

A

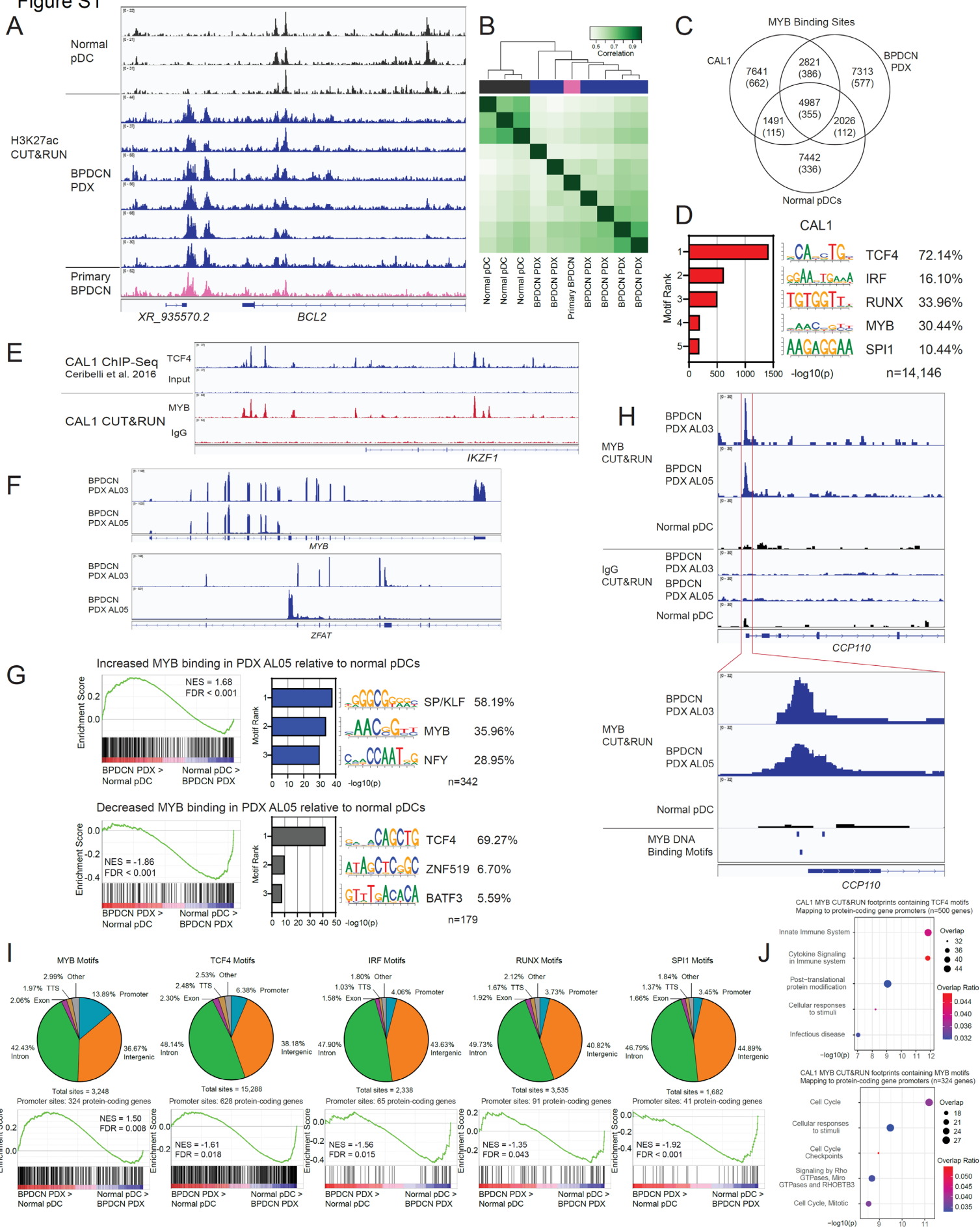
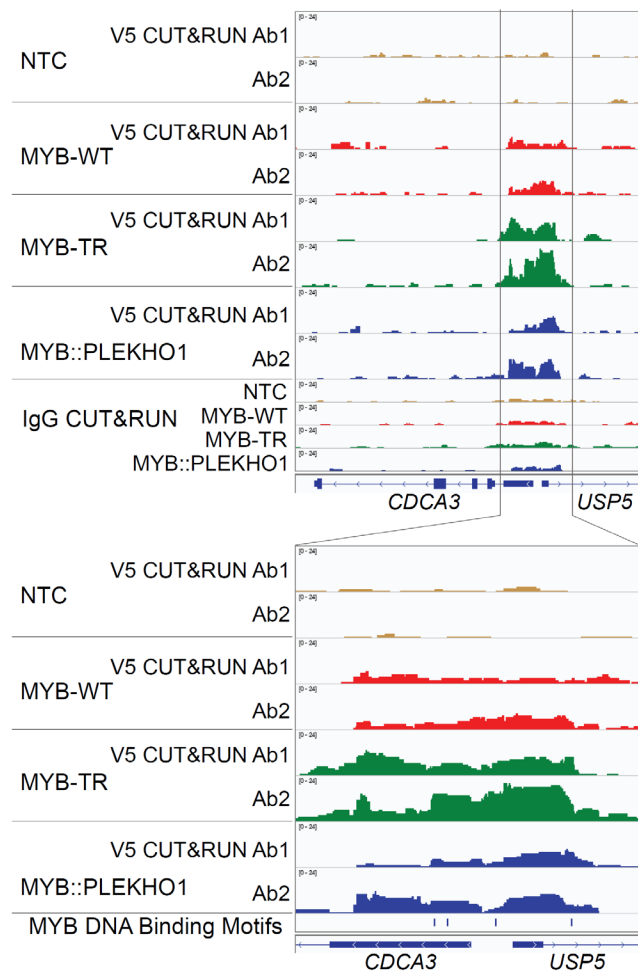


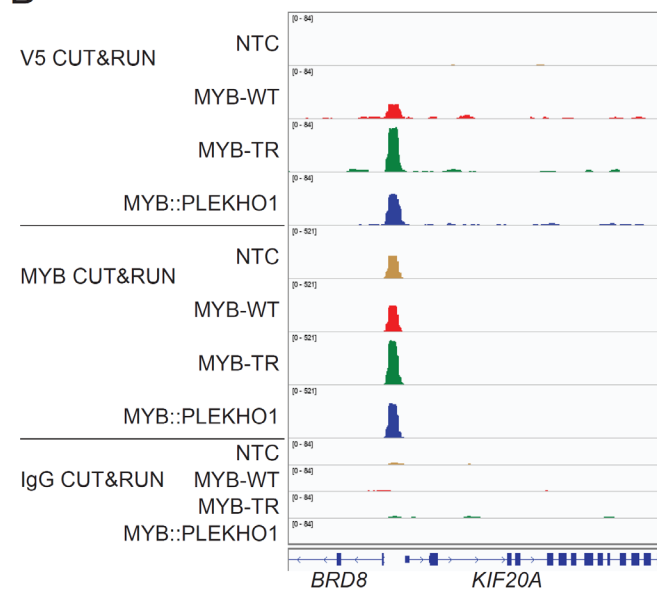
Figure S1: MYB aberrantly regulates G2/M cell cycle genes in BPDCN.

(A) H3K27ac CUT&RUN tracks at the *BCL2* locus in all samples from the indicated cell types (normal pDCs n=3, BPDCN PDX n=6, primary BPDCN n=1). (B) Clustering of H3K27ac CUT&RUN data from all samples from the indicated cell types (normal pDCs n=3, BPDCN PDX n=6, primary BPDCN n=1). Clustering was performed after counting of reads within pooled H3K27ac peaks and normalization to the total number of reads inside peaks for each sample. (C) Venn diagram showing number of MYB CUT&RUN peaks and, in brackets, number of peaks mapping to protein-coding gene promoters in the indicated cell types. (D) Top 5 ranked motifs enriched in MYB CUT&RUN peaks in CAL1 cells, and percent of peaks containing each motif. (E) TCF4 ChIP-seq (36) and MYB CUT&RUN tracks in CAL1 cells at the *IKZF1* locus. (F) Tracks showing RNA-sequencing read density at the *MYB* and *ZFAT* loci in AL03 and AL05 BPDCN PDX cells. (G) (Left) GSEA comparing normal pDCs and BPDCN PDX cells for genes showing differential MYB binding at promoter regions in BPDCN PDX AL05 relative to normal pDCs (n=346 genes increased and n=182 genes decreased MYB binding). (Right) Top 3 ranked motifs enriched in differentially bound MYB sites mapping to promoters, and percent of sites containing each motif. (H) MYB and IgG CUT&RUN tracks at the *CCP110* locus in the indicated cell types. (I) (Top) Locations of MYB CUT&RUN footprints containing the indicated motifs in CAL1 cells. (Bottom) GSEA comparing normal pDCs and BPDCN PDX cells for genes showing MYB CUT&RUN footprints containing the indicated motifs at promoter regions in CAL1 cells. (J) Top 5 ranked Reactome gene sets overlapping with genes showing MYB CUT&RUN footprints containing the indicated motifs at promoter regions in CAL1 cells.

A Figure S2



B



C

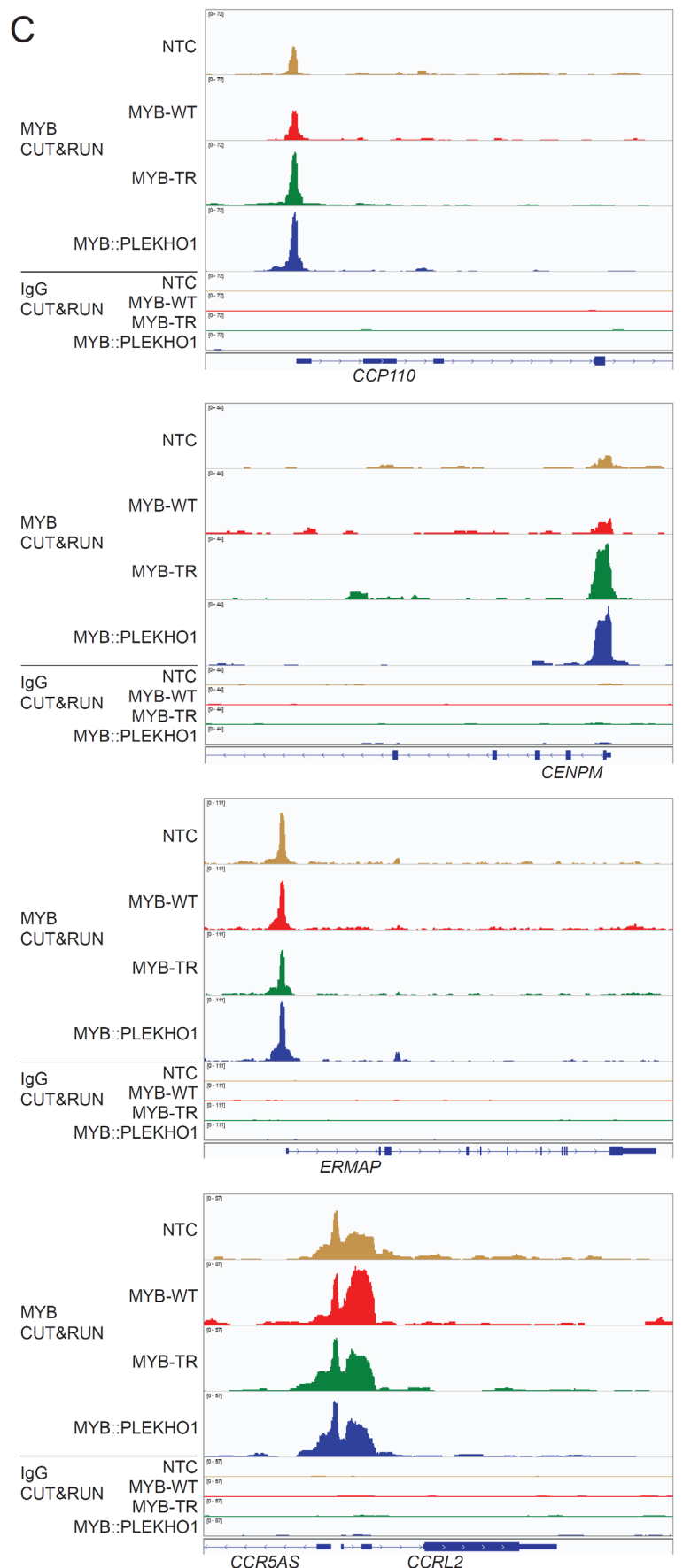
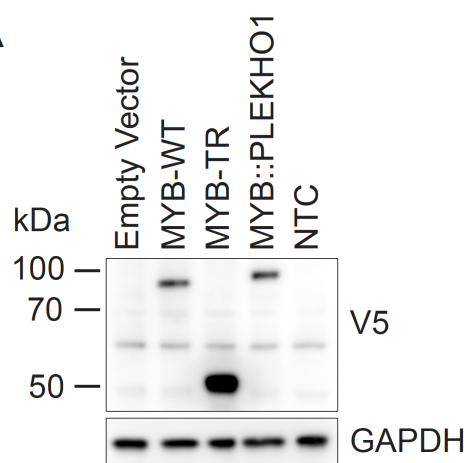


Figure S2: BPDCN MYB fusions cause increased binding to G2/M cell cycle genes.

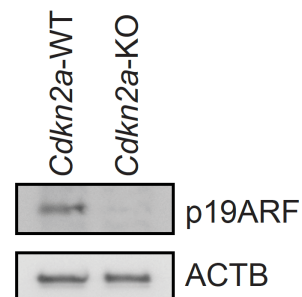
(A) V5 and IgG CUT&RUN tracks at the *CDCA3/USP5* locus in MYB-V5 knock-in K562 cells of the indicated genotypes, for two different V5 antibodies. (B) V5, MYB and IgG CUT&RUN tracks at the *BRD8/KIF20A* locus in MYB-V5 knock-in K562 cells of the indicated genotypes. (C) MYB and IgG CUT&RUN tracks at the *CCP110*, *CENPM*, *ERMAP* and *CCRL2* loci in MYB-V5 knock-in K562 cells of the indicated genotypes.

Figure S3

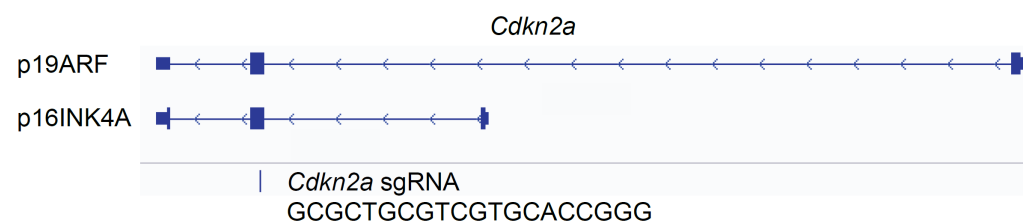
A



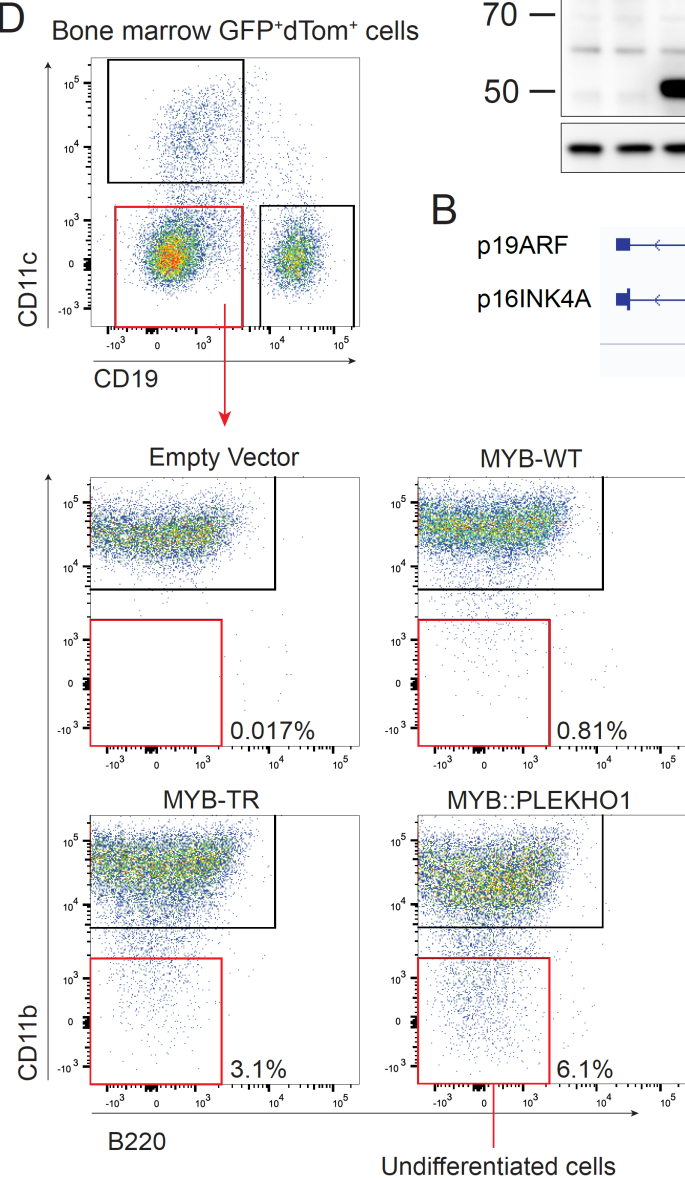
C



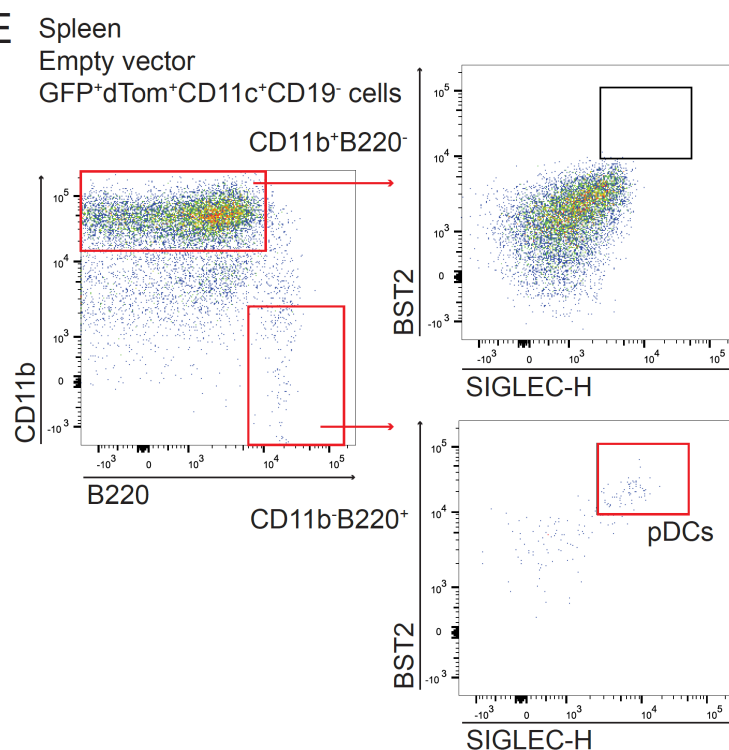
B



D



E



F

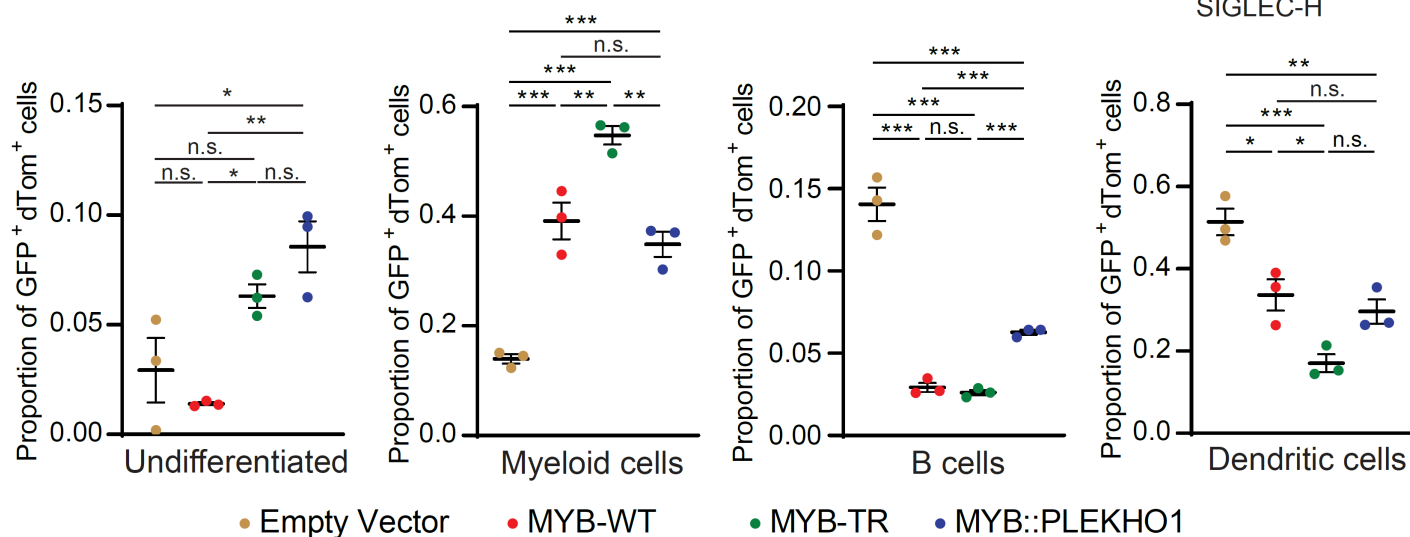


Figure S3: BPDCN MYB fusions impair differentiation in dendritic progenitor cells.

(A) Western blots showing expression of V5-MYB constructs in Hoxb8-FL cells. (B) Schematic showing mouse *Cdkn2a* locus p19ARF and p16INK4A open reading frames, with target location and sequence of *Cdkn2a*-KO sgRNA. (C) Western blot showing knockout of p19ARF in *Cdkn2a*-KO Hoxb8-FL cells. p16INK4A expression was too low to detect in undifferentiated Hoxb8-FL cells. (D) Representative flow cytometry plots of GFP⁺dTom⁺ bone marrow cells at day 7 of in vivo differentiation. Upper plot shows empty vector cells, lower plots show the indicated genotypes. (E) Representative flow cytometry plots of empty vector GFP⁺dTom⁺CD11c⁺CD19⁻ spleen cells at day 7 of in vivo differentiation. (F) Number of GFP⁺dTom⁺ spleen cells of the indicated cell types as a proportion of total GFP⁺dTom⁺ bone marrow cells (n=3 for each genotype). Undifferentiated CD19⁻CD11c⁻CD11b⁻B220⁻, myeloid cells CD11c⁻CD19⁻CD11b⁺, B cells CD11c⁻CD19⁺CD11b⁻B220⁺, dendritic cells CD11c⁺CD19⁻. Data represent mean $\pm$ SEM. Significance determined by one-way ANOVA with Tukey correction for multiple comparisons. * p<0.05, ** p<0.01, *** p<0.001.

A Figure S4

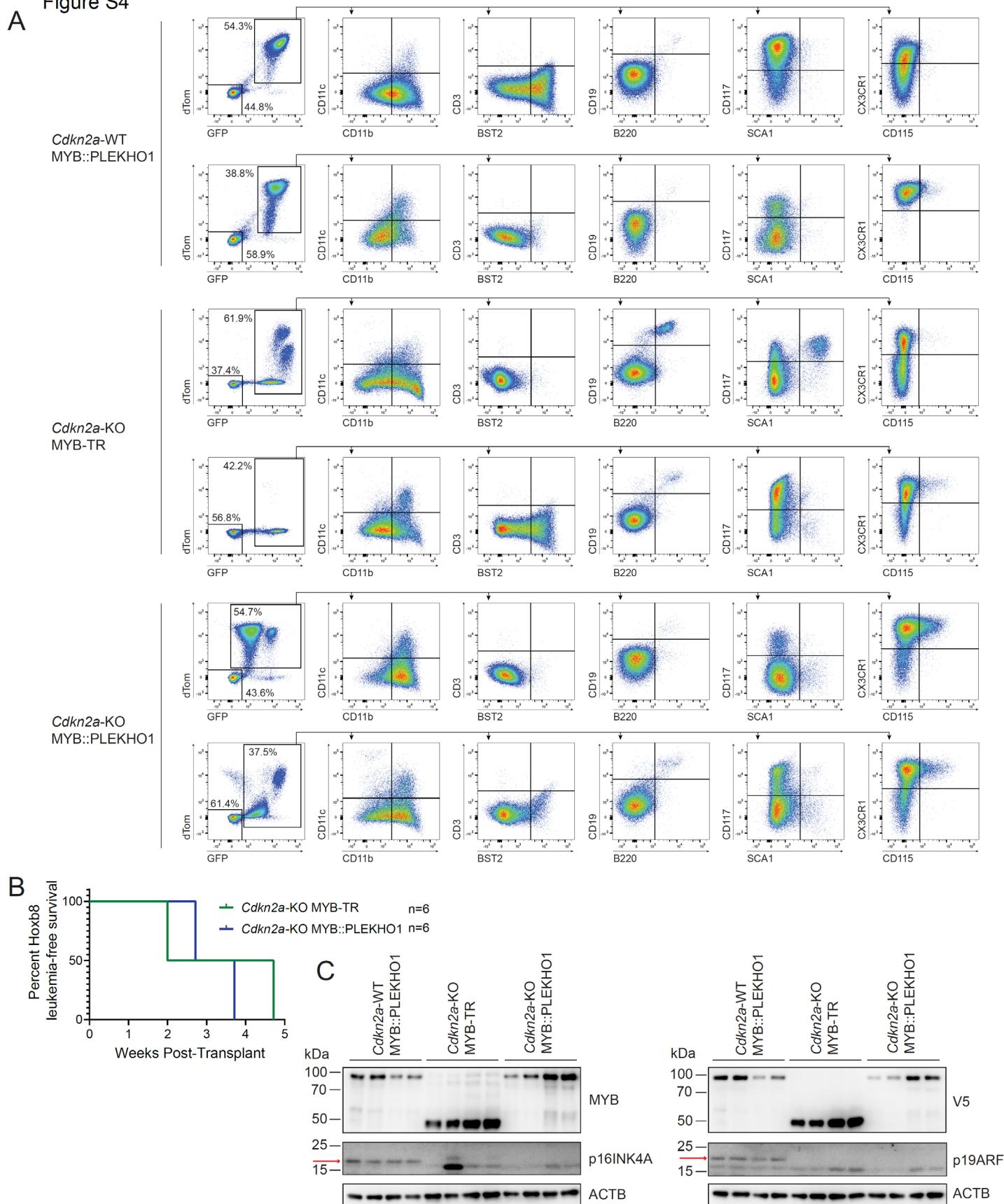


Figure S4: BPCDN MYB fusions expressed in hematopoietic progenitor cells induce myeloid-dendritic leukemia in vivo.

(A) Spleen flow cytometry plots for 6 leukemic Hoxb8-FL recipient mice of the indicated genotypes. (B) Hoxb8-FL cell-derived leukemia-free survival curves for secondary recipient mice of two primary *Cdkn2a*-KO MYB-TR and two primary *Cdkn2a*-KO MYB::PLEKHO1 leukemias (n=3 secondary recipients per primary leukemia). (C) Western blots showing expression of V5-MYB constructs, p16INK4A and p19ARF in unfractionated bone marrow cells from leukemic recipient mice of the indicated genotypes (n=4 per genotype).

Figure S5

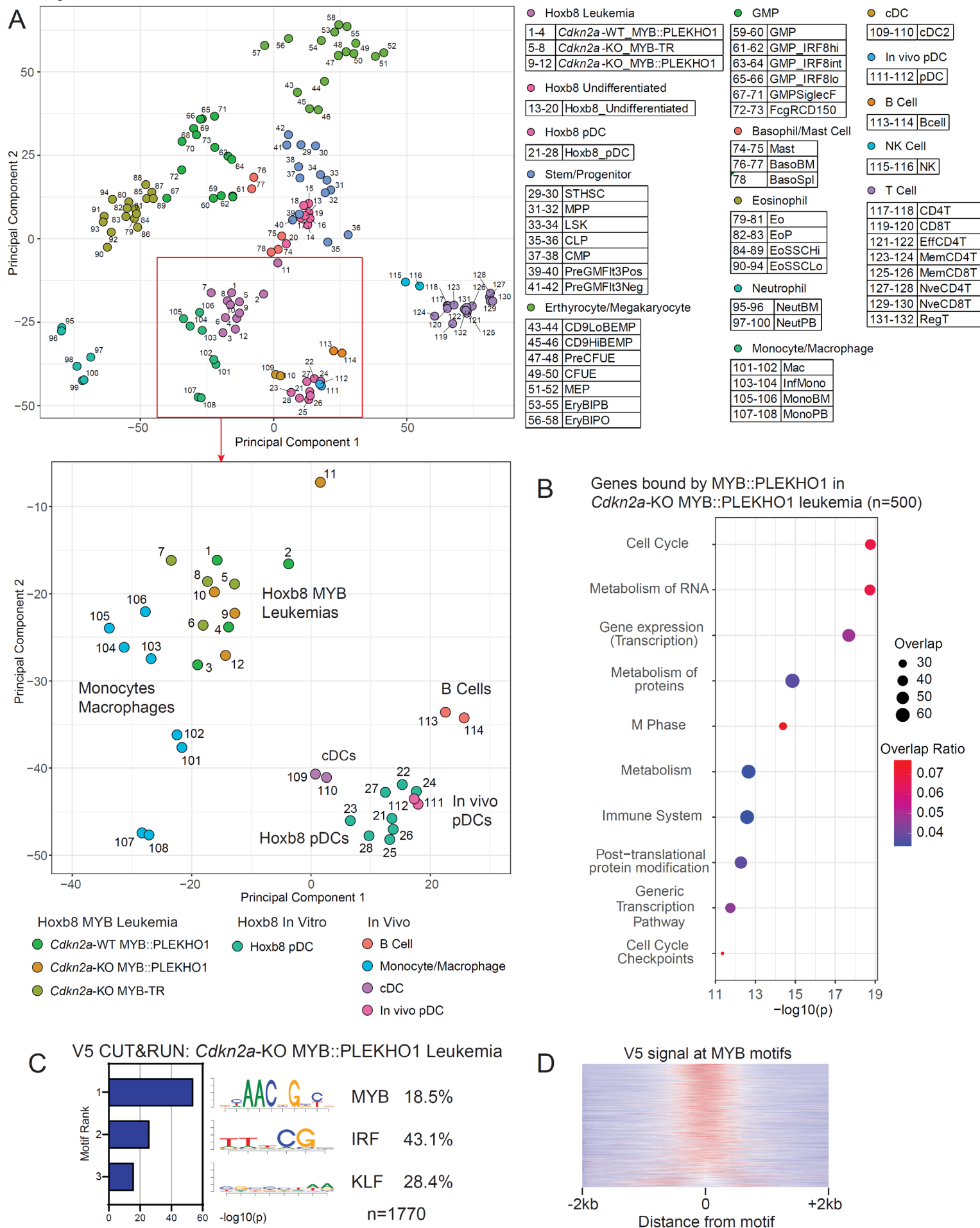


Figure S5: BPDCN MYB fusions bind to G2/M cell cycle genes in myeloid-dendritic leukemia.

(A) Principal component analysis of RNA-seq data from Hoxb8-FL cell-derived leukemias of the indicated genotypes, wild-type undifferentiated Hoxb8-FL cells and Hoxb8-FL cell-derived pDCs, and hematopoietic cell populations from the Haemopedia dataset (45). Lower panel shows the highlighted section zoomed in with points recolored to differentiate leukemia genotypes. (B) Top 10 ranked Reactome gene sets overlapping with the 500 unique genes bound by MYB::PLEKHO1 nearest to the transcription start site in *Cdkn2a*-KO MYB::PLEKHO1 leukemia cells. (C) Top 3 ranked motifs enriched in V5 CUT&RUN peaks in *Cdkn2a*-KO MYB::PLEKHO1 leukemia cells, and percent of peaks containing each motif. (D) Heatmap showing V5 CUT&RUN signal centered on MYB motifs inside V5 binding peaks located within 10 kb of a transcription start site, ordered by signal strength at the motif (n=2100 motifs).

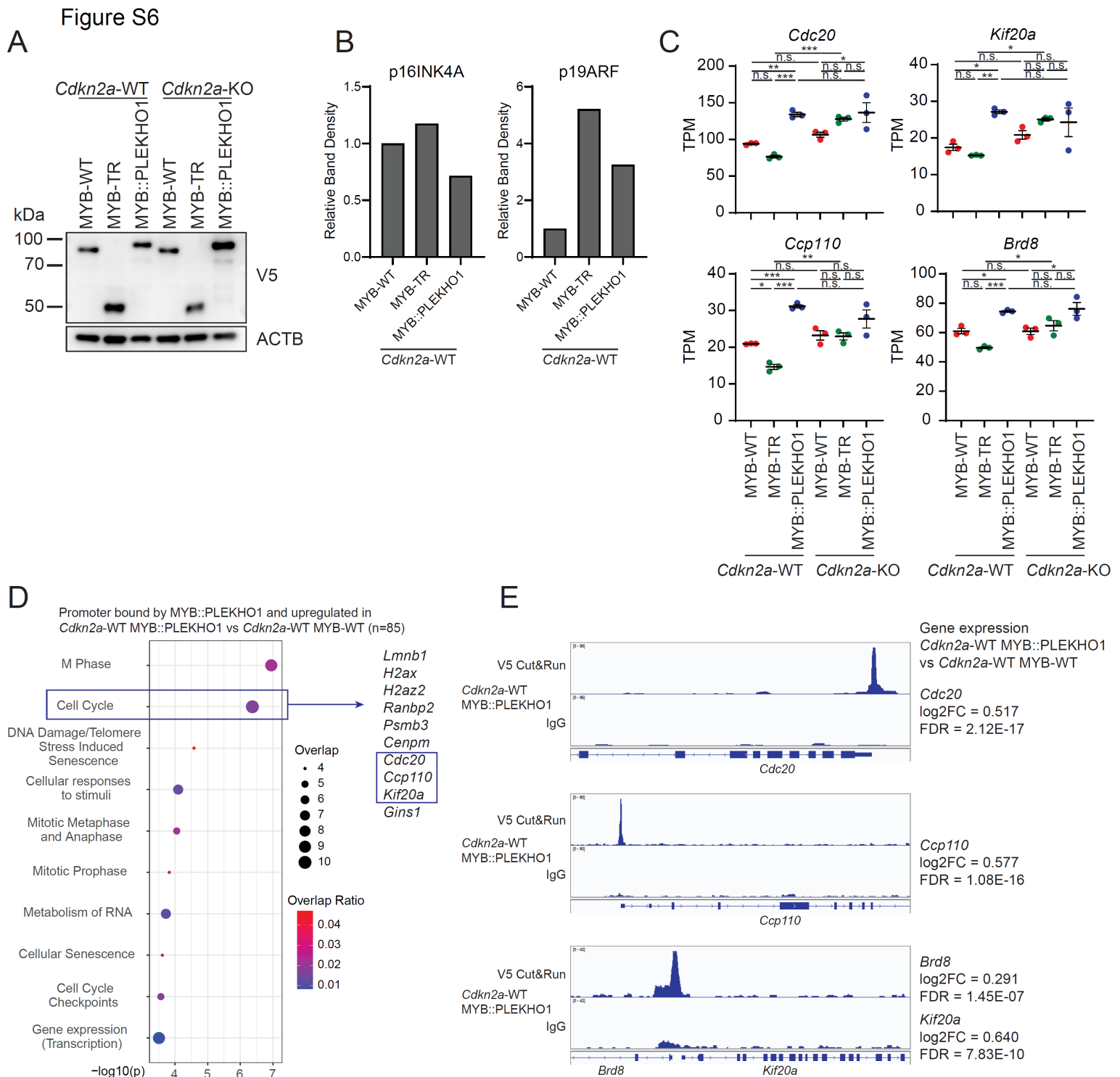


Figure S6: BPCDN MYB fusions bind to and activate G2/M cell cycle genes in Hoxb8-FL.MS5 cells.

(A) Western blot showing expression of V5-MYB constructs in Hoxb8-FL.MS5 cells of the indicated genotypes. (B) Quantification of western blot bands for p16INK4A and p19ARF stains shown in Figure 5D, normalized to ACTB signal and shown relative to MYB-WT. (C) TPM of *Cdc20*, *Ccp110*, *Kif20a* and *Brd8* in Hoxb8-FL.MS5 cells of the indicated genotypes (n=3 per genotype). Data represent mean $\pm$ SEM. Significance determined by one-way ANOVA with Tukey correction for multiple comparisons. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. (D) Top 10 ranked Reactome gene sets overlapping with genes showing both increased expression in *Cdkn2a*-WT MYB::PLEKHO1 relative to *Cdkn2a*-WT MYB-WT Hoxb8-FL.MS5 and MYB::PLEKHO1 binding at promoter regions in *Cdkn2a*-WT MYB::PLEKHO1 Hoxb8-FL.MS5 cells (n=85). All overlapping genes in the “Cell Cycle” gene set (n=10) are shown. (E) V5 CUT&RUN tracks at the *Cdc20*, *Ccp110* and *Brd8*/*Kif20a* loci in *Cdkn2a*-WT MYB::PLEKHO1 Hoxb8-FL.MS5 cells. Gene expression fold changes and FDR values determined by DESeq2 are shown for *Cdkn2a*-WT MYB::PLEKHO1 Hoxb8-FL.MS5 cells relative to *Cdkn2a*-WT MYB-WT Hoxb8-FL.MS5 cells.

Figure S7

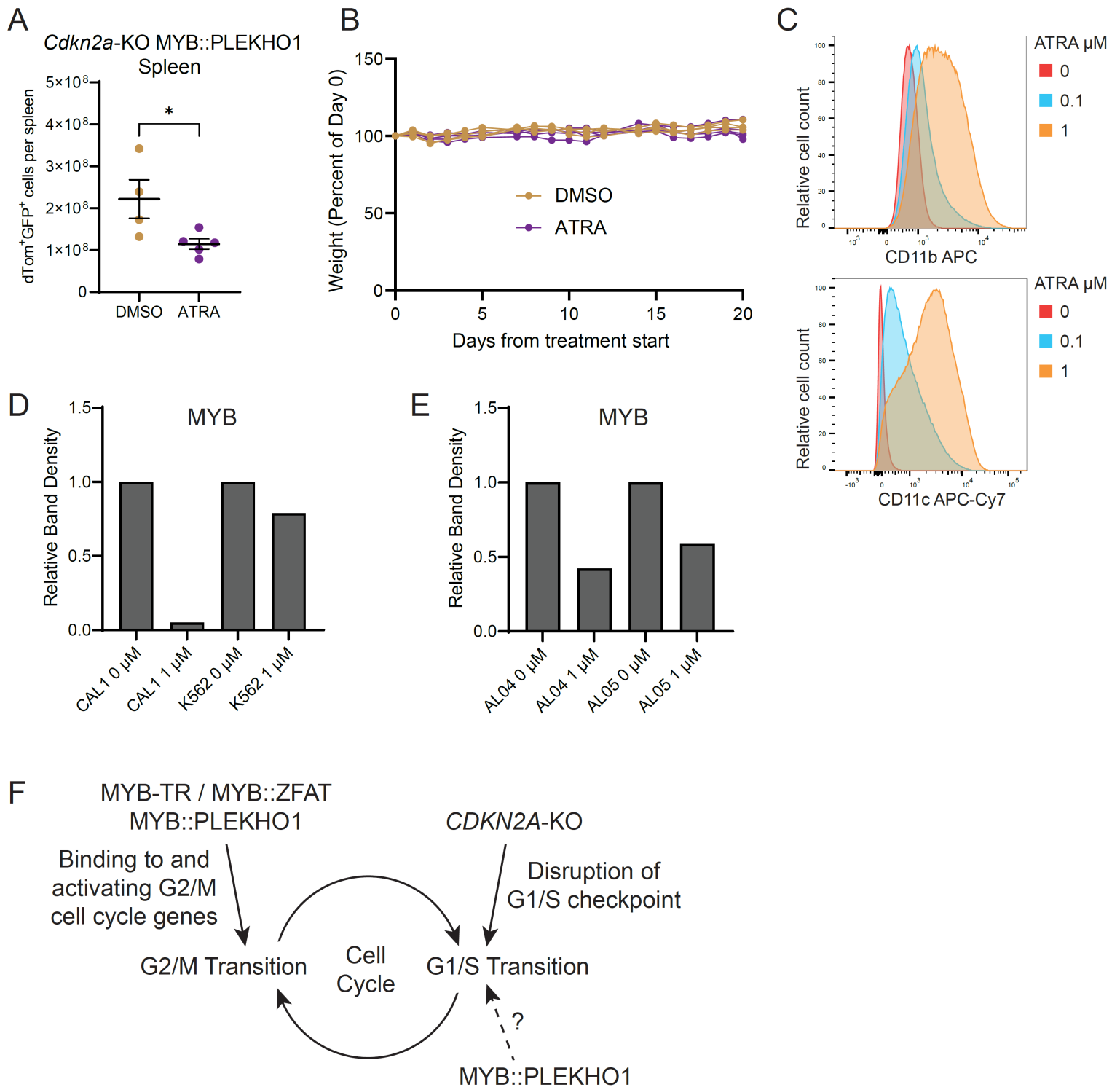


Figure S7: BPDCN cells undergo loss of MYB and cell death upon ATRA treatment.

(A) Number of viable GFP⁺dTom⁺ cells per spleen of *Cdkn2a*-KO MYB::PLEKHO1 secondary recipient mice treated daily with 20 mg/kg ATRA for 21 days (DMSO n=4, ATRA n=5). Data represent mean $\pm$ SEM. Significance determined by t test. * p<0.05. (B) Body weight during treatment period of mice in (A), shown as percentage of starting weight. (C) Representative fluorescence intensity histograms showing cell frequency relative to mode for CD11b APC and CD11c APC-Cy7 in CAL1 cells after 2 days of ATRA treatment at the indicated dose. (D) Quantification of western blot bands for MYB stain shown in Figure 6H, normalized to ACTB signal and shown relative to 0 μ M ATRA. (E) Quantification of western blot bands for MYB stain shown in Figure 6I, normalized to ACTB signal and shown relative to 0 μ M ATRA. (F) Model showing proposed mechanism of MYB fusion oncogenesis in BPDCN.