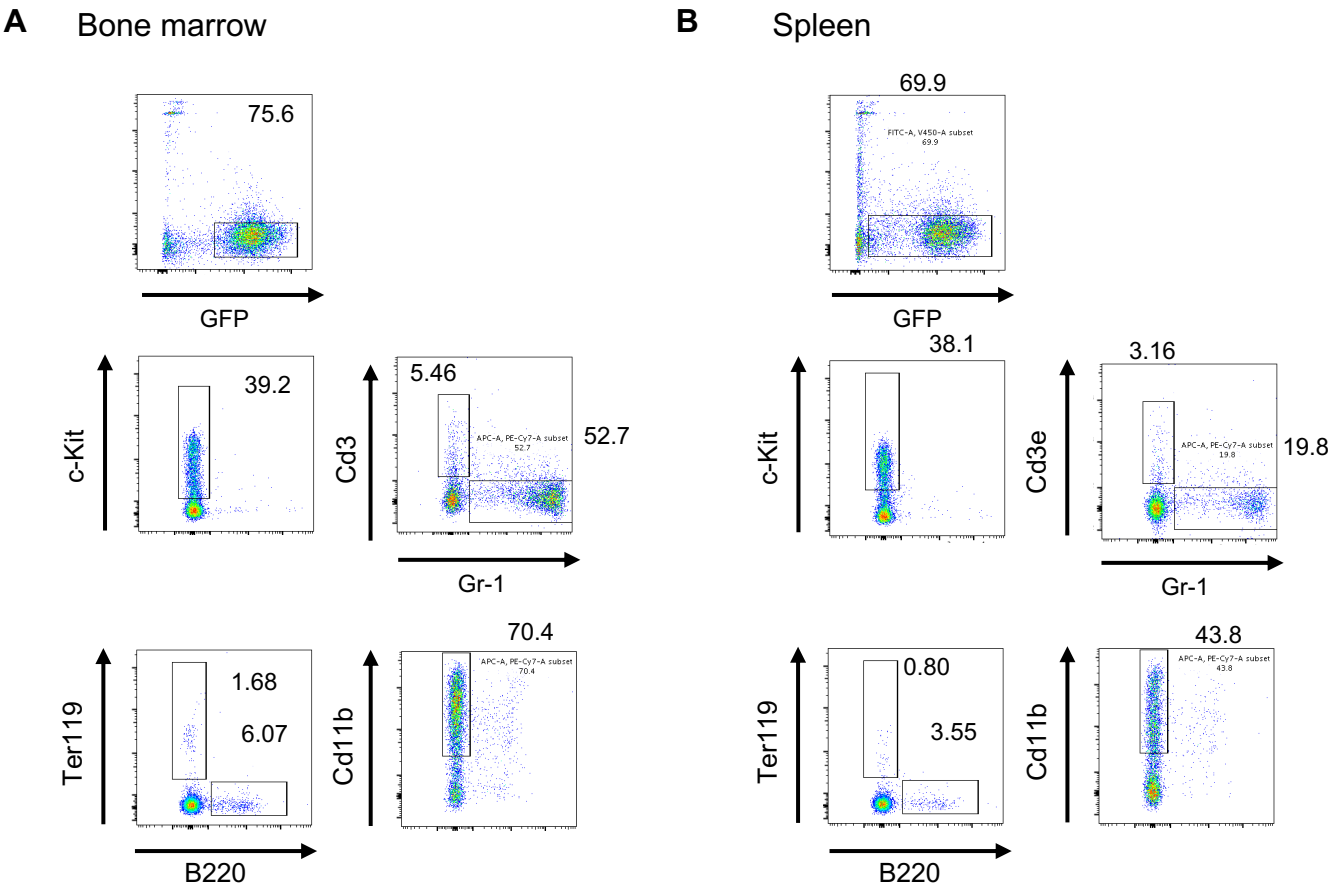
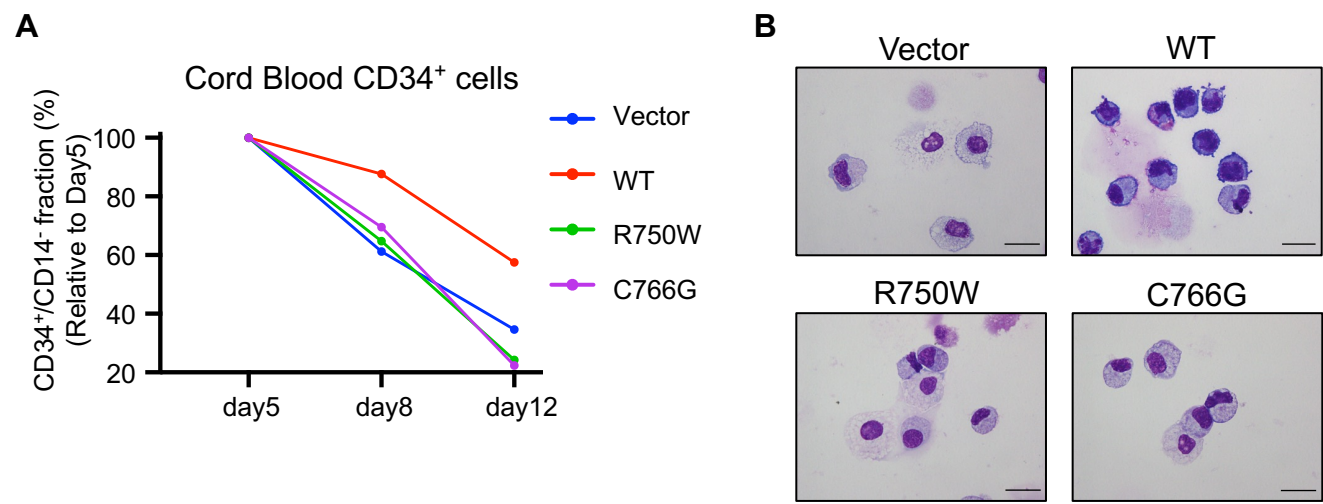


Supplementary Figure 1



Flow cytometric profiles of MECOM-driven leukemia.
A, B. FACS analysis of GFP⁺ (MECOM-transduced) cells in bone marrow (**A**) and spleen (**B**).

Supplementary Figure 2

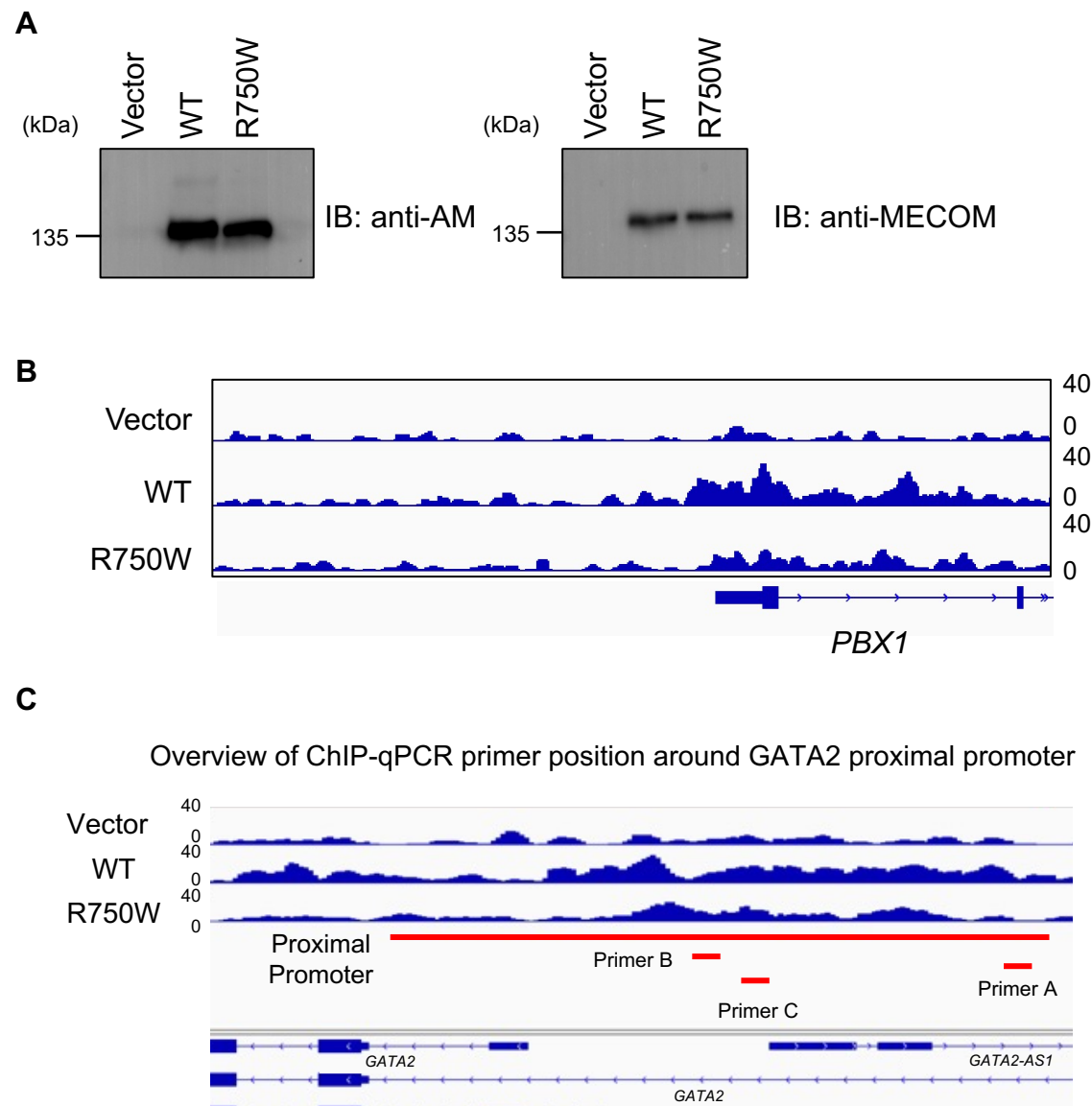


Effects of wild-type and mutant MECOM on self-renewal of cord blood cells.

A. Human cord blood (CB) CD34⁺ cells were transduced with vector, wild-type (WT) or mutant (R750W, C766G) MECOM coexpressing GFP. The cells were cultured in myeloid skewing medium and analyzed by FACS to measure the frequency of CD34⁺/CD14⁻ cells. See also Figure 2H.

B. Representative images of CB cells transduced with the indicated construct at day12.

Supplementary Figure 3

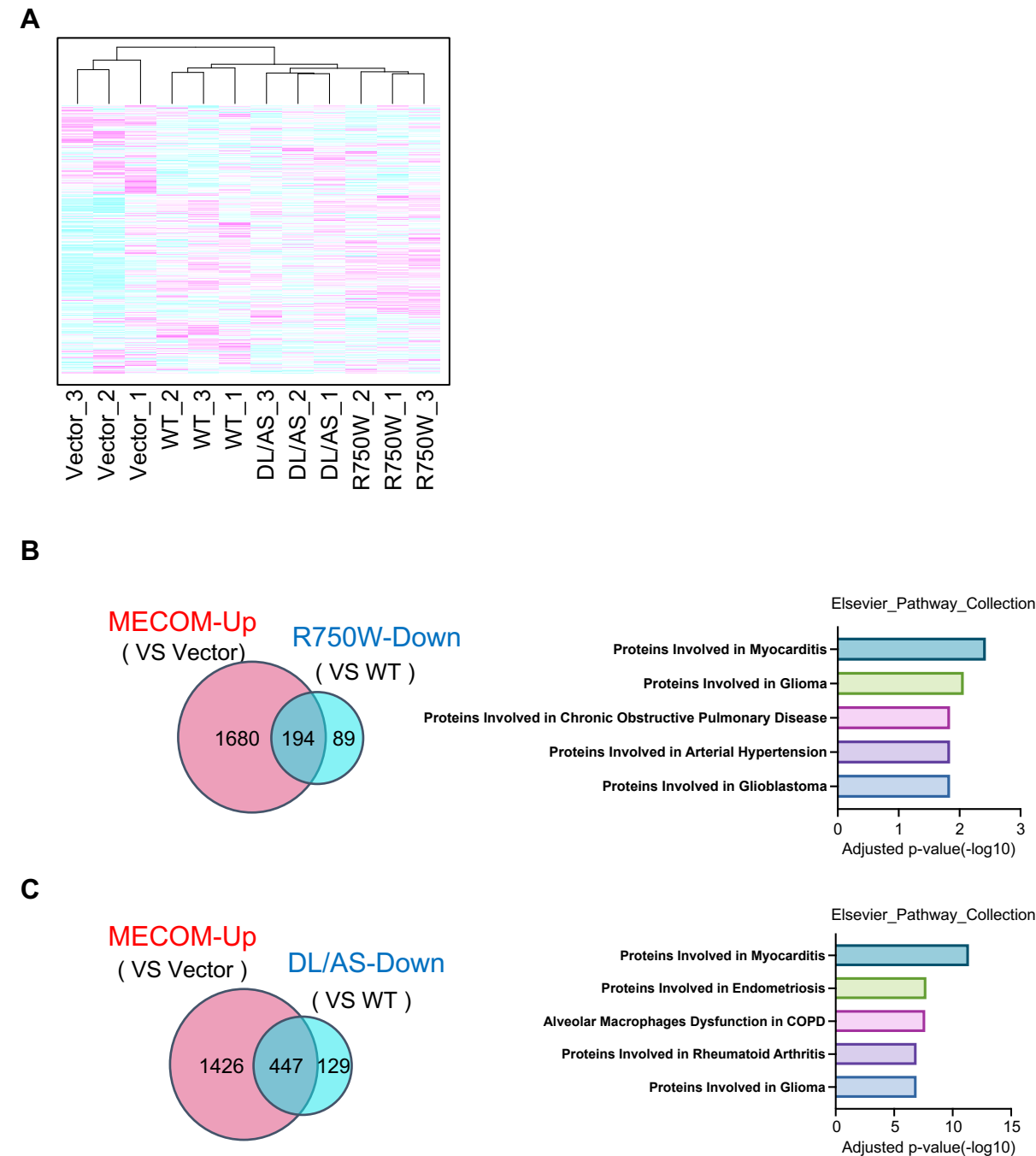


ChIP-seq analysis of 293T cells expressing wild-type (WT) MECOM or MECOM-R750W.

A. Expression of AM-tagged MECOM-WT and MECOM-R750W was confirmed by Western blotting. **B.** Visualization of MECOM-WT and MECOM-R750W bound to the *PBX1*.

C. Scheme of the position of the primers (Primer A, B, C) used for the ChIP-qPCR for the *GATA2* proximal promoter.

Supplementary Figure 4



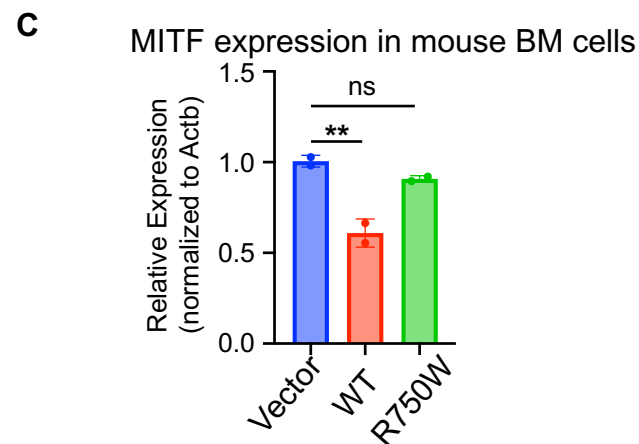
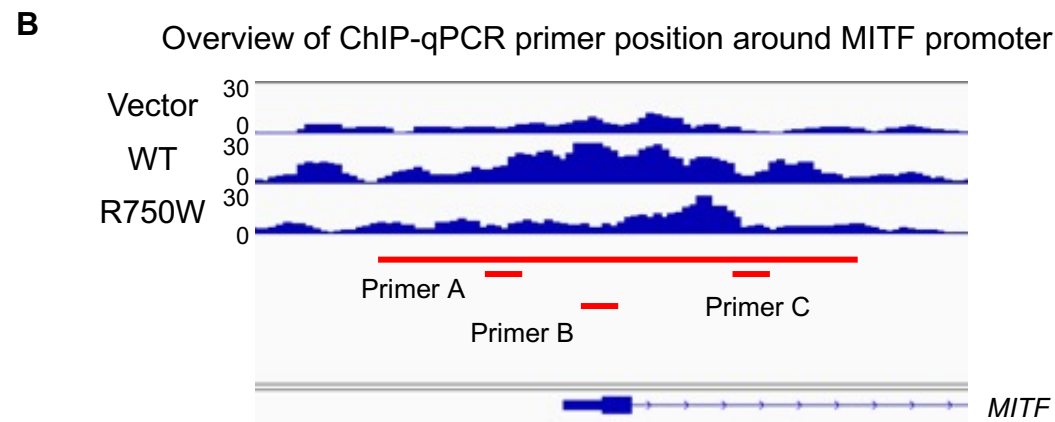
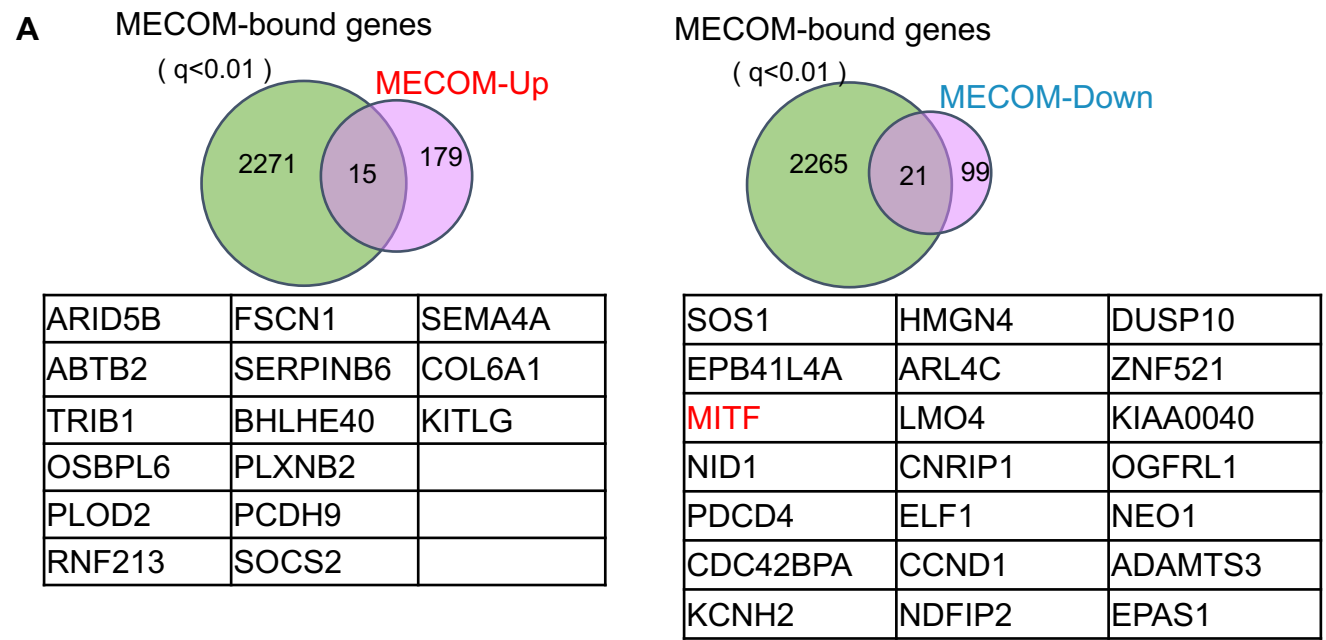
RNA-seq analysis of wild-type and mutant MECOM-transduced human cord blood cells.

A. Hierarchical clustering of the RNA-Seq data from human cord blood (CB) cells transduced with vector, wild-type (WT) or mutant (DL/AS, R750W) MECOM. n = 3 for each group.

B. (Left) Venn diagram showing the genes upregulated in MECOM-WT transduced CB cells compared to vector-transduced CB cells (red circle) and genes downregulated in MECOM-R750W transduced CB cells compared to MECOM-WT transduced CB cells (blue circle). Genes with FDR<0.05 were considered as up- or down-regulated genes. (Right) The 194 overlapping genes were used for enrichment analysis using Elsevier Pathway Collection.

C. (Left) Venn diagram showing the genes upregulated in MECOM-WT transduced CB cells compared with vector-transduced CB cells (red circle) and genes downregulated in MECOM-DL/AS transduced CB cells compared with MECOM-WT transduced CB cells (blue circle). Genes with FDR<0.05 were considered as up- or down-regulated genes. (right) The 447 overlapping genes were used for enrichment analysis using Elsevier Pathway Collection.

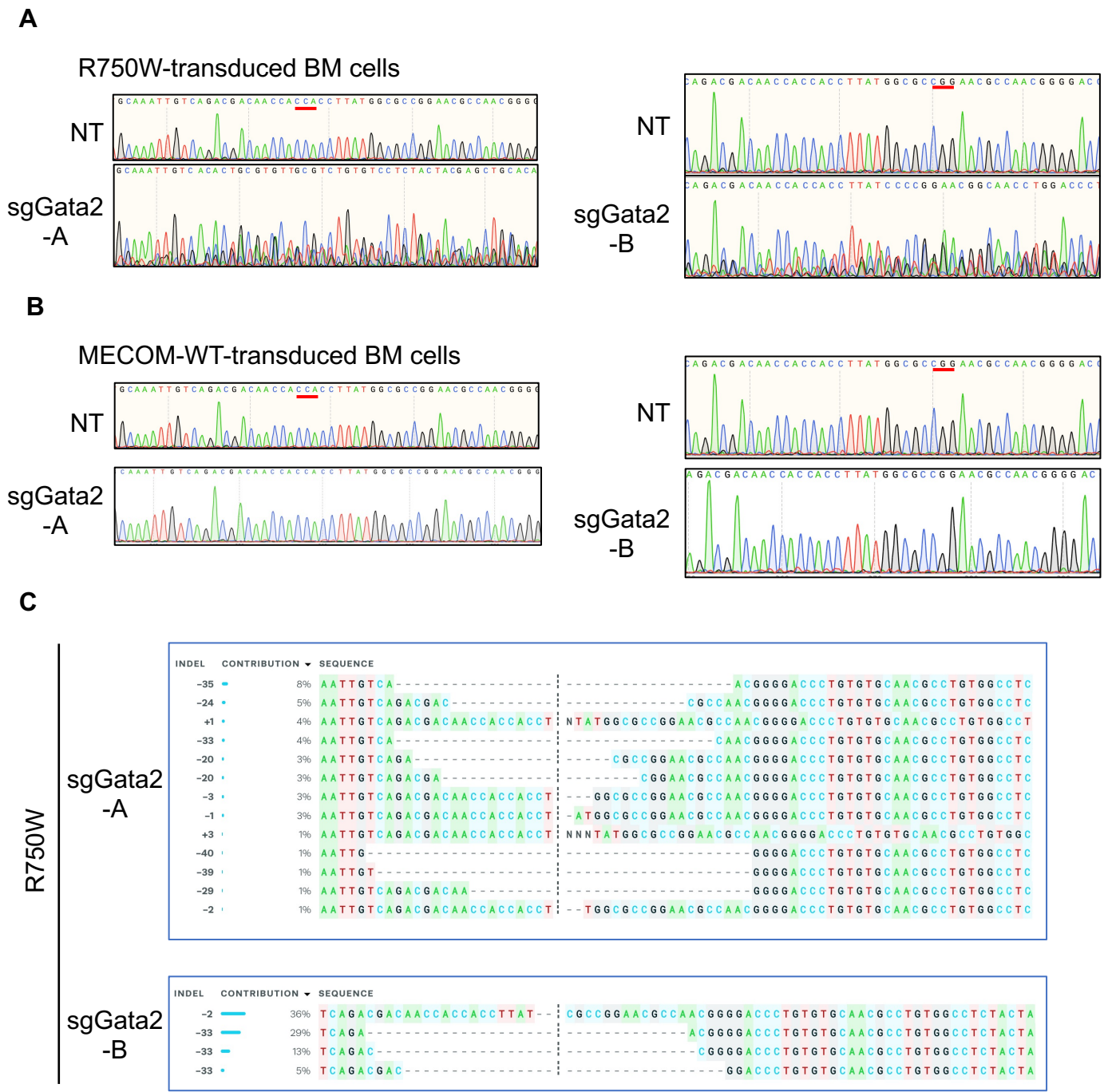
Supplementary Figure 5



Identification of MECOM target genes.

A. Venn diagram showing the overlap between 2286 genes bound by MECOM (green circle) and 194 genes upregulated by MECOM (red circle: left) or 120 genes downregulated by MECOM (red circle: right). The 15 MECOM-activated and 21 MECOM-repressed genes are shown in the lists. **B.** Scheme of the position of the primers (Primer A, B, C) used for the ChIP-qPCR for the MITF promoter. **C.** Mouse bone marrow c-Kit⁺ cells were transduced with vector, MECOM-WT or MECOM-R750W and were cultured in semi-solid medium. RNA was extracted on day 8 and Mitf levels were analyzed by qRT-PCR. Results were normalized to Actb, with the relative mRNA level in vector-transduced cells set to 1. Data are presented as mean with SD of two biologically independent experiments. ** $P < 0.01$, one-way ANOVA with Dunnett's multiple comparisons test.

Supplementary Figure 6

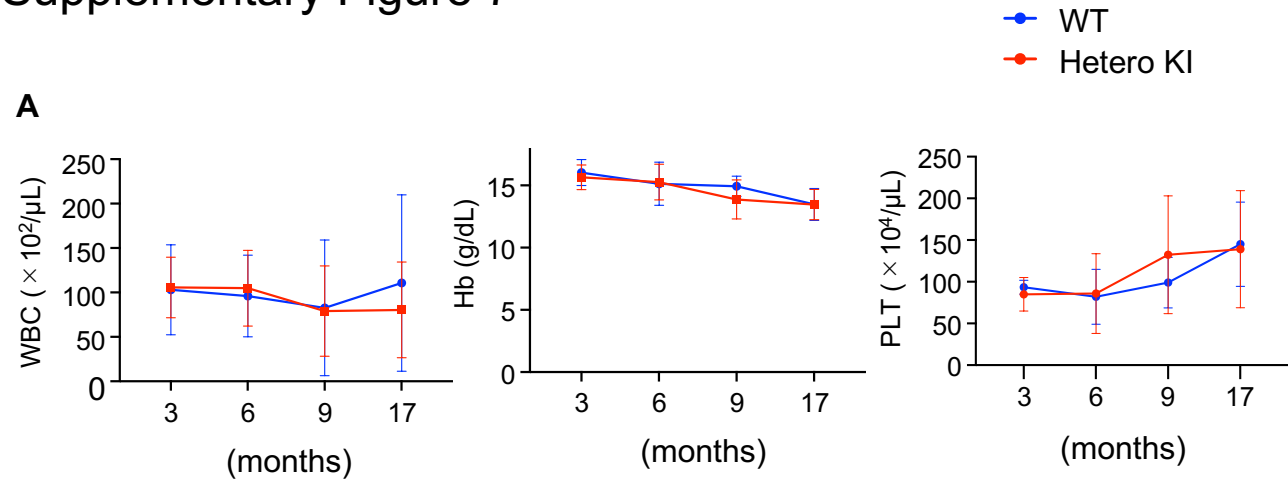


Confirmation of Gata2 depletion in MECOM-R750W transduced cells

A, B. DNA was extracted from Cas9⁺ mouse bone marrow cells transduced with wild-type MECOM (**A**) or MECOM-R750W (**B**) together with non-targeting (NT) or two independent Gata2-targeting sgRNAs (sgGata2-A or sgGata2-B). Sequences near the PAM sequence (red line) were evaluated. Note that R750W/sGata2-transduced cells show the mixture of several different sequences, while other cells show only a single sequence.

C. Sequence results of the R750W/sGata2-transduced cells were submitted to the Synthego website (<https://www.synthego.com/>), showing various indels in the *Gata2* gene.

Supplementary Figure 7



Peripheral blood analysis of MECOM^{WT/ R751W} mice.

A. White blood cells (WBC), hemoglobin (Hb), and platelets (PLT) were measured at 3, 6, 9, and 17 months of age. Data are presented as mean with SD (WT: n = 9, MECOM^{WT/ R751W} mice: n = 13 at 3 and 6 months. WT: n = 9, MECOM^{WT/ R751W} mice: n = 12 at 9 months. WT: n = 7, MECOM^{WT/ R751W} mice: n = 10 at 17months.).