Tumor suppressor function of *WT1* in acute promyelocytic leukemia

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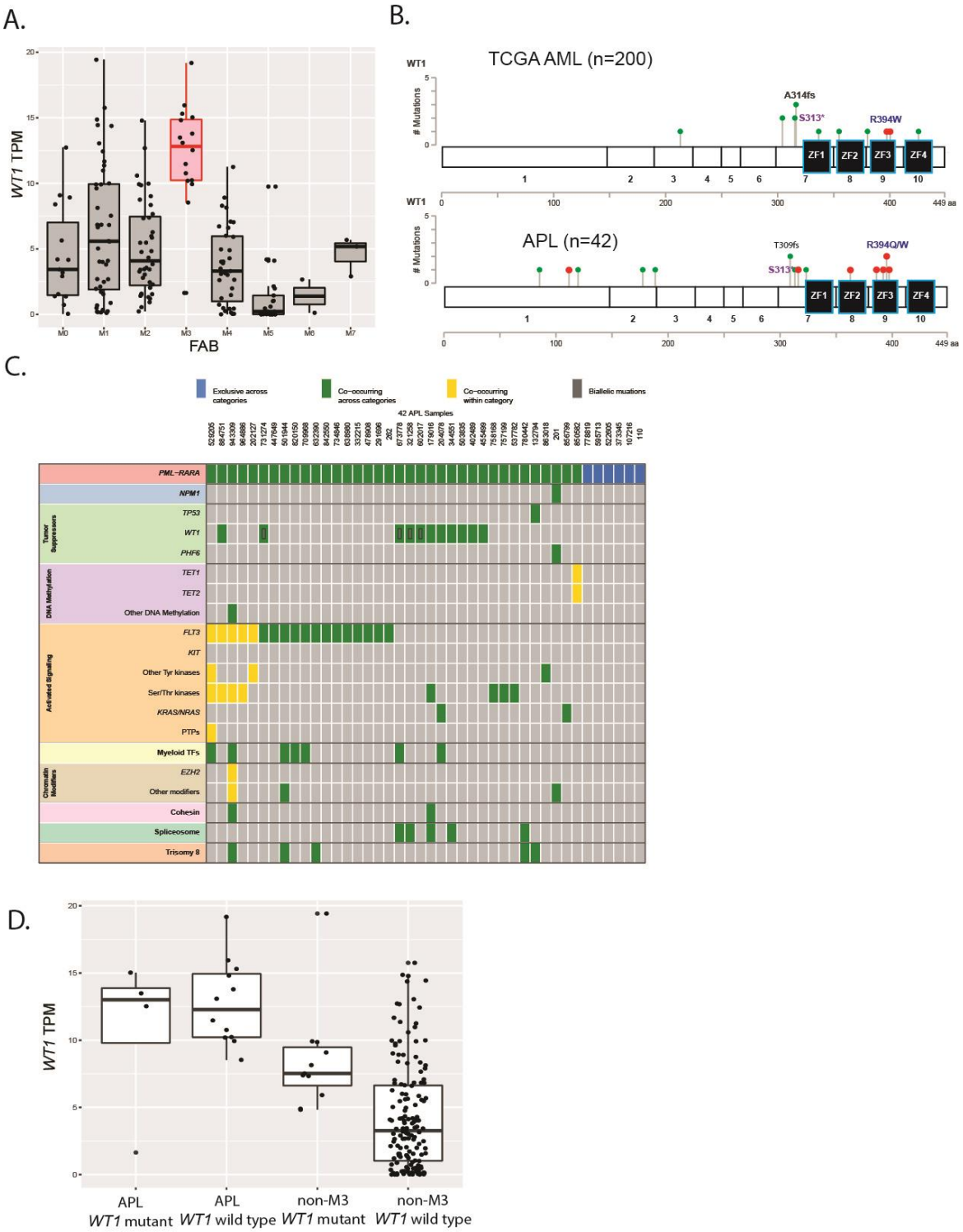
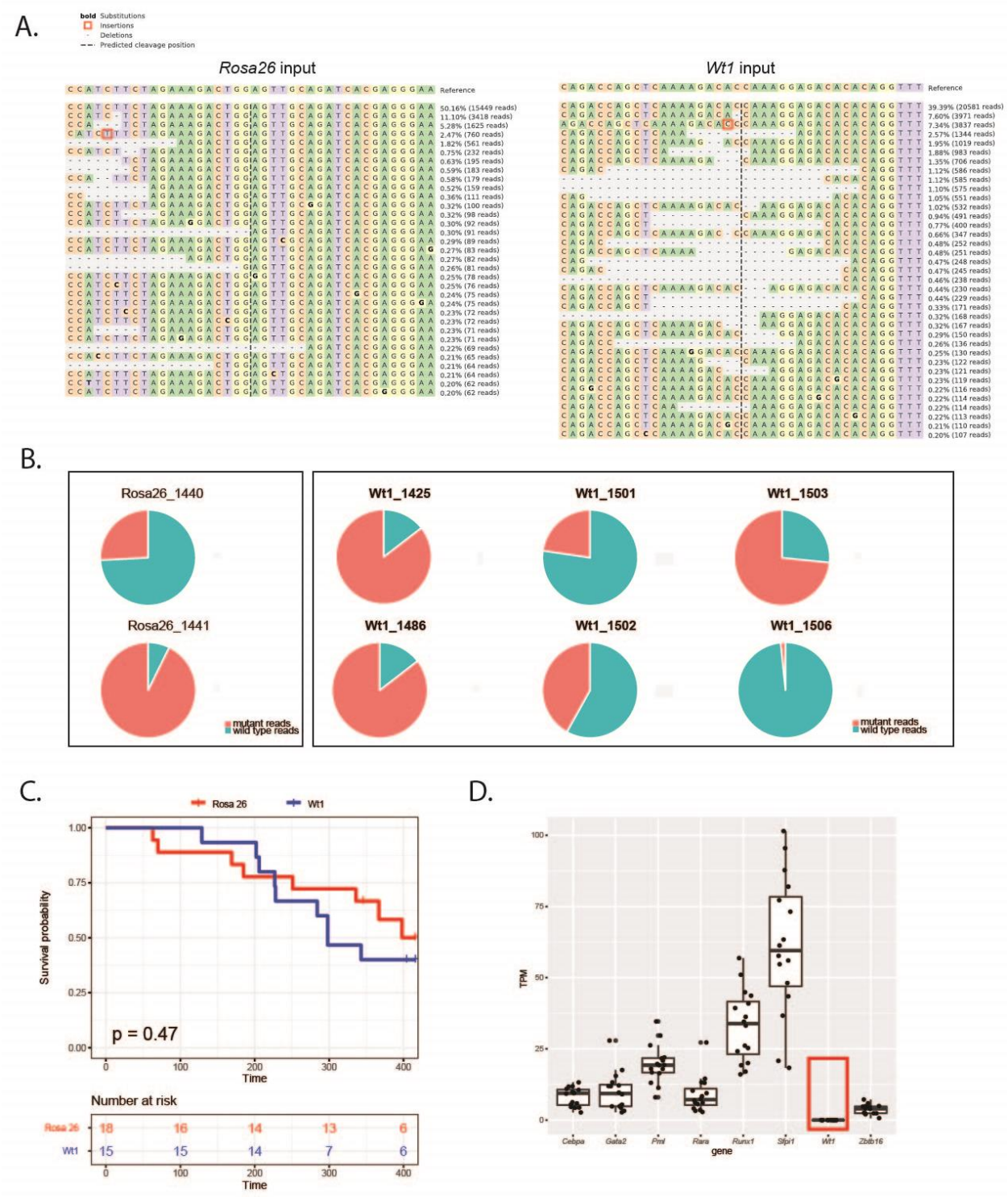
Supplementary Figures and Legends



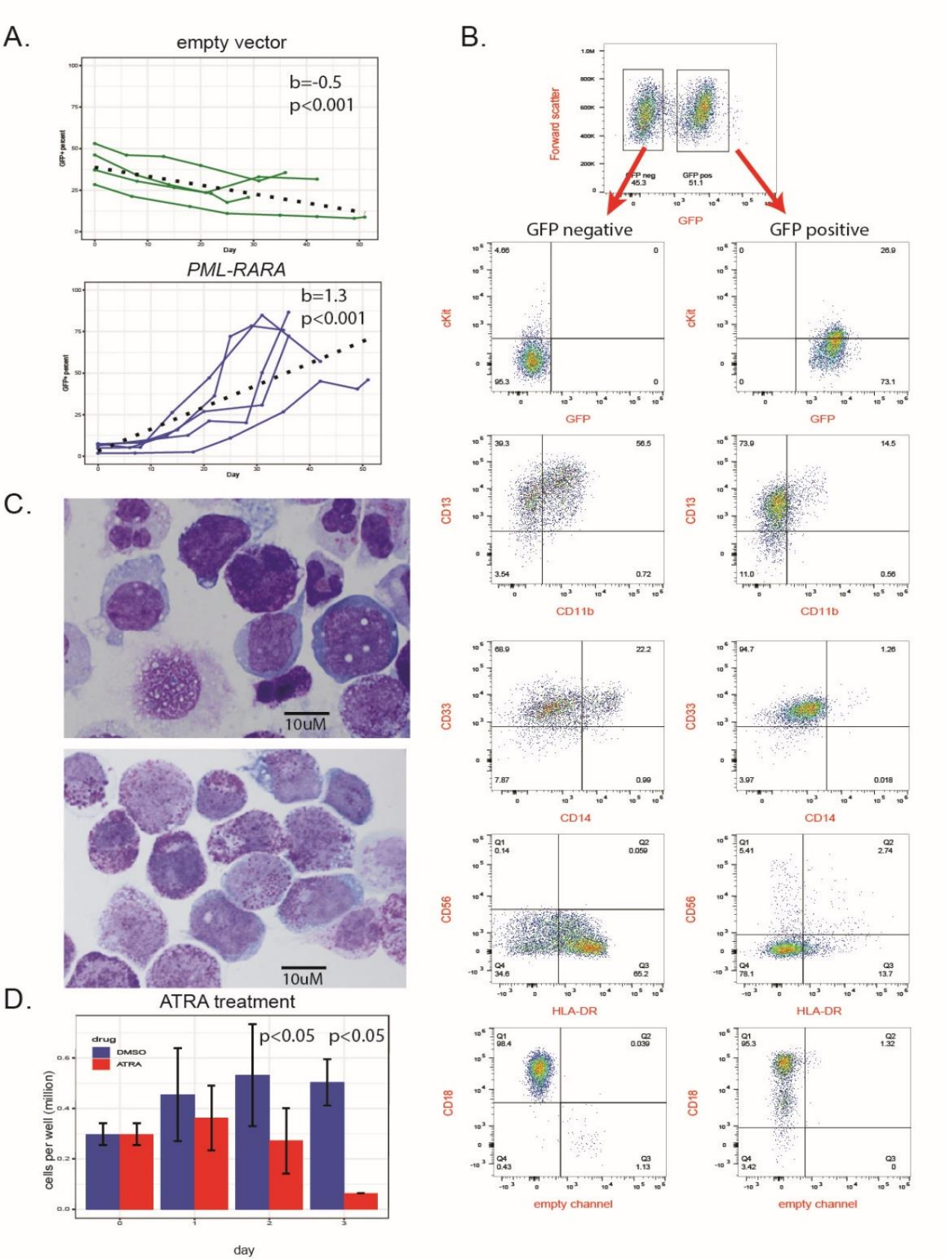
Supplementary Figure 1.

Supplementary Figure 1. *WT1* is both highly expressed and frequently mutated in APL. (A) RNA expression data from the AML TCGA dataset showing expression of *WT1* across all FAB subtypes. *WT1* expression is significantly higher in M3 AML compared to all other subtypes with p-values ranging from 0.04 (vs. M7) to 2.43×10^{-13} (vs. M5) using Tukey's Range Test. TPM, Transcripts Per Kilobase Million. (B) Schematics showing *WT1* mutations in AML samples from the TCGA dataset (N=200 cases, top) and in the APL cases from this study (N=42 cases, bottom). Green circles represent nonsense or frameshift mutations, red circles represent missense mutations, numbers indicate exon number, ZF1-ZF4 are zinc finger domains 1-4. Hotspot mutations (e.g. R394Q/W) are labelled. (C) Plot showing gene mutations co-occurring with the *PML-RARA* translocation in 42 cases of APL. Yellow boxes indicate mutations that co-occur within a biologic category and black inset boxes indicate mutations that occur in more than one allele. Blue boxes indicate cases where no cooperating mutations were identified with a capture panel containing the recurrently mutated genes in the TCGA AML study (2). (D) RNA expression data from the AML TCGA dataset showing expression of *WT1* in WT1 mutant (first and third bars) compared to WT1 wild type cases (second and fourth bars). Cases are stratified as APL (two left bars) or non-M3 (right two bars). WT1 expression is significantly lower in the non-M3, WT1 wild-type cases as a group ($p<0.01$ for all comparisons using Tukey's Range Test.) TPM, Transcripts Per Kilobase Million.



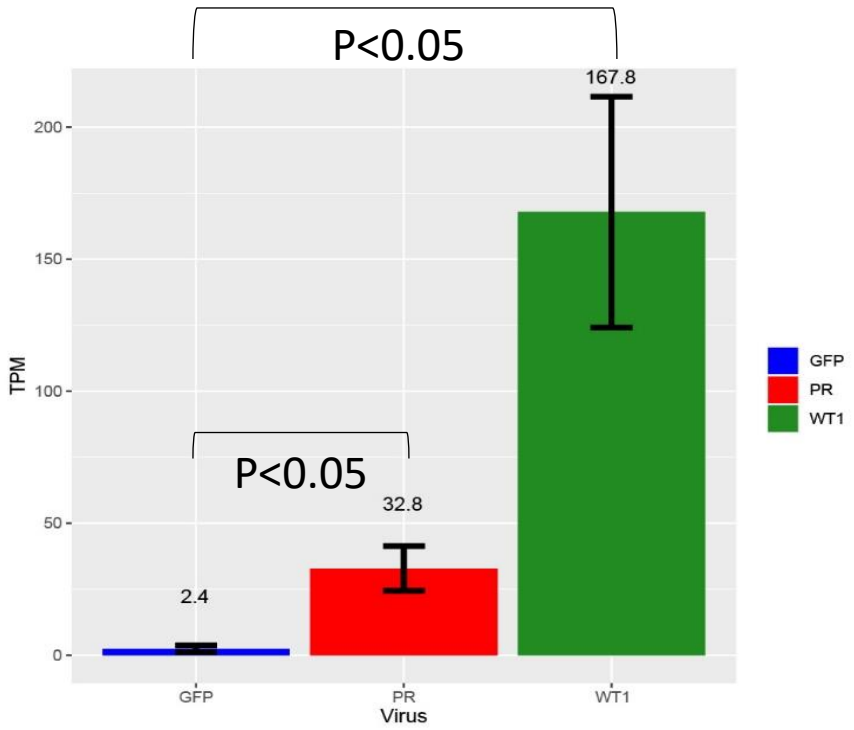
Supplementary Figure 2

Supplementary Figure 2. *Wt1* loss-of-function mutations do not cooperate with *PML-RARA* in a mouse APL model. (A) Amplicon sequencing of the gRNA target regions from *Ctsg-PML-RARA* lineage depleted bone marrow cells after CRISPR editing, prior to transplantation into lethally irradiated recipients. Shown is the mutational spectrum of hematopoietic cells edited at *Rosa26* (left) or *Wt1* (right) loci. Bolded missense variants are presumed to be PCR artifacts generated during amplification. (B) Mice that died during the tumor watch succumbed to clonal APL arising from either a mutated or an unmutated hematopoietic progenitor cell. DNA was isolated from unfractionated spleen cells from mice with viable tissue at the time of death (N=8), PCR was performed using primers flanking the *Rosa26* or *Wt1* CRISPR gRNA site, and mutant reads were quantified by digital sequencing. Shown is the proportion of wild type to mutant *Rosa26* (left) or *Wt1* (right) reads in each sequenced sample. These APLs all contained cells without *Wt1* mutations, suggesting that these cells were not selected for in this model system. (C) Survival of mice (N=15-18 recipients in each group) transplanted with BM from *Ctsg-PML-RARA*$^{+/-}$ knock-in mice genetically altered using CRISPR/Cas9 to create indels in *Wt1* (blue) or control mutations in *Rosa26* (red). The log rank test for difference in survival gives a p-value of $p = 0.47$. (D) RNA expression of selected transcription factors in 16 previously banked mouse APL tumors. Red box highlights *Wt1* expression.



Supplementary Figure 3

E.



Supplementary Figure 3. Human CD34+ cells transduced with *PML-RARA* have a growth advantage in long term culture, and phenotypically resemble APL cells. Umbilical cord blood-derived CD34+ cells were transduced with a GFP-tagged retrovirus expressing *PML-RARA* and cultured in cytokines for up to 8 weeks. (A) Expansion of *PML-RARA* (right) or empty vector-transduced (left) human CD34+ cells *in vitro*. Shown is percent of GFP+ cells in culture versus time. Black dotted lines show line of best fit calculated by linear regression. Transduction with *PML-RARA* leads to expansion of GFP+ cells (slope b=1.3% per day, p<0.001), while empty vector-transduced cells gradually decrease in culture (slope b=-0.05, p<0.001). P values were calculated using a linear regression model, and represent the probability that the slope of the best fit line equals zero. (B) Representative flow cytometry plots showing GFP positive and negative populations in a culture of *PML-RARA*-transduced CD34+ cells maintained in culture for 5 weeks (top plot). Other plots show KIT vs GFP, CD13 vs CD11b, CD33 vs CD14, CD56 vs HLA-DR, and CD18 vs empty channel gated on the GFP positive (right) or GFP negative (left) populations. (C) Representative photomicrographs of CD34+ cells expressing *PML-RARA* (bottom) or empty vector (top) after 6-8 weeks of culture.

Empty vector-transduced cells display a full range of maturing myeloid cells (top), while cultures with *PML-RARA*-transduced cells (bottom) contain predominantly promyelocytic cells with primary granules overlying the nucleus, consistent with a myeloid maturation arrest. (D) *PML-RARA*-transduced CD34+ cells are sensitive to all-trans retinoic acid (ATRA) *in vitro*. PML-RARA-transduced CD34+ cells were expanded 6 weeks in culture and then treated *in vitro* for up to three days with ATRA or vehicle. Shown is total cell number on each day of treatment. (E) RNA sequencing showing relative level of WT1 expression 7 days after lentiviral transduction of CD34+ cells with KTS+ isoform of WT1 ("WT1", green bar) compared to cells transduced with PML-RARA or empty vector ("PR" and "GFP", red and blue bars). N=2 experiments each construct. $p<0.05$ in both comparisons comparisons by Student T test. TPM, transcripts per kilobase million.