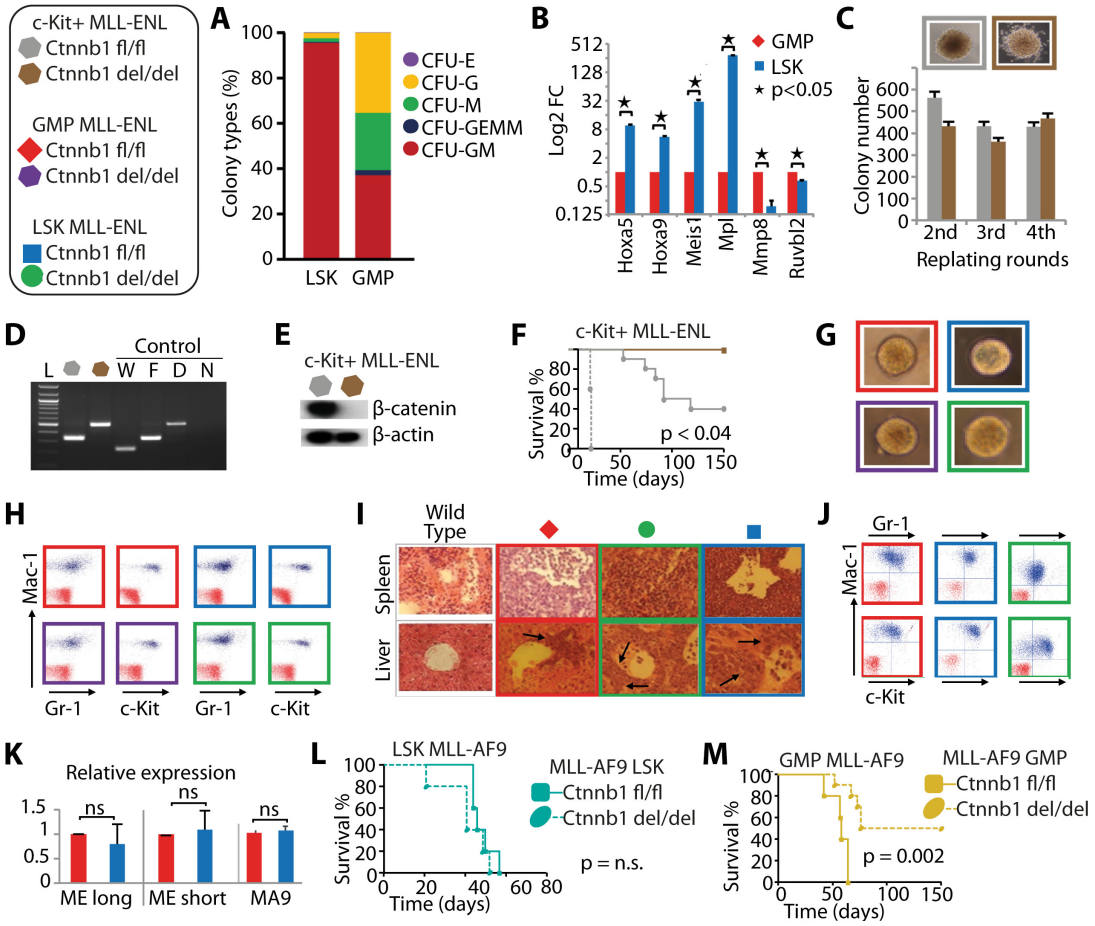
**Appendix – table of contents:**

# Appendix figures




**Appendix Figure S1 related to Figure 1.** Keys and colour codes in the top left corner indicate the origin and the β-catenin status of MLL-ENL transduced cells as well as the controls used throughout this figure. **(A)** FACS-sorted LSK and GMPs were plated in semi-solid medium and incubated for 14 days, after which colony types were scored (%). Typical colony morphologies displayed to the right. **(B)** qRT-PCR with RNA isolated from FACS-sorted normal LSKs and GMPs using primers specific for the indicated genes. Data are represented as mean ± SD. **(C)** Colony numbers in serial replating assay of MLL-ENL transduced c-Kit+ cells, and images of typical 4$^{th}$ round colonies. **(D)** PCR validation of Ctnnb1 deletion on genomic DNA isolated from indicated MLL-ENL transduced c-Kit+ cells. **(E)** Cell lysates from indicated MLL-ENL transduced cells after the fourth round of plating were blotted with anti- $\beta$ -catenin (top) and anti-actin (bottom) antibodies. **(F)** Kaplan-Meier survival curve of indicated c-kit+ MLL-ENL cells transplanted into primary recipient (solid lines) and secondary recipient mice (dotted lines). **(G)** Colony morphology of indicated GMP-MLL-ENL and LSK-MLL-ENL transduced cells after the 3$^{rd}$ round of plating. **(H)** Immunophenotypic analysis of MLL-ENL transformed cells of different cellular origins and genetic backgrounds. Unstained cells are shown in red and stainings obtained with antibodies against surface markers are shown in blue. **(I)** hematoxylin and eosin (H&E) stained spleen and liver sections from wild type or leukemic mice

injected with the indicated MLL-ENL transduced GMP and LSK cells, black arrows indicate infiltrated myeloid blasts. **(J)** Immunophenotypic analysis of leukemic mice. Unstained cells are shown in red and stainings obtained with antibodies against surface markers are shown in blue. **(K)** qRT-PCR with RNA isolated from GMP-MLL-ENL cells with the indicated Ctnnb1 genotypes using primers specific for the MLL-ENL long form (left), MLL-ENL short form (middle) or MLL-AF9 (right) normalized to Gapdh. Data are represented as mean ± SD. N=3, two-tailed t-test was performed. **(L, M)** Kaplan-Meier survival curve of indicated LSK (L) or GMP MLL-ENL (M) cells transplanted into primary recipient.

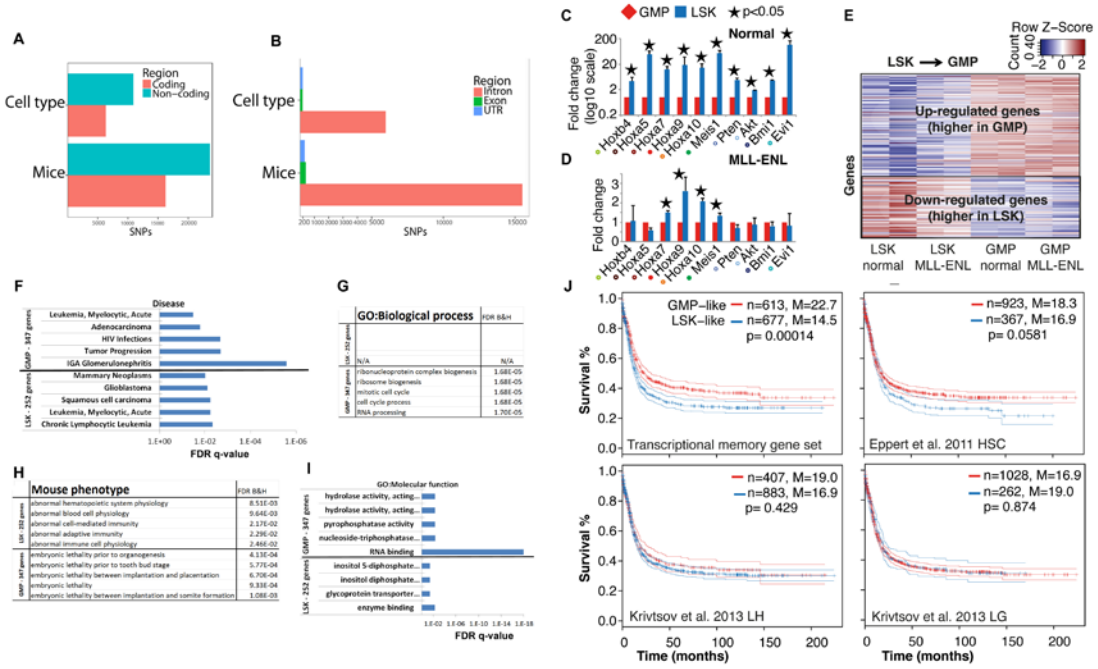



**Figure S2 related to figure 2. (A, B)** The unique SNPs present within cell type or mice (as indicated) are divided into coding and non-coding regions (A). The coding region is further divided into exon, intron and UTR regions (B). **(C, D)** The bar charts show the number of SNPs falling into these regions for cell type and mice a indicated.q-RT-PCR validation of selected genes normalized to mGAPDH (GMP expression set to 1). Normal GMP and LSK are shown in (C) LSK-MLL-ENL and GMP-MLL-ENL are shown in (D). Data are represented as mean ± SD. N=3, two-tailed t-ted was performed. **(E)** Heatmap of transcriptional memory candidate genes (after conversion from mouse genes to human orthologs). **(F-I)** Toppgene functional annotation (https://toppgene.cchmc.org/enrichment.jsp)analysis was carried out for the 252 gene LSK- and the 347 gene GMP transcriptional memory signatures. Four different categories, “Disease” (F), “GO:Biological process” (G), “mouse phenotype” (H) and “GO:Biological function” (I) are shown. The top 5 associations and only those results with an FDR q-value < 0.05 are reported. **(J)** Comparison of the transcriptional memory signature (Fig. 2E) with published candidate signatures. For several patient datasets, patients (n=1290) were classified by k-means clustering as high or low similarity to the indicated signal, and the survival differences between the patient groups analyzed. For each patient group, (M) the median survival (in months), (n) number of patients and (p) statistical significance is indicated. The dotted lines indicate 0.95% confidence intervals.

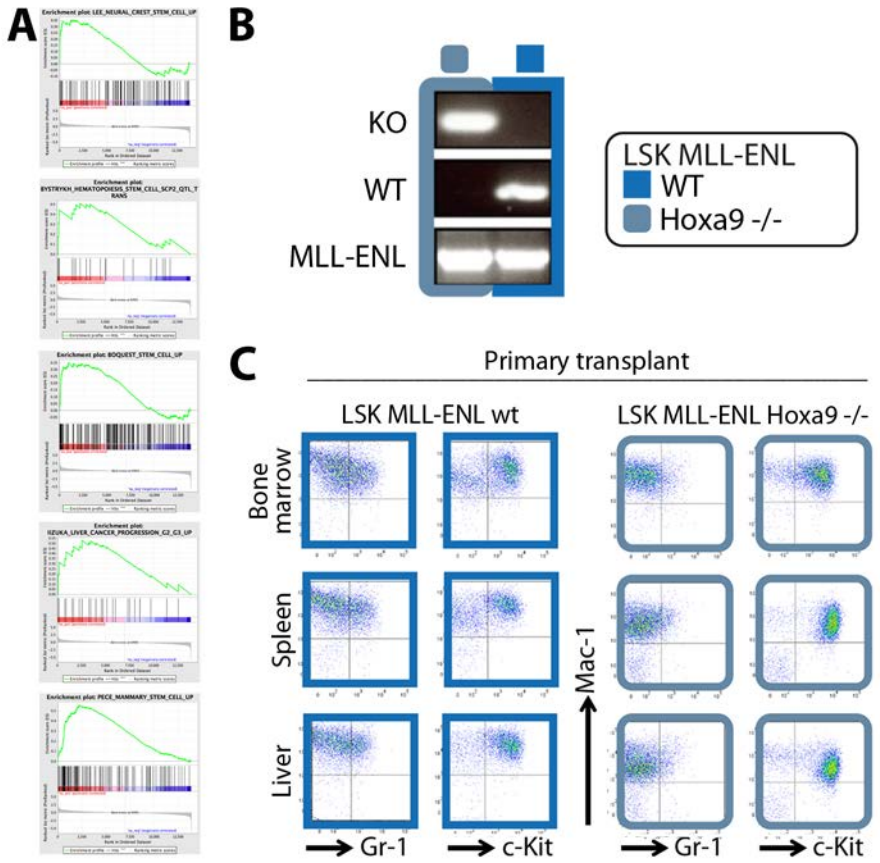



**Figure S3 related to figure 3. (A)** GSEA plots of the indicated 'stem_cell' gene sets positively enriched in LSK-MLL-ENL compared to GMP-MLL-ENL upon b-catenin inactivation. **(B)** PCR validation of Hoxa9 deletion in indicated LSK-MLL-ENL cells. **(C)** Immunophenotypic analysis of indicated tissues of primary/secondary LSK-MLL-ENL wt and Hoxa9$^{-/-}$ leukemic mice.

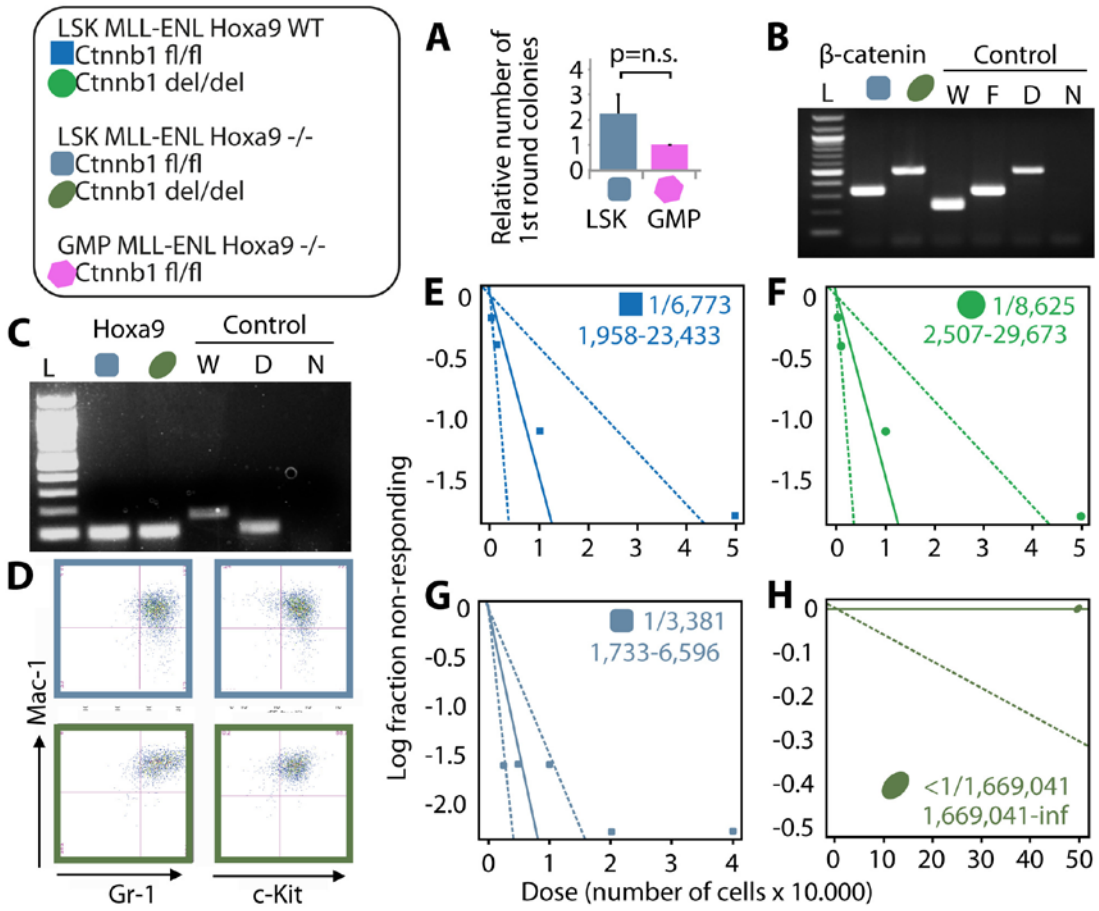



**Figure S4 related to figure 4.** Keys and colour codes in the left hand corner indicate the origin and the β-catenin status of MLL-ENL transduced cells **(A)** Number of first round colonies obtained of indicated MLL-ENL transduced LSKs and GMPs isolated from Hoxa9-/- Ctnnb1fl/fl mouse bone marrow. Data are represented as mean ± SD. N=3, two-tailed t-test was performed **(B, C)** PCR validation of Ctnnb1 (B) and Hoxa9 (C) genotypes on genomic DNA isolated from the indicated cells. Controls and expected bands are indicated. W, wild type; F, floxed; D, deleted; N, negative. **(D)** Immunophenotypic analysis of LSK-MLL-ENL Hoxa9$^{-/-}$Ctnnb1$^{fl/fl}$ with or without 72h Tamoxifen treatment. **(E-H)** The solid lines in the graphs show the estimated frequencies of LSC in the indicated cells obtained by in vivo limiting dilution analysis. The dotted lines represent the 95% confidence intervals.

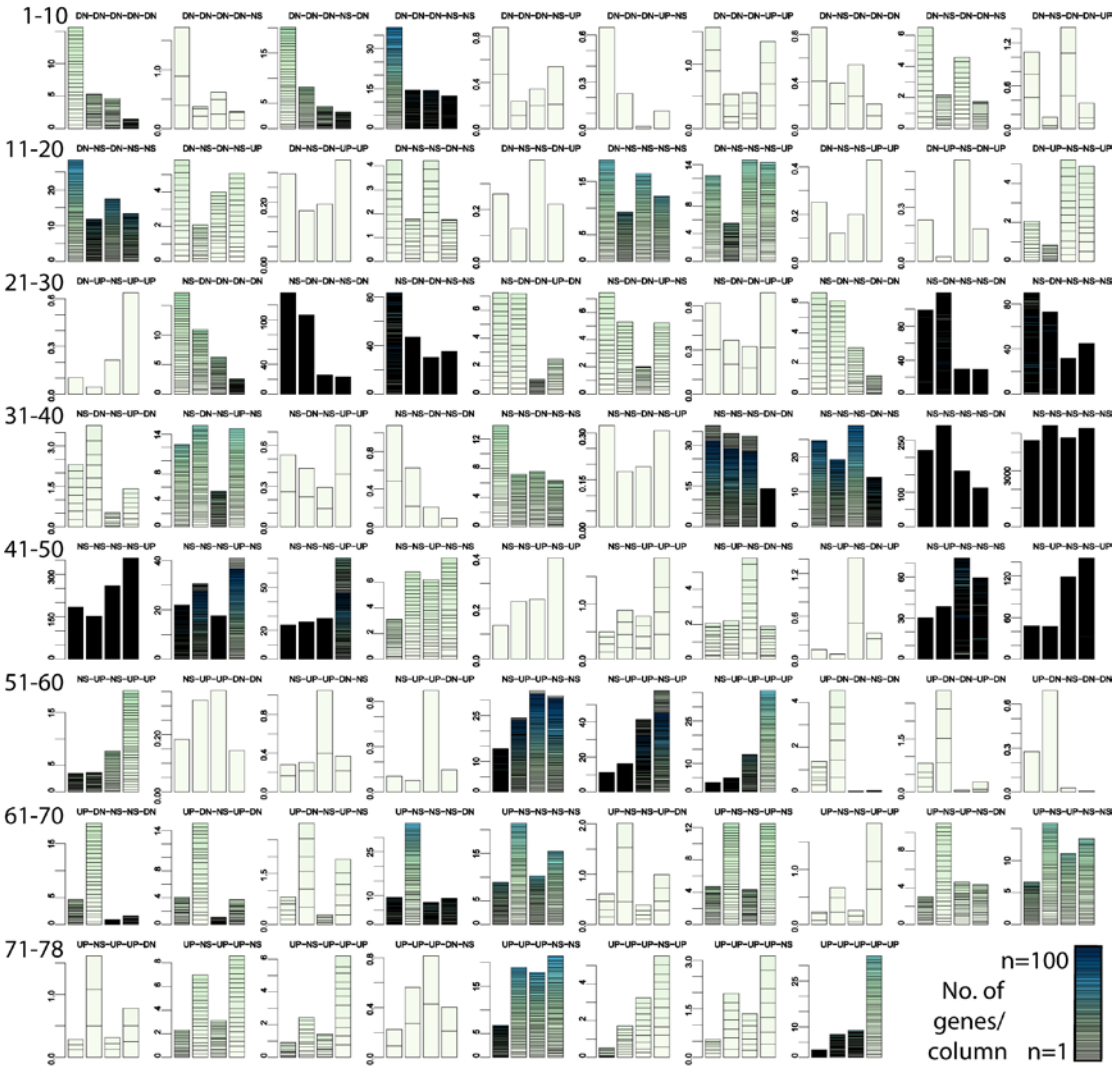



**Figure S5 related to figure 5.** Genes which show, or do not show a synergistic effect of simultaneous knockout of Cbnnt1 and Hoxa9, all genes are grouped based on 5 differential expression comparisons (1. LSK WT to Ctnnb1 KO, 2. LSK WT to HoxA9 KO, 3. Additive effect LSK WT to Ctnnb1 KO and Hoxa9 KO, 4. LSK Ctnnb1 KO to Ctnnb1:HoxA9 interaction and 5. LSK HoxA9 KO to Ctnnb1:HoxA9 interaction). Selected results are illustrated in Fig. 5C, and full results illustrated above. For each graph, results have been normalized per gene. The four columns in each graph represents library-normalised expression values of: Wild type (Ctnnb1 fl/fl), Ctnnb1 del/del, Hoxa9 -/- , both Ctnnb1 del/del and Hoxa9 -/-. More detailed results are in Supplementary Table S4C. For each graph, "Graph number", "n=Number of genes": 1 n=27, 2 n=3, 3 n=36, 4 n=79, 5 n=2, 6 n=1, 7 n=4, 8 n=2, 9 n=15, 10 n=3, 11 n=71, 12 n=17, 13 n=1, 14 n=12, 15 n=1, 16 n=57, 17 n=47, 18 n=1, 19 n=1, 20 n=13, 21 n=1, 22 n=37, 23 n=292, 24 n=196, 25 n=18, 26 n=20, 27 n=2, 28 n=17, 29 n=277, 30 n=240, 31 n=8, 32 n=48, 33 n=2, 34 n=2, 35 n=35, 36 n=1, 37 n=119, 38 n=87, 39 n=788, 40 n=38440, 41 n=953, 42 n=111, 43 n=149, 44 n=24, 45 n=1, 46 n=4, 47 n=12, 48 n=2, 49 n=202, 50 n=360, 51 n=34, 52 n=1, 53 n=2, 54 n=1, 55 n=103, 56 n=127, 57 n=57, 58 n=6, 59 n=4, 60 n=1, 61 n=26, 62 n=24, 63 n=6, 64 n=61, 65 n=56, 66 n=4, 67 n=34, 68 n=3, 69 n=23, 70 n=47, 71 n=3, 72 n=21, 73 n=11, 74 n=2, 75 n=65, 76 n=11, 77 n=7, 78 n=52. Combination of differential expression; DN=significantly down-regulated, UP=significantly up-regulated, NS=non-significant difference.

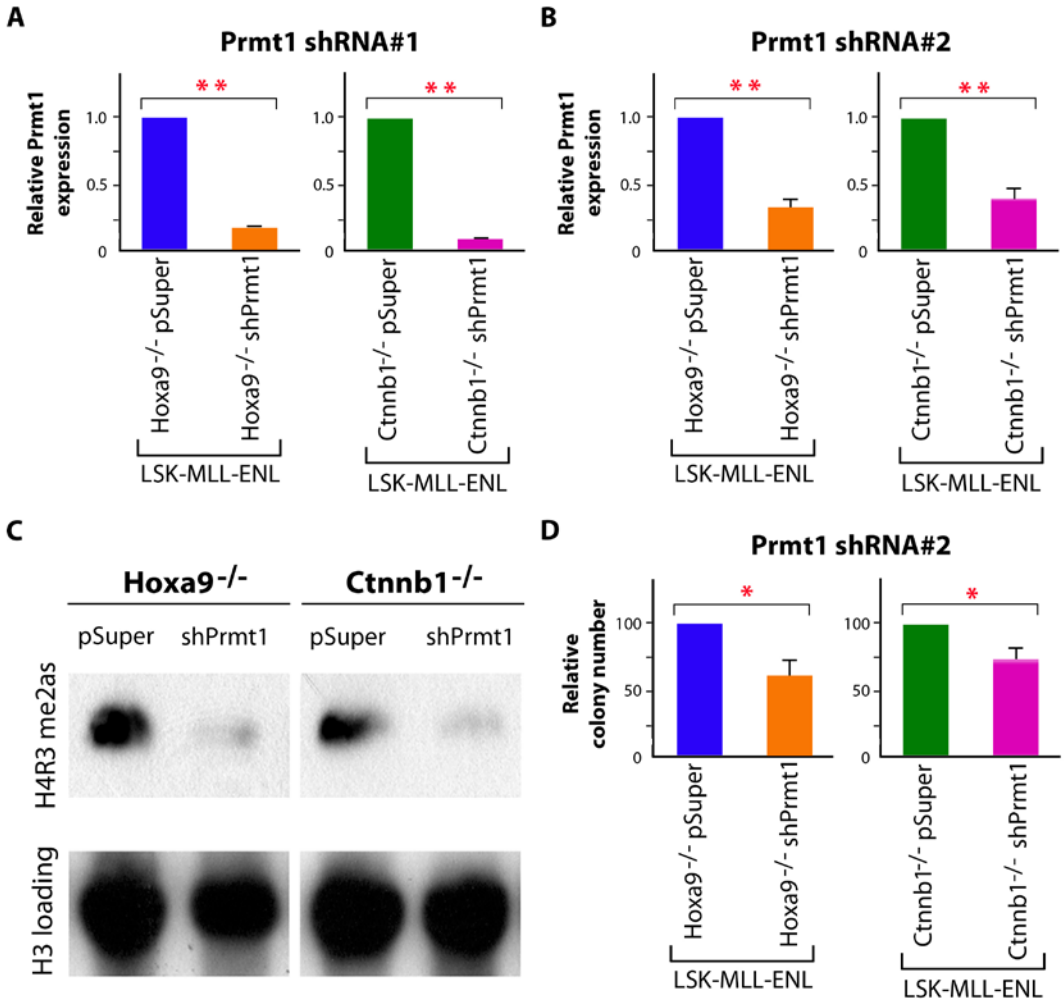



**Figure S6 related to figure 7.** (**A-B)** q-RT-PCR of Prmt1 (normalized to Gapdh) on RNA isolated from LSK-MLL-ENL cells with the indicated genotypes transduced with independent shRNA 1 (A) or shRNA 2 (B) against PRMT1 or control. Data are represented as mean ± SD. **(C)** Western blot showing suppression of H4R3me2as marks by shPrmt1 in Ctnnb1$^{-/-}$ or Hoxa9$^{-/-}$ LSK-MLL-ENL cells. **(D)** The relative number of colonies from LSK-MLL-ENL leukemic cells of the indicated genotypes transduced with vector control or PRMT1 shRNA 2. Data are represented as mean ± SD. Keys and colour codes indicate the origin, $\beta$ -catenin and Hoxa9 status of MLL-ENL transduced cells

**Appendix Table S1**

**q-RT-PCR**

Primer and probe sequences used for PCR throughout the manuscript:

| Gene | | sequence 5' to 3' | qPCR chemistry |
|---|---|---|---|
| Hoxb4 | forward primer | CTGGATGCGCAAAGTTCACGTG | Sybr green |
| | reverse primer | GCGTCAGGTAGCGATTGTAGTG | |
| Hoxa9 | forward primer | GCCTTCTCCGAAAACAATGCCG | Sybr green |
| | reverse primer | TTCCGAGTGGAGCGAGCATGTA | |
| Hoxa10 | forward primer | CTGTCTCCAGCCCCTTCAGAAA | Sybr green |
| | reverse primer | TCTGGTGCTTCGTGTAAGGGCA | |
| Meis1 | forward primer | GCAGTTGGCACAAGATACAGGAC | Sybr green |
| | reverse primer | ACTGCTCGGTTGGACTGGTCTA | |
| Pten | forward primer | TGAGTTCCCTCAGCCATTGCCT | Sybr green |
| | reverse primer | GAGGTTTCCTCTGGTCCTGGTA | |
| Akt | forward primer | GGACTACTTGCACTCCGAGAAG | Sybr green |
| | reverse primer | CATAGTGGCACCGTCCTTGATC | |
| Bmi1 | forward primer | ACTACACGCTAATGGACATTGCC | Sybr green |
| | reverse primer | CTCTCCAGCATTCGTCAGTCCA | |
| Evi1 | forward primer | CCTCCATGTTCAGCTTCCGAGC | Sybr green |
| | reverse primer | AAGTGCCGTGTTAGGTTCGCAG | |
| Prmt1 | forward primer | ACCCTCACATACCGCAACTC | Sybr green |
| | reverse primer | CAGCAAACATGCAGAGGATG | |
| Mpl | forward primer | TCACCTTGGTGACTGCTCTG | Sybr green |
| | reverse primer | CCACAAAGCATGCCTCAGTC | |
| Mmp8 | forward primer | CTTTCAACCAGGCCAAGGTA | Sybr green |
| | reverse primer | GAGCAGCCACGAGAAATAGG | |
| Ruvbl2 | forward primer | TCCGAGAAGGGAAGATTGC | Sybr green |
| | reverse primer | TGGCTGTGAATGGTGTGTC | |
| Hoxa5 | forward primer | TAGTTCCGTGAGCGAACAATTC | Taqman |
| | reverse primer | GCTGAGATCCATGCCATTGTAG | |
| | probe | CTCGGCGAGCATGCACTCCG | 5'FAM, 3'TAMRA |
| Hoxa7 | forward primer | ACGCGCTTTTTAGCAAATATACG | Taqman |
| | reverse primer | GGGTGCAAAGGAGCAAGAAG | |
| | probe | CTTCTCTCTTCCAAAATGCCGAGCCG | 5'FAM, 3'TAMRA |