Supplementary data:

Novel Inhibitors of the Histone-Methyltransferase DOT1L Show Potent Antileukemic Activity in Patient-derived Xenografts.

Figure S1

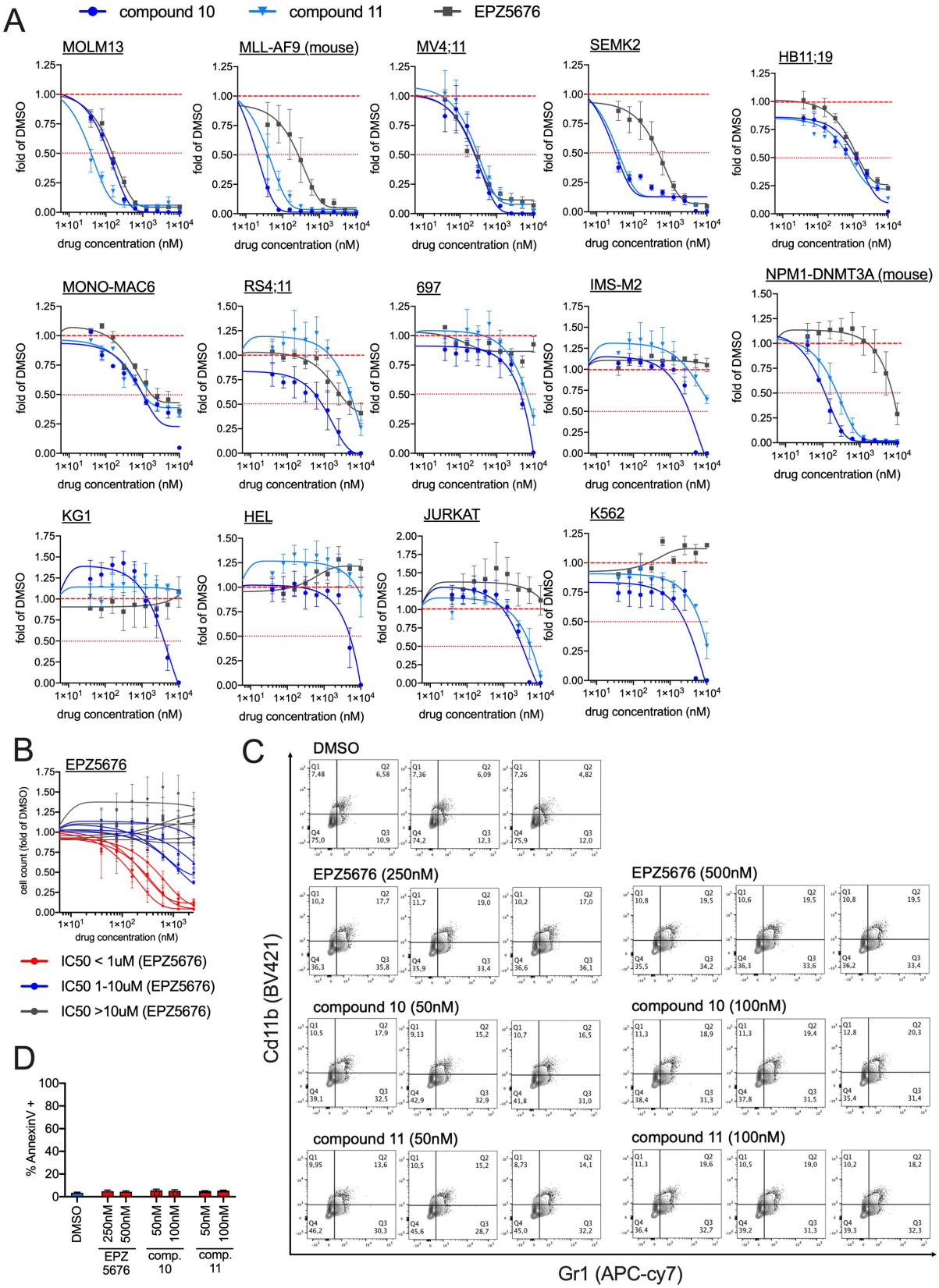

Figure S1:

A) Single cell line plots of dose-response curves in 14 leukemia lines treated with EPZ5676, compound 10 and compound 11 by counting of viable cells using flow cytometry (dead cell exclusion by DAPI-staining). **B)** Dose-response curves of cell lines towards EPZ5676-sensitive (red), -intermediate (blue) und -insensitive (gray) cell lines. **C)** FACS contour plots showing changes in CD11b and Gr1 expression in mouse MLL-AF9 leukemia cells after treatment with DOT1L inhibitors (corresponding statistics shown in Figure 1C). Plots were generated using Flowjo v.10. **D)** Quantification of apoptosis induction using AnnexinV/DAPI staining. Measured is the percentage of AnnexinV+ cells among the cells that are DAPI- to accurately quantify specific induction of apoptosis while excluding cells that underwent non-apoptotic cell death.

Figure S2

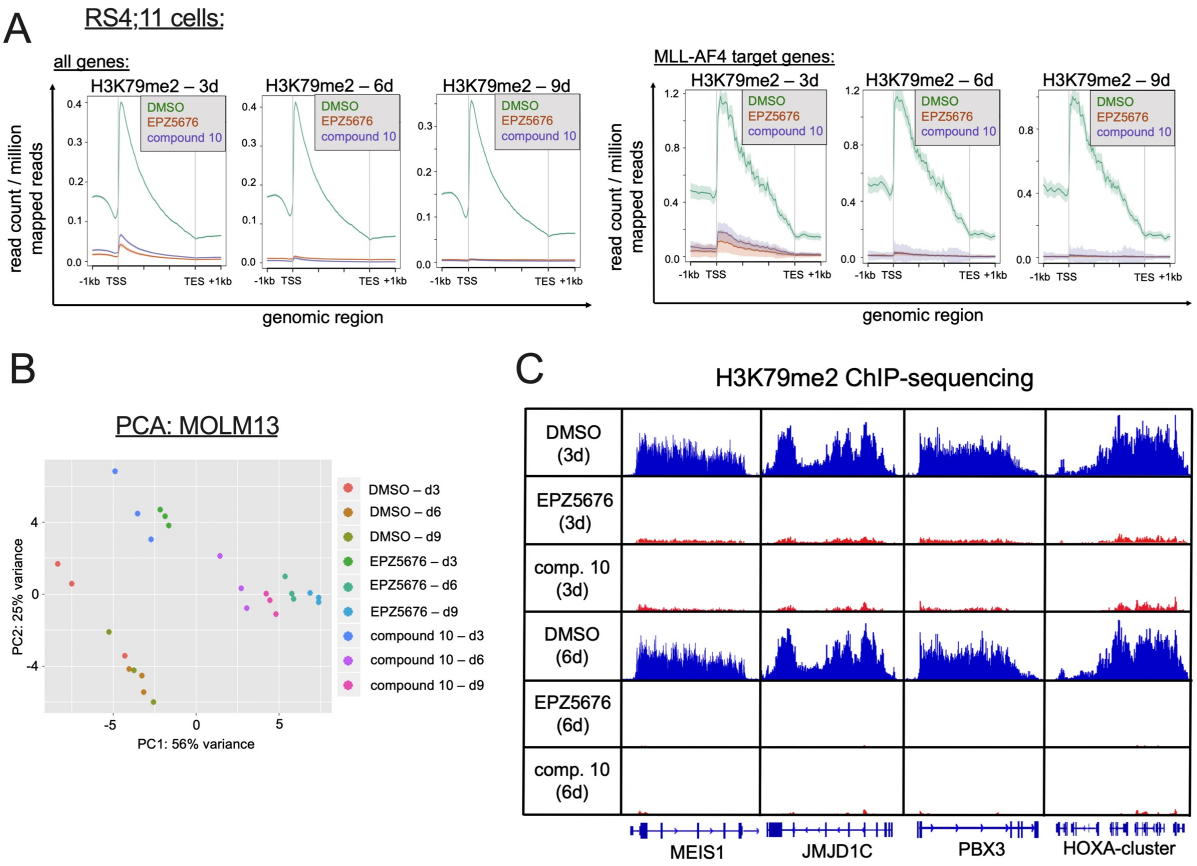

Figure S2:

A) Meta-plots of H3K79me2-ChIPseq in RS4;11 cells after treatment with EPZ5676 (1µM), compound 10 (100nM) or DMSO over the gene body of all protein coding genes (top) and MLL-AF9 target genes (bottom). ChIP-sequencing signal was normalized using Drosophila chromatin spike-in (dm6). **B)** Principle component analysis (PCA) of RNA sequencing after compound 10, EPZ5676 or DMSO in MOLM13 cells. Corresponding to the data shown in Figure 1H, I, and J. **C)** ChIP-sequencing signal of H3K79me2 at selected MLL-AF9-target loci 3d and 6d after inhibition of DOT1L with EPZ5676 (1µM) or compound 10 (100nM). ChIP-sequencing signal was normalized using Drosophila chromatin spike-in (dm6).

Figure S3

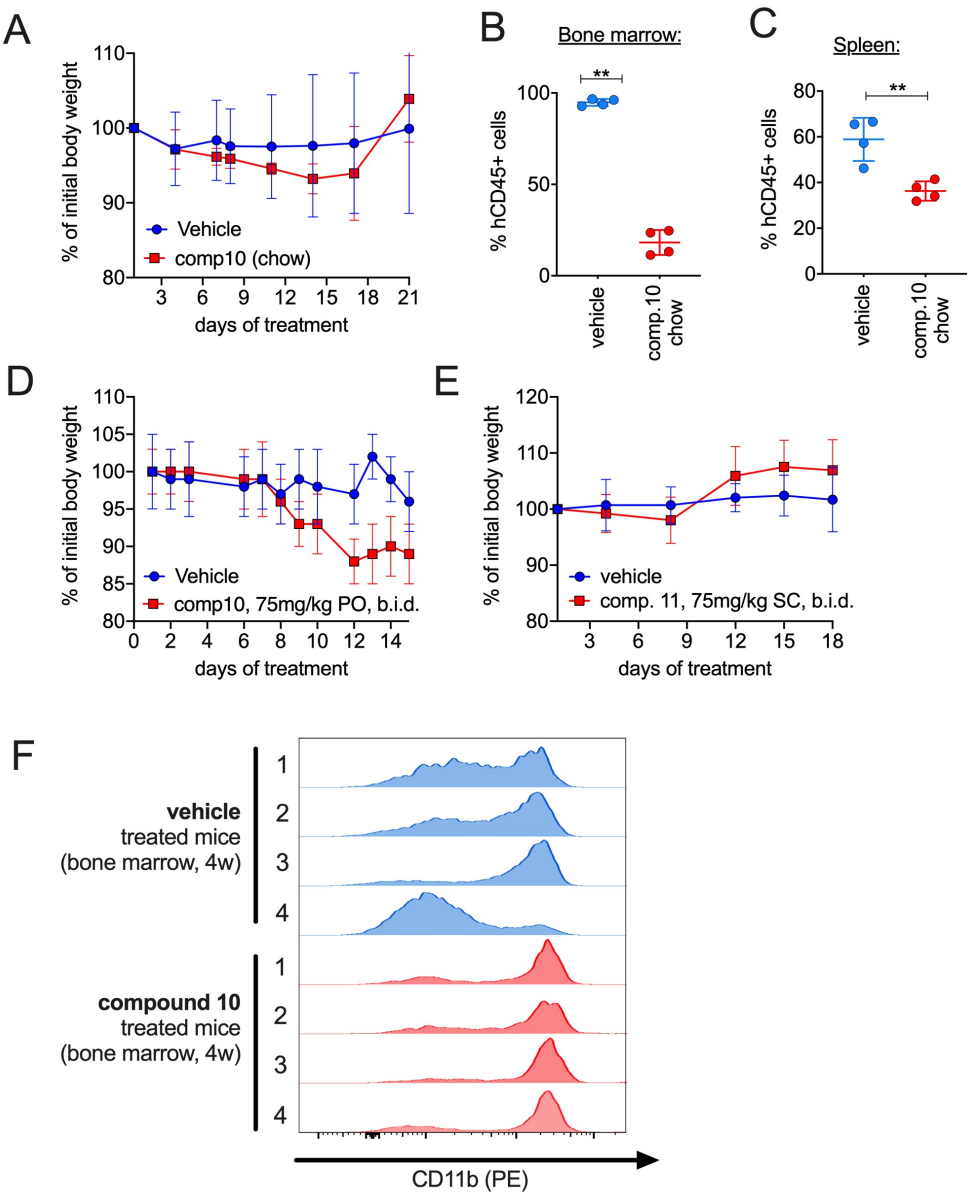

Figure S3:

A) Assessment of mouse body weights during compound 10 treatment via drug supplemented chow (0.05%) over the course of 3 weeks as a measure of tolerability. **B, C)** Detection of the percentage of human cells in the **B)** bone marrow and **C)** spleen of PDX#1 engrafted mice treated with compound 10 chow over the course of 4 weeks. **D, E)** Assessment of mouse body weights during **D)** compound 10 treatment via oral gavage (75mg/kg, BID) or **E)** compound 11 via subcutaneous injections (75mg/kg, BID). Subcutaneous administration resulted in local toxicity at the injection sites both in the compound 11 as well as the vehicle treated animals. Therefore, the application was switched to intraperitoneal administration for further experiments which was better tolerated. **F)** Histograms of CD11b expression in mice transplanted with PDX1 and treated with compound 10 for 4 weeks. Measurements shown here correspond to the data points shown in Figure 2L.