Figure S1

A

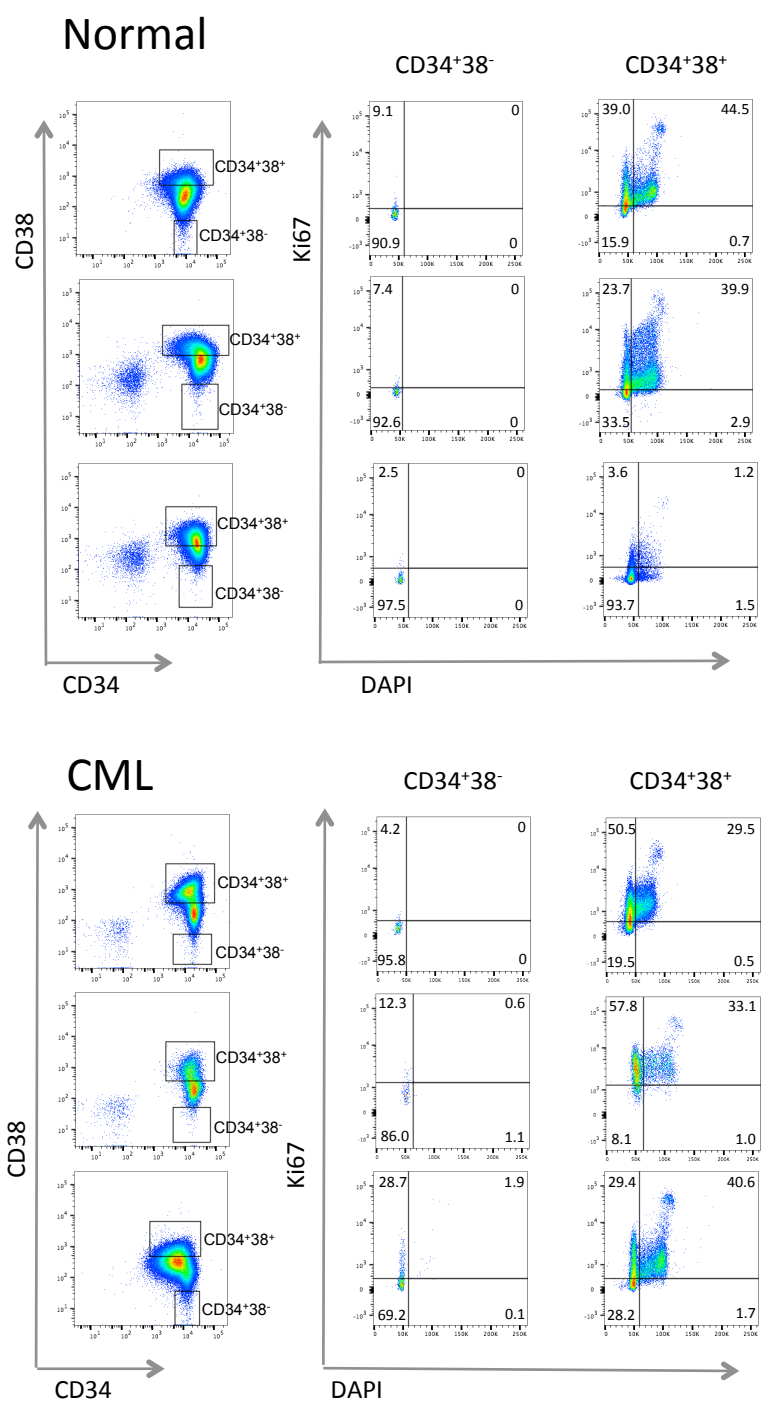
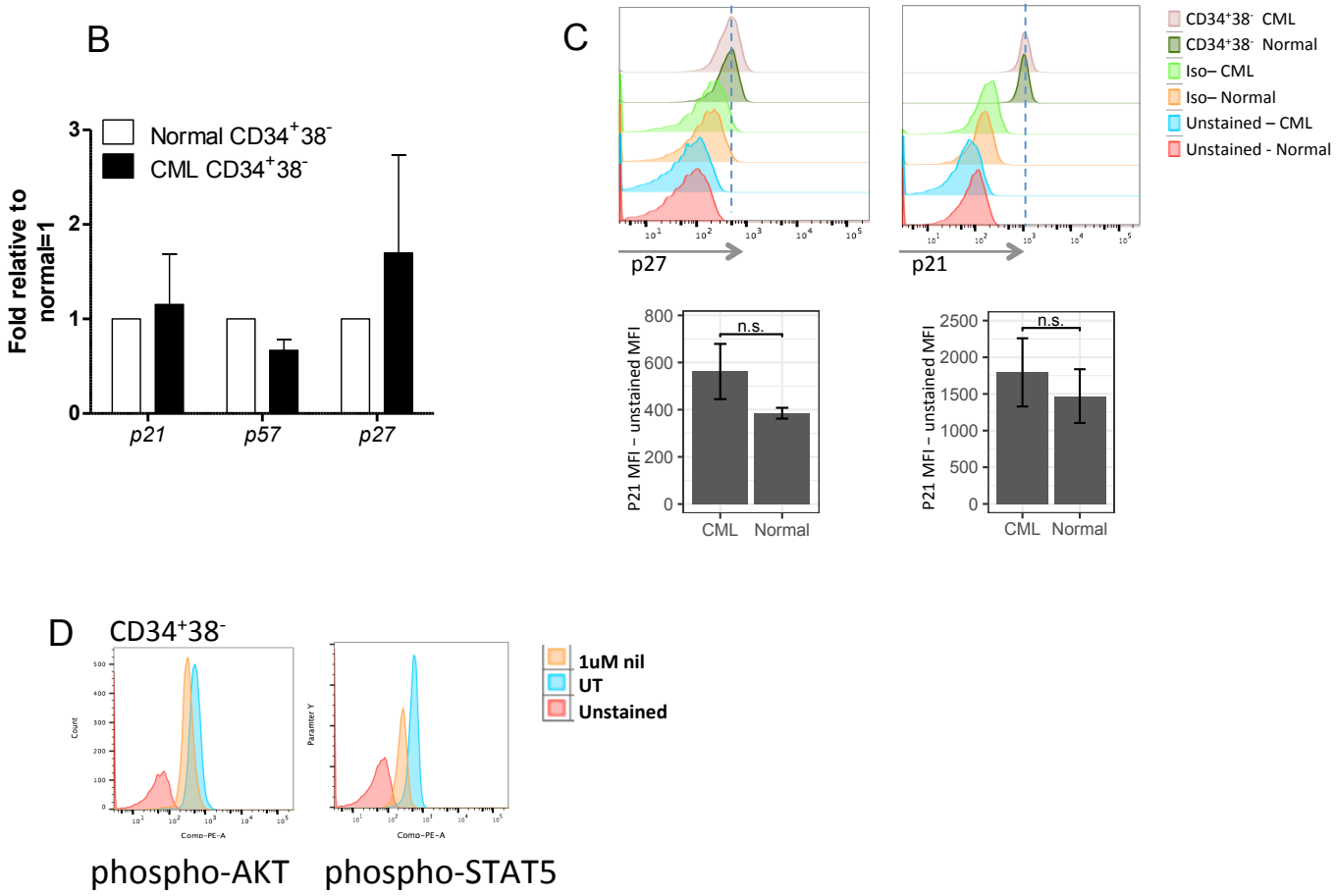

Normal
CD34+38-
CD34+38+
CD38
CD34+38+
CD34+38-
CD34+38+
CD34+38-
CD34+38+
CD34+38-
9.1
0
90.9
0
39.0
44.5
15.9
0.7
7.4
0
92.6
0
23.7
39.9
33.5
2.9
2.5
0
97.5
0
3.6
1.2
93.7
1.5
CD34
Ki67
DAPI
CML
CD34+38-
CD34+38+
CD38
CD34+38+
CD34+38-
CD34+38+
CD34+38-
CD34+38+
CD34+38-
4.2
0
95.8
0
50.5
29.5
19.5
0.5
12.3
0.6
86.0
1.1
57.8
33.1
8.1
1.0
28.7
1.9
69.2
0.1
29.4
40.6
28.2
1.7
CD34
Ki67
DAPI

Figure S1



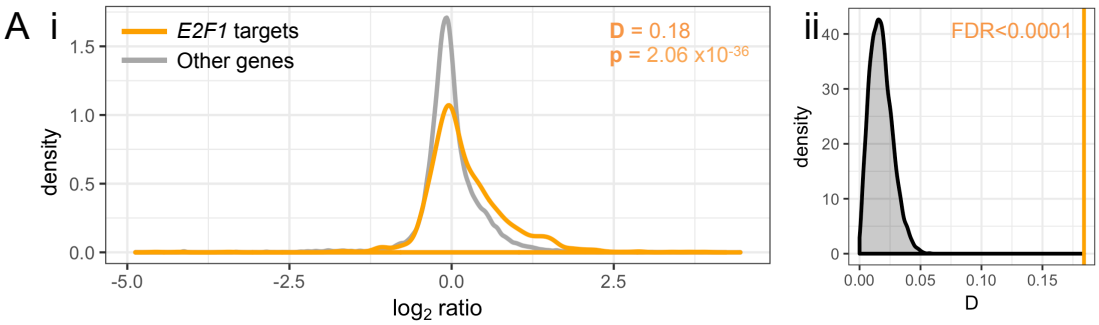
E

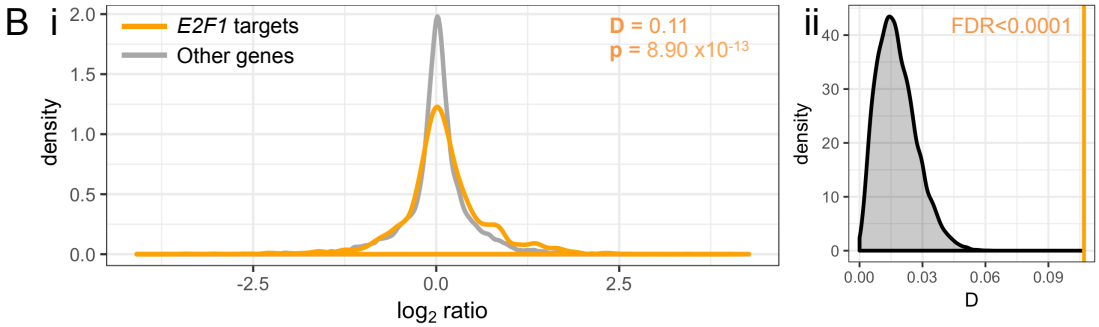
hsa-mir183 sequence	**3' CUUAAGAUGGUCACGGUAU 5'**
Pairing sequence in EGR1-3'UTR	**5' GTACAGTGTCTGTGCCATG 3'**

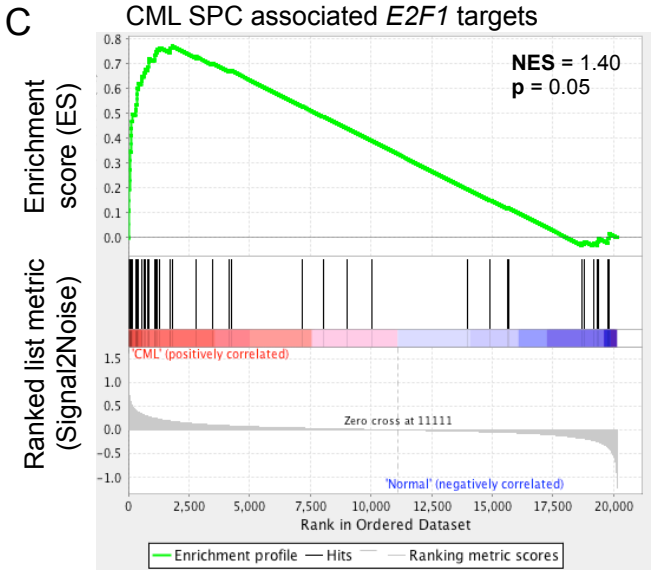
Reporters	**Oligos**
hsa-mir183 forward primer	**gggtttaaacctggagtggaaggtctattggc**
hsa-mir183 reverse primer	**Gggtcgac-ccatggcacagacactgtacaag**
hsa-mir183 reverse primer-Mut	**Gggtcgac-cGGatCCcacagacactgtacaag**

Supplemental Figure S1. (A) Representative dot plots showing gating strategy and cell cycle phases in normal and CML $CD34^+38^-$ and $CD34^+38^+$ cells measured by Ki-67/DAPI staining. N=3 biological samples. (B) Levels of *p21, p57* and *p27* mRNA measured in normal and CML SPC**.** N=3 biological samples. (C) Levels of *p27* and *p21* protein measured in normal and CML SPC by FACS. N=3 biological samples. Representative FACS plots for 1 biological replicate shown. (D) Levels of phospho-AKT and phospho-STAT5 protein after 24hrs treatment with 1 uM nilotinib in CML SPC measured by FACS. N=1 biological samples. (E) (top) The sequence of *hsa-mir183* and its pairing sequence within the 3'UTR region of *EGR1* (underlined). (bottom) Primer sequences for the oligos used to amplify *EGR1* 3'UTR (wild type, WT) and the mutant version of the binding site (mutant, MUT). The insertion of a BAMHI restriction enzyme site in the mutant form is shown by the underlined sequence (cGGatCC). (Nil:nilotinib; Iso: Isotype control; n.s.: Not significant).

Figure S2







Supplemental Figure S2. (A) Transcriptional deregulation in CML G0 v normal G0 cells (ArrayExpress accession E-MTAB-2508; N=2 normal, N=5 CML): (i) $\log_2$ ratios for *E2F1* targets (orange) and all other genes (grey); (ii) distribution of Kolmogorov-Smirnov D statistic for 10,000 random samplings (grey) and for *E2F1* targets (orange vertical line). (B) As (A) but for a second, complementary CML G0 v normal G0 dataset (GEO accession GSE24739; N=4 normal, N=8 CML). (C) GSEA enrichment plot for the CML SPC associated *E2F1* targets (Figure 3Aii), in the second, complementary CML G0 v normal G0 dataset mentioned in (C) (NES=1.40, p=0.05). (D) Microarray data for *E2F2* and *E2F3* (2 probesets) in normal and CML $CD34^{+}PY^{-}Ho^{-}$ cells (N=2 normal, N=5 CML).

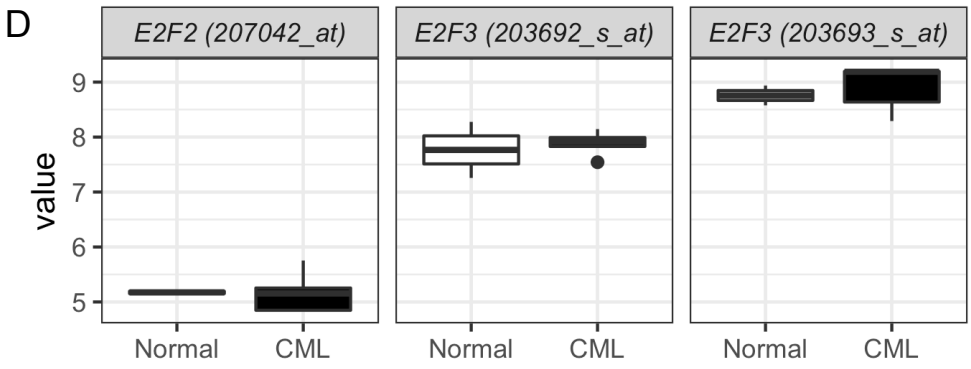
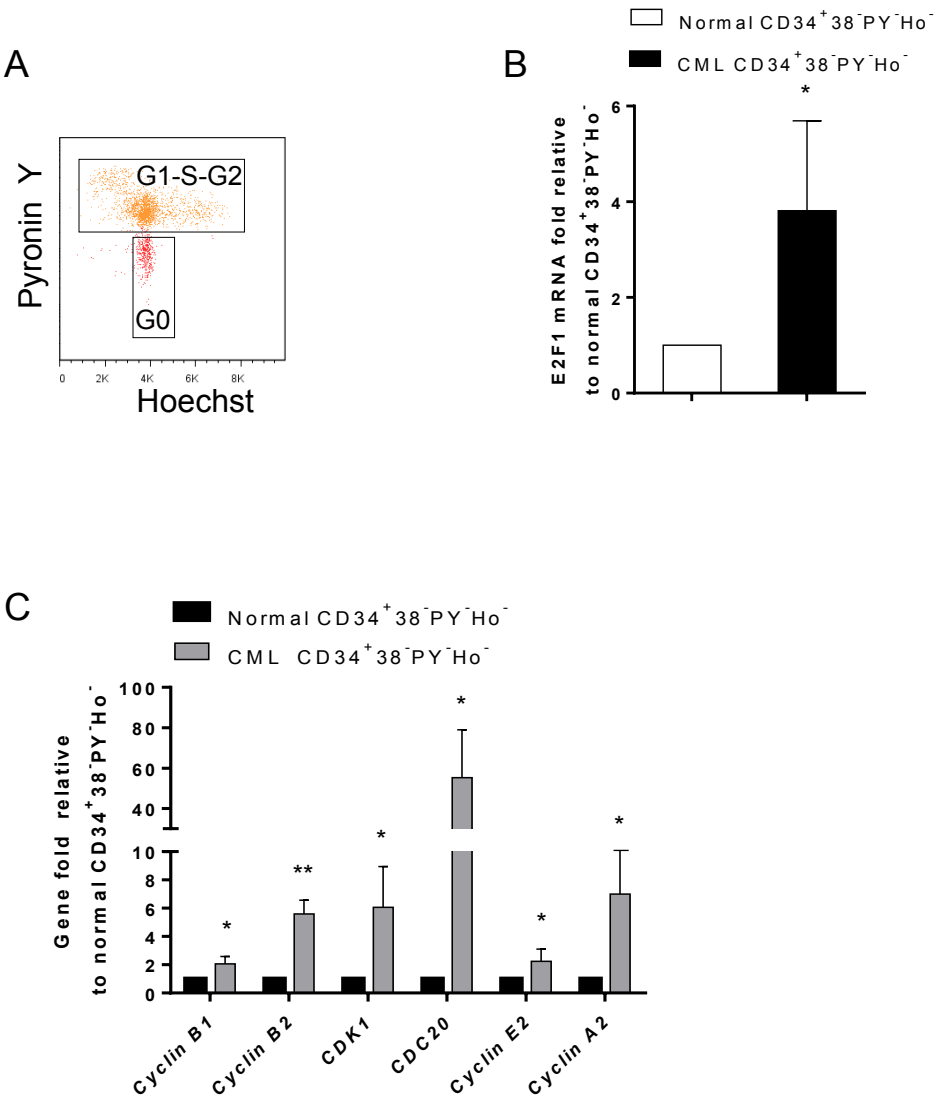
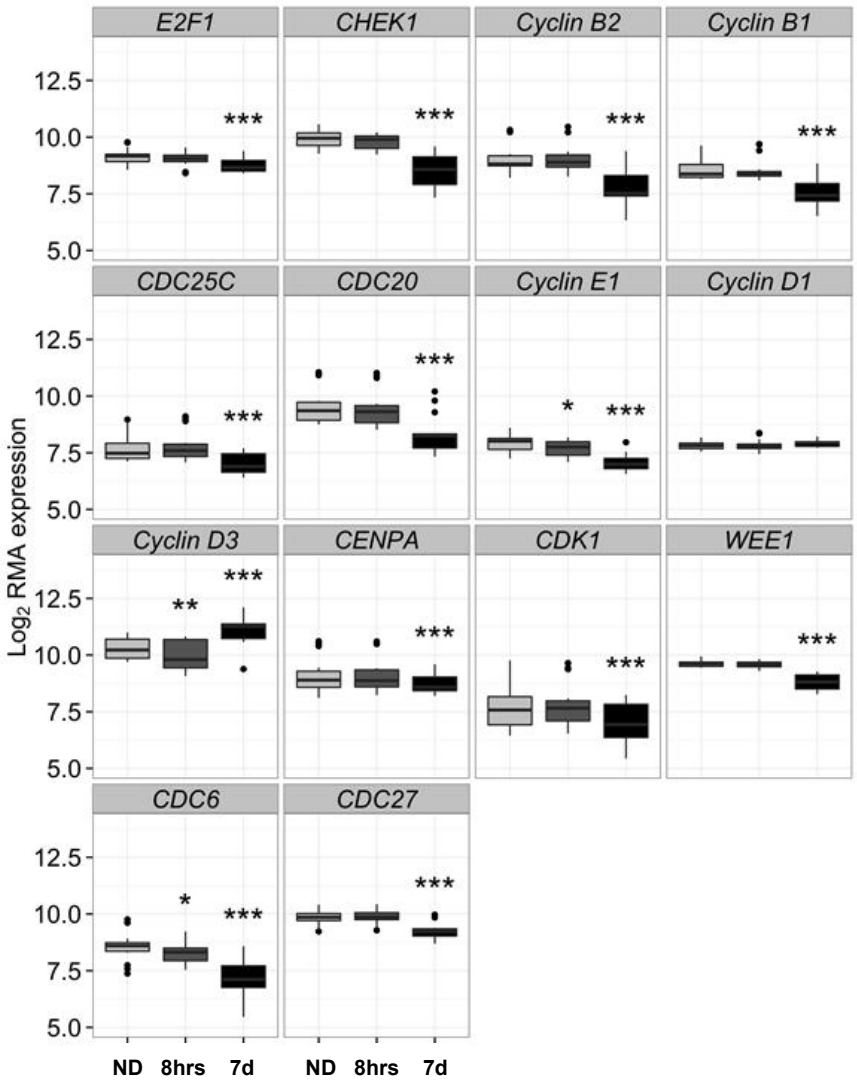


Figure S3



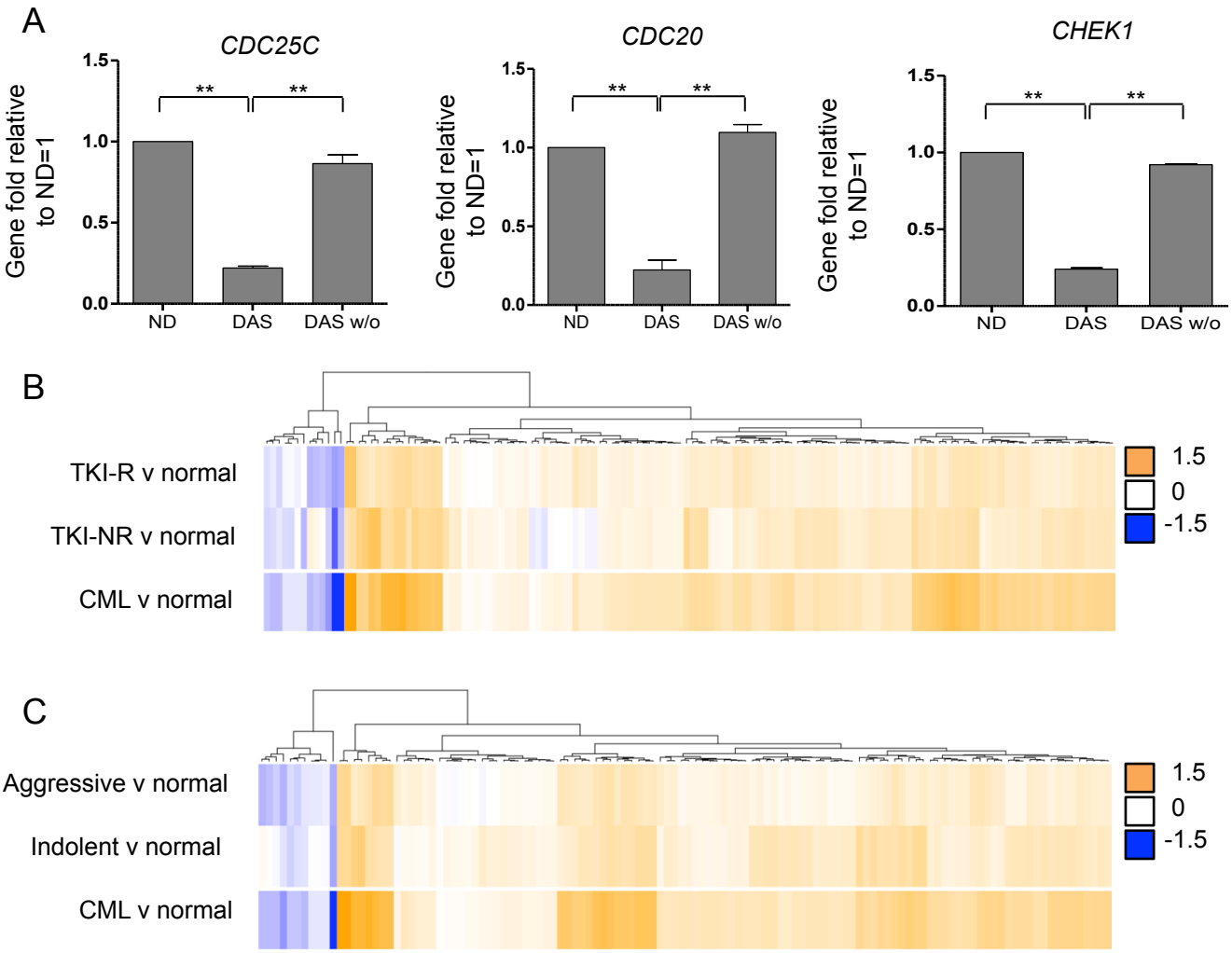
Supplemental Figure S3. (A) G0 cells ($PY^{-}Ho^{-}$) within the $CD34^{+}38^{-}$ SPC from normal and CML populations were isolated by FACS. The mRNA profile of *E2F1* (B) and its downstream genes (C) were analysed by Q-PCR using a Fluidigm platform. N=3 technical replicates; N=1 biological replicate (*P<.05, ** P<.01).

Figure S4



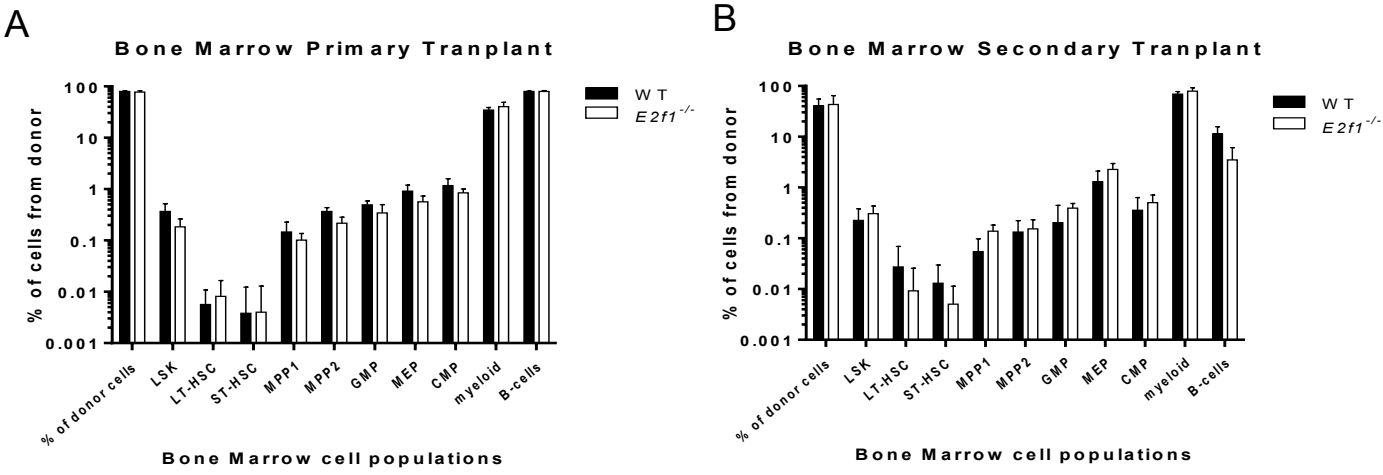
Supplemental Figure S4. Global gene expression array data for E2F1 and representative downstream genes in CML SPC treated with TKI imatinib (5uM), dasatinib (150nM) or nilotinib (5uM) at 8hrs and 7d in the absence of growth factors (N=6 biological replicates). ND=no drug (* *P*<.05; ** *P*<.001).

Figure S5



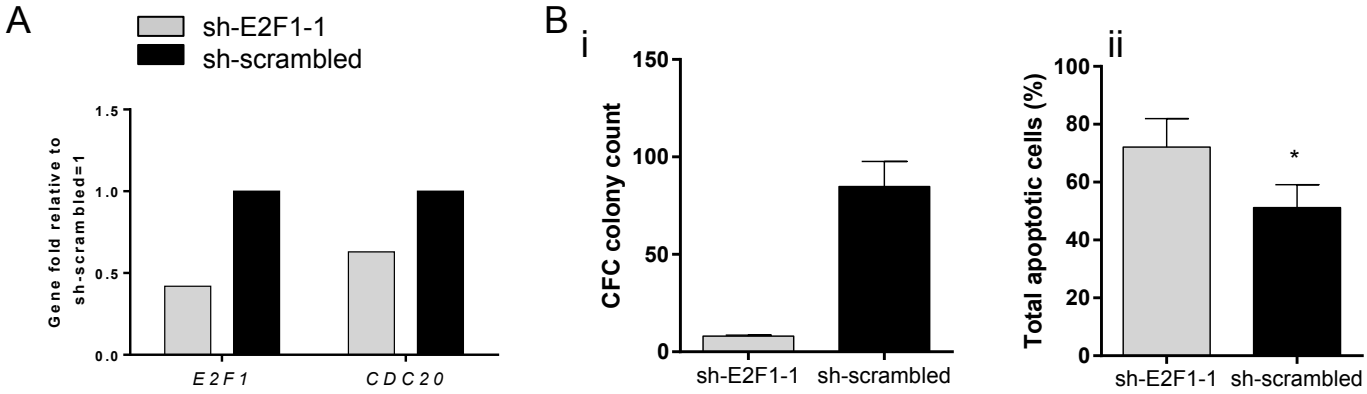
Supplemental Figure S5. (A) mRNA expression data for *CDC25C, CDC20*, and *CHEK1* (left to right) in CML SPC before drug treatment (ND=no drug), treated for 7d with dasatinib (DAS) and washed and cultured for a further 3d without drug (DAS w/o). N=3 biological replicates (***P*<.01). (B) Transcriptional deregulation (logFC) of E2F1 targets in three different CML v normal comparisons of $CD34^{+}$ cells from PB: TKI-responders (TKI-R) versus normal (top lane); TKI-non-responders (TKI-NR) versus normal (middle lane) and CML versus normal (bottom lane). Down-/up-regulation are indicated by blue/orange respectively (colors as indicated to the right). (C) As (B) but for the comparisons: aggressive versus normal (top lane); indolent versus normal (middle lane); and CML versus normal (bottom lane).

Figure S6



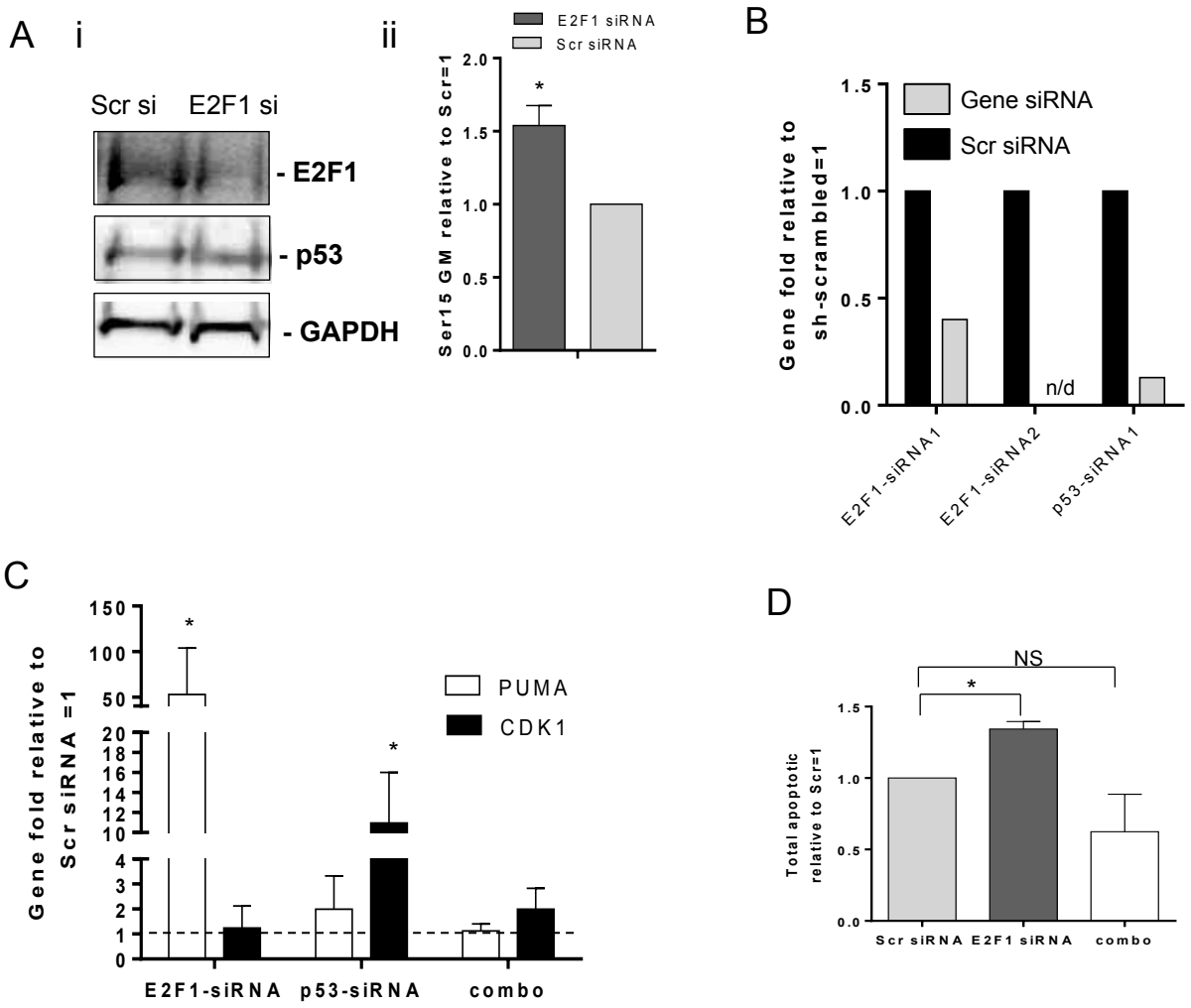
Supplemental Figure S6. (A) Characterization of the BM of primary and (B) secondary transplanted mice. 1000 LSK cells were transplanted into lethally irradiated 8-10 wk-old C SJL C57/B6 CD45.1 recipient mice together with 2×10^5 CD45.1$^+$ syngeneic competitor BM cells. The primary recipients were analysed every 4 weeks after transplantation. 2000 CD45.2$^+$ LSK cells were sorted from their BM and transplanted into secondary recipients together with competitor BM cells. Secondary recipients were analysed every 4 weeks after transplantation. N=5 recipients per donor. Statistical analysis showed no significant differences between samples.

Figure S7



Supplemental Figure S7. (A) To confirm the efficiency of our lentiviral system in primary cells, we knocked-down *E2F1* in CML CD34$^+$ cell samples using a shRNA (sh-E2F1-1) specific for different regions of the *E2F1* sequence. (B) (i) CFC colony count and (ii) total apoptosis (Annexin-V$^+$/7-AAD$^+$) following E2F1 knock-down. N=3 for technical replicates; N=2 biological replicates (*P<.05).

Figure S8



Supplemental Figure S8. (A) (i) Western blotting indicating levels of E2F1 and p53 proteins in *E2F1* knock-down CML SPC by siRNA (E2F1 si). Scrambled siRNA (Scr si) was used as a control in the transfections. GAPDH was used as a loading control in the western blotting. (ii) Phosphorylation of p53 at Ser-15 measured by FACS in *E2F1* knock-down CML SPC. (B) mRNA levels of *E2F1* and *p53* following *E2F1* knock-down (using E2F1-siRNA1 and E2F1-siRNA2) and *p53* knock-down (using p53-siRNA1) (N=1 technical replicate; N=1 biological replicate). Scrambled: Scr; not detected: n/d. (C) Levels of representative E2F1 and p53 downstream target genes measured after *E2F1* and *p53* single knock-down (E2F1-siRNA and p53-siRNA) or in an *E2F1/p53* double knock-down (combo) in CML SPC. Scrambled siRNA (Ctr-siRNA) was used as control. (D) Percentage of total apoptosis after *E2F1* or *E2F1/p53* double (combo) knock-down for 72h in SPC indicated by Annexin-V$^+$/7-AAD$^+$ cells. N=3 biological and technical replicates for all experiments (*P <.05; *** P <.001; NS: not significant with $P>.05$).