5; and 3' untranslated regions (UTR), non-conding RNA (ncRNA) isoforms (nc Isoforms), ncRNAs, introns and intergenic sequences. (**B, C**) P-site position and periodicity after P-site position correction (**B**; 12nt), and (**C**) P-site profile across all read lengths. (B-C) are of CD42- megakaryocytes (replicate 1) and are representative for all 6 samples.

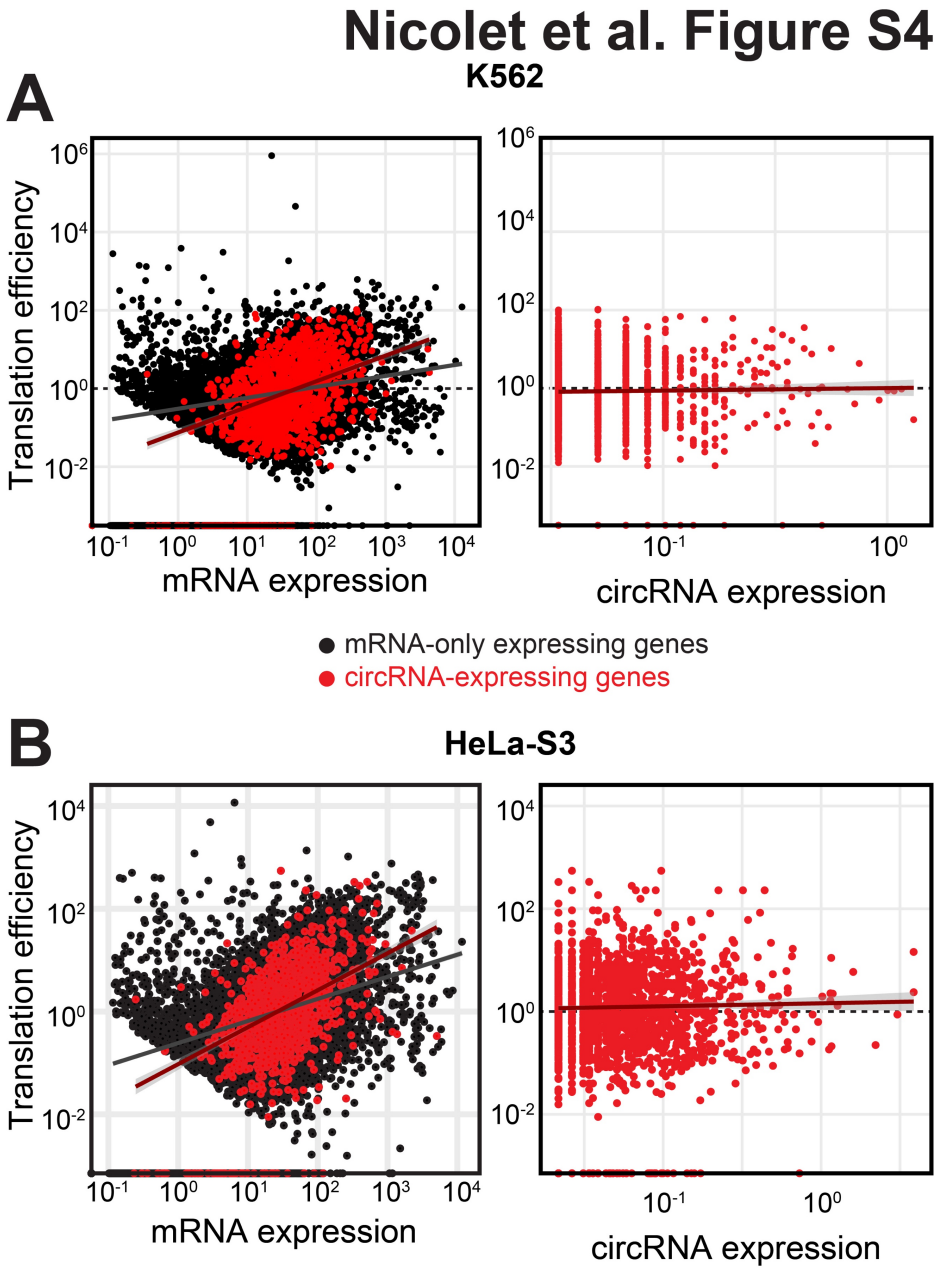

Figure S4: CircRNA-mediated regulation of mRNA translation is limited in cell lines

(**A-B**) mRNA translation efficiency was calculated for (**A**) K562 and (**B**) HeLa-S3 cell lines, from previously published data (see methods), and plotted against mRNA expression (left panels) or circRNA expression (right panels). Red dots represent circRNA-expressing genes and black dots represent mRNA-only expressing genes. Regression line for circRNA-expressing gene is shown in dark-red while this of mRNA-expressing gene is shown in black.

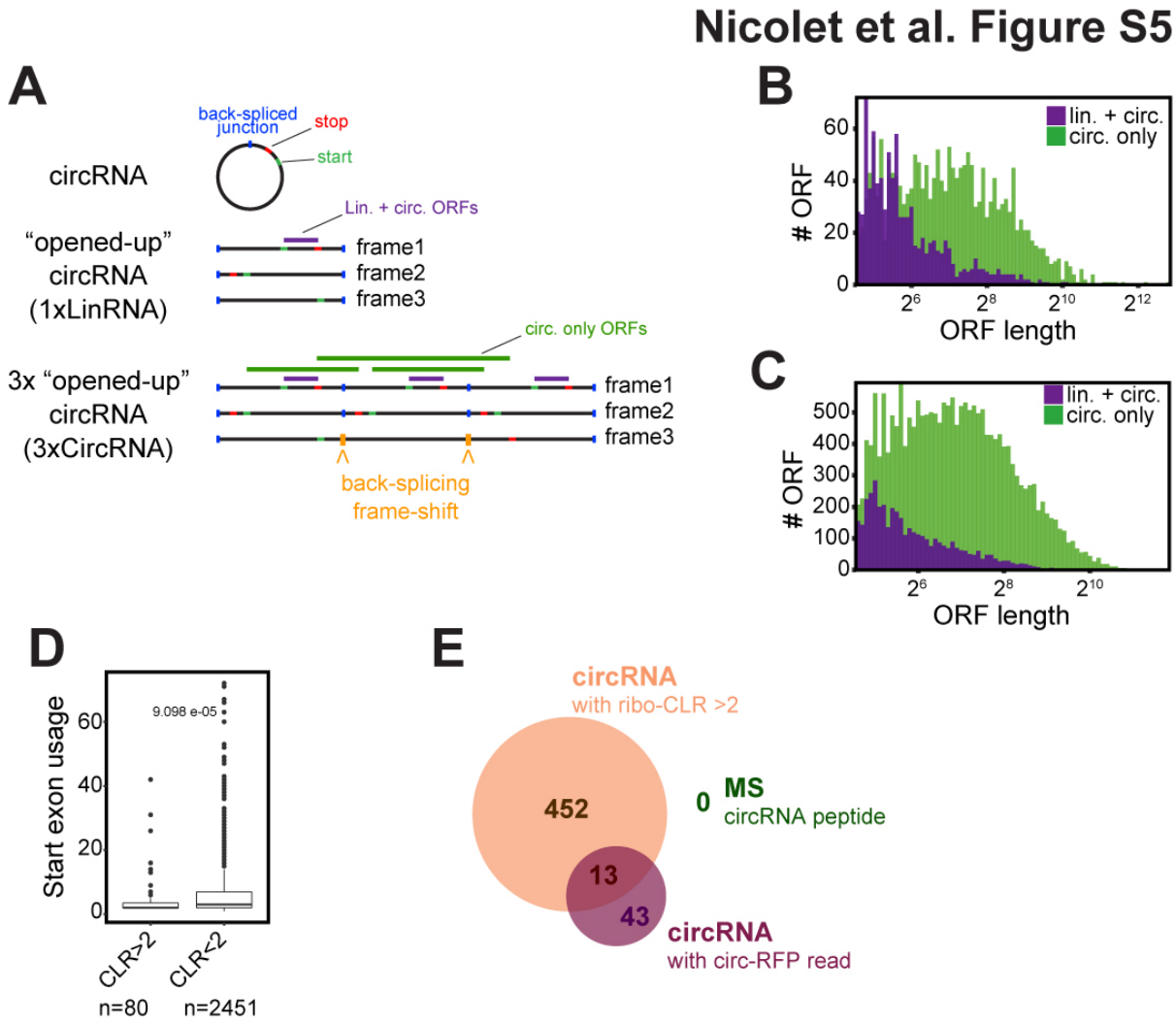

Figure S5: circRNA-specific ORFs are abundant in erythroid cells and platelets

(**A-C**) Open reading frames (ORFs) were computed for high confidence circRNA sequences that were linearized at the back-spliced junction (1xLinRNA), and for tripled juxtaposed linearized sequence of high confidence circRNA to include circularized junction areas (3xCircRNA). (**A**) Schematic representation of the circRNA, 1xLinRNA and 3xCircRNA. Start (green) and stop (red) codon position can results in LinRNA + circRNA (Lin. + circ.) ORFs (purple), or circRNA-only (circ. only) ORFs (green) when the ORFs span over the back-spliced junction (blue), which can results in back-slice induced frame-shift (orange). (**B-C**) Bar graph depicting the length and frequency of the ORFs found in both 1xLinRNA and 3xCircRNA sequences (purple) or specifically in 3xCircRNA (green) in (**B**) differentiating erythroblasts and (**C**) platelets. (**D**) Box plot depicting the start exon usage of circRNA with a ribo-CLR >2 or <2 (Wilcoxon signed rank test; p = 9.098e-05). (**E**) Venn diagram showing the overlap in platelets of circRNAs with ribo-CLR >2, circRNAs with circ-RFP and circRNA-specific peptide detected by mass spectrometry (no peptide detected; MS; data from (Van Oorschot et al., 2019)).