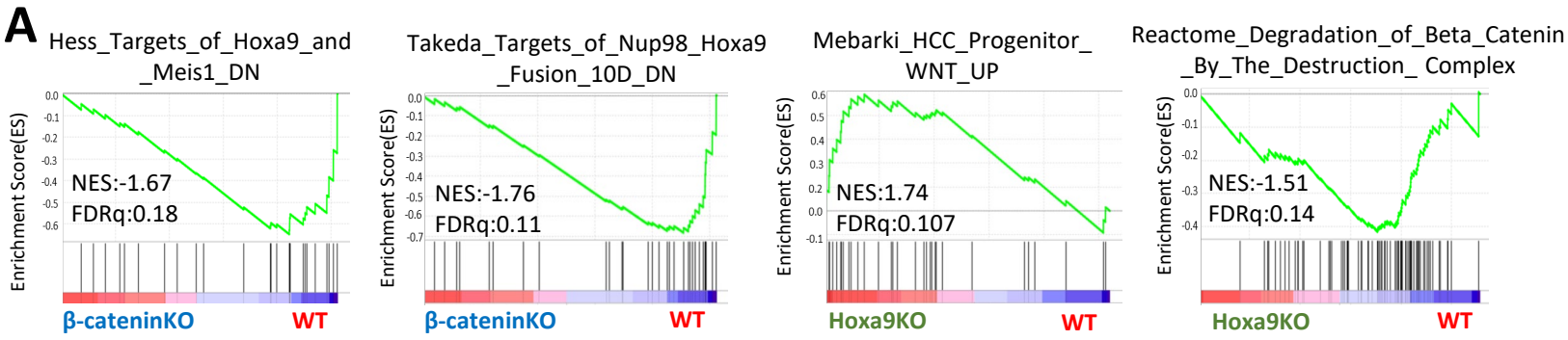
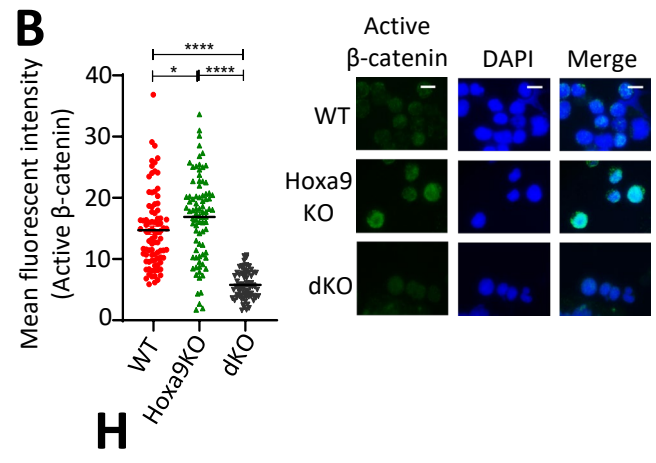
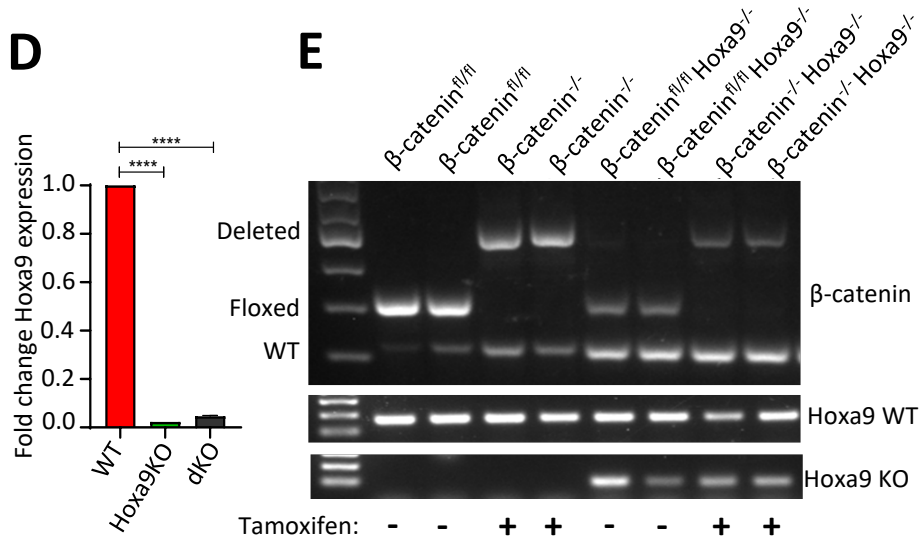
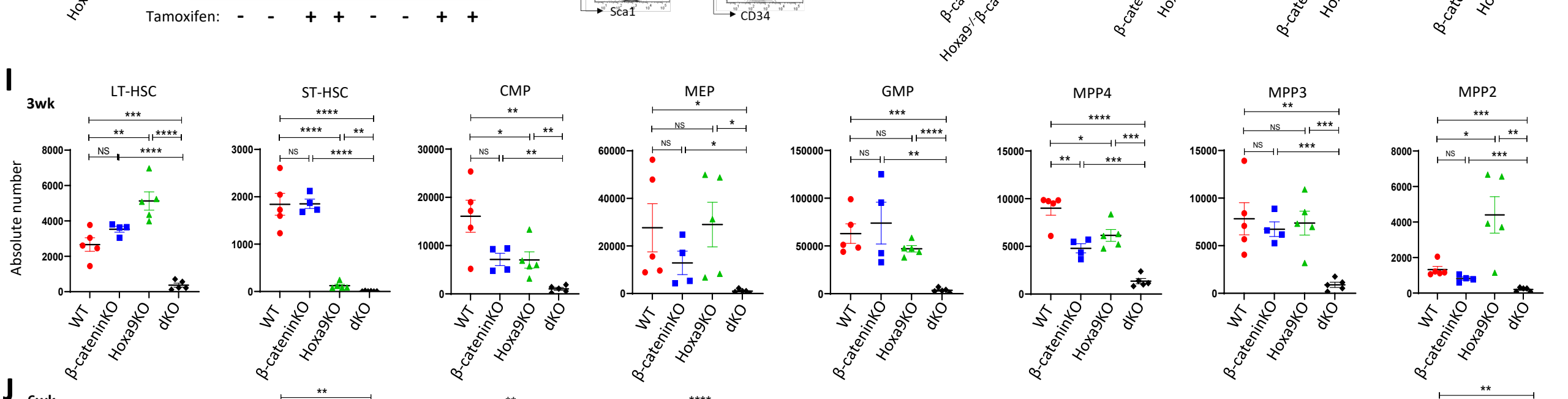
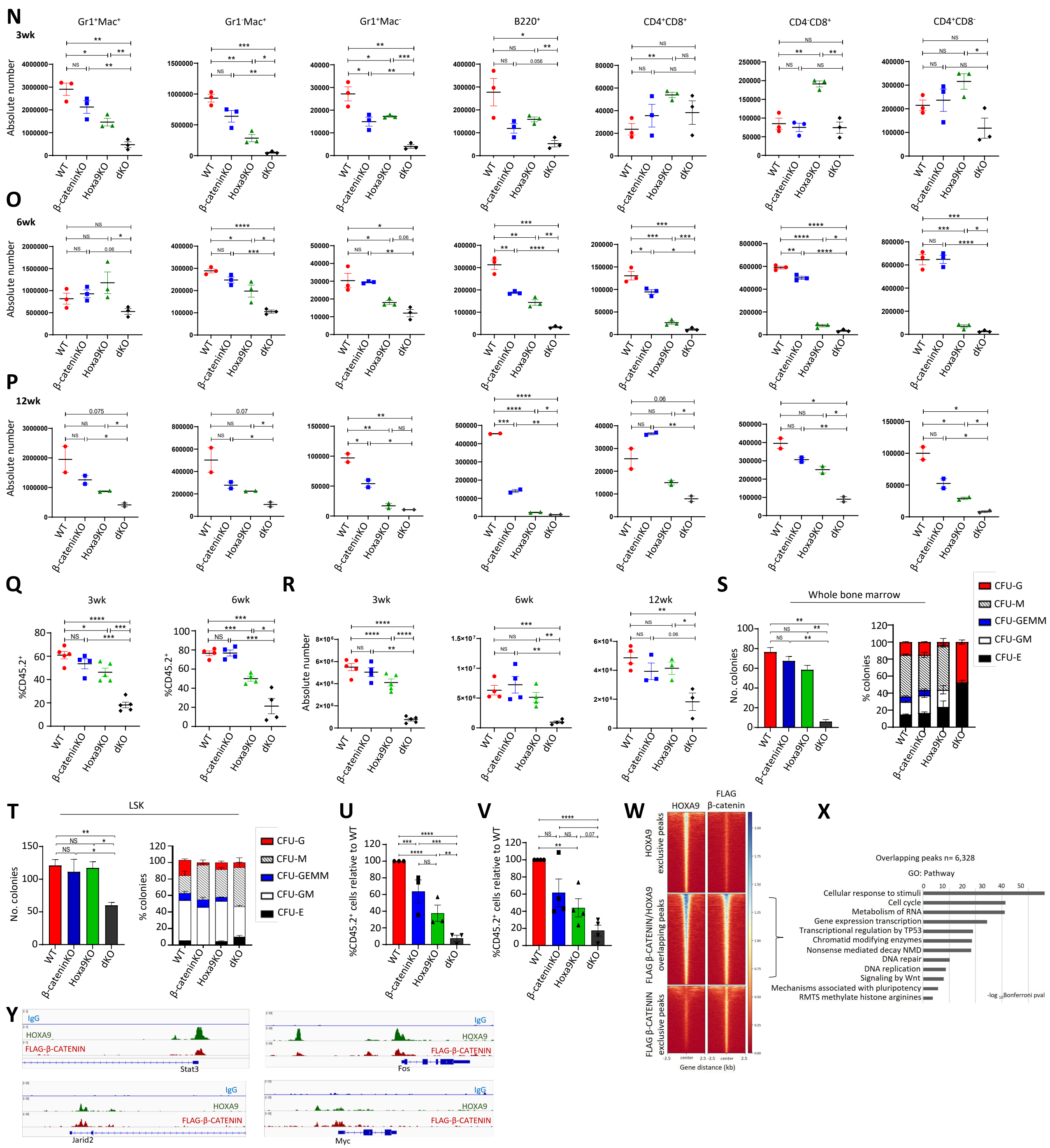
Supplemental Figure 1





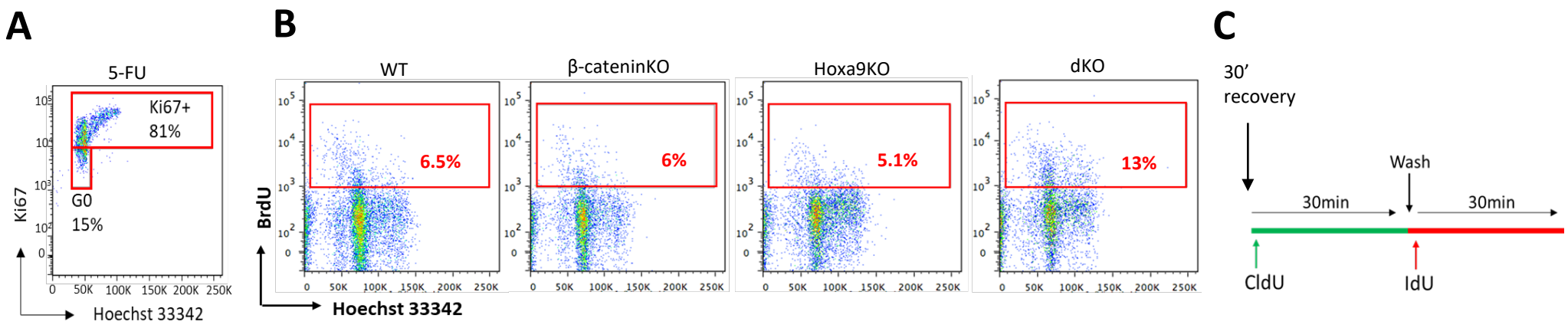


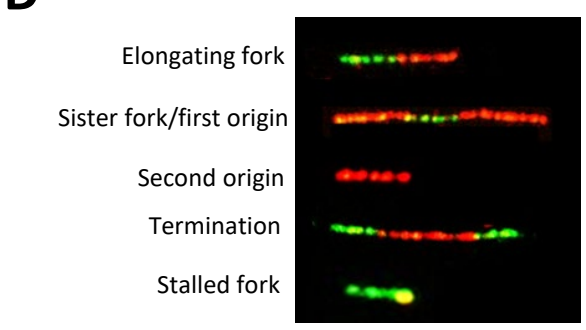


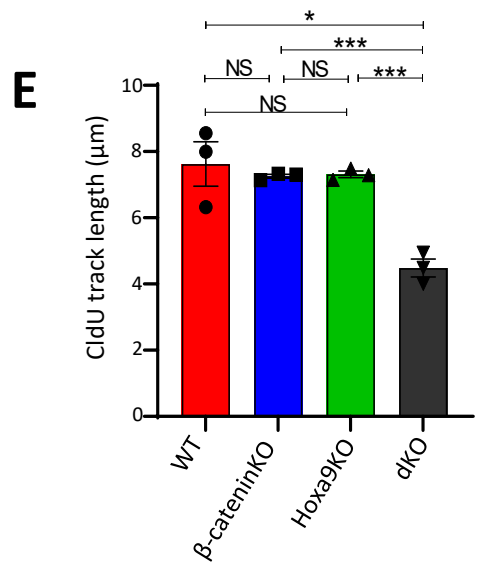


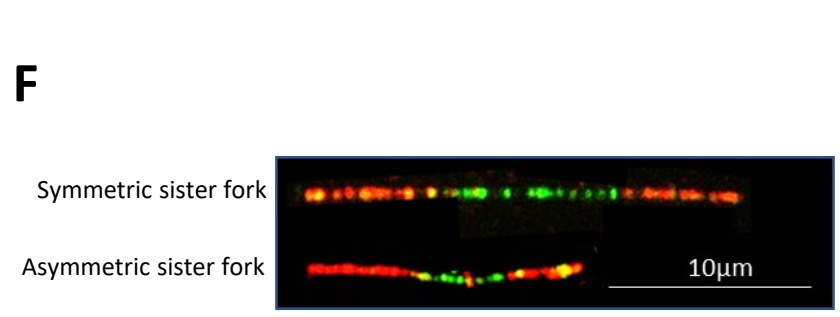
(A) GSEA analysis of RNA-seq data from β-catenin KO and Hoxa9 KO LSKs. Mean fluorescent intensity (B) of WT, Hoxa9KO and dKO HSPCs immunostained with non-phosphorylated (active) β-catenin antibody and representative images of active β-catenin signal (green) and DAPI (blue). Scale bar, 10 μm. mRNA expression levels of β-catenin (C) and Hoxa9 (D) in indicated bone marrow cells post Tamoxifen treatment. (E) Representative gel electrophoresis of genotyping PCR from whole bone marrow isolated from primary transplanted mice 6 weeks post injection with Tamoxifen or corn oil (vehicle) indicated as +/- Tamoxifen (n=2 mice per genotype is shown). Floxed, deleted and WT (from recipient mice) bands are shown. (F) Gating strategy utilized to identify stem and progenitor populations by FACS analysis. (G) Percentage CD45.2^{+} donor cells present in the peripheral blood of *β-catenin*$^{fl/fl}$ and *Hoxa9KO β-catenin*$^{fl/fl}$ transplanted mice three weeks post transplantation (pre-Tamoxifen treatment). (H) Absolute number of MPP populations in bone marrow of indicated mice 12 weeks post Tamoxifen treatment. At least n=3 mice per group. Absolute number of LT-HSC, ST-HSC, CMP, MEP and GMP and MPP populations from indicated mice at 3 week (I) (n=5 mice/group) and 6 week (J) (n=4 mice/group) time points. Percentage of indicated stem and progenitor populations present in bone marrow of transplanted mice at 3 week (n=5 mice/group) (K), 6 week (n=4 mice/group) (L) and 12 week (n=3 mice/group) (M) time points. Absolute number of myeloid cell, B-cell and T-cell lineages in the CD45.2^{+} bone marrow fraction of transplanted mice at 3 week (N), 6 week (O) and 12 week (P) time points. At least n=2 mice/group per time point is shown. (Q) Percentage CD45.2^{+} donor cells in the bone marrow of indicated WT and KO mice at 3 and 6 week post Tamoxifen treatment. At least 4 mice/group per time point in shown. (R) Absolute number of CD45.2^{+} donor cells in the bone marrow of indicated mice 3, 6 and 12 weeks post Tamoxifen treatment. (S) Total number and composition of colonies formed from whole bone marrow (S) and LSKs (T) from *β-catenin*$^{fl/fl}$, *Hoxa9-/-β-catenin*$^{fl/fl}$ mice treated with Tamoxifen or vehicle control in *in vitro* clonogenic assay. Data from three independent experiments are shown. Percentage CD45.2^{+} donor cells in peripheral blood (U) and bone marrow (V) of secondary recipient mice 4 weeks post transplantation. At least n=3 mice per group is shown. (W) Heatmap of HOXA9 and FLAG-tagged β-CATENIN CUT&RUN signal in HSPCs showing 2.5 kb regions flanking the HOXA9 and β-CATENIN peak centers. HOXA9/β-CATENIN exclusive and overlapping peaks are shown. (X) Gene ontology signature associated with HOXA9/β-CATENIN overlapping peaks. (Y) HOXA9/β-CATENIN co-occupancy at HSC regulator gene loci.

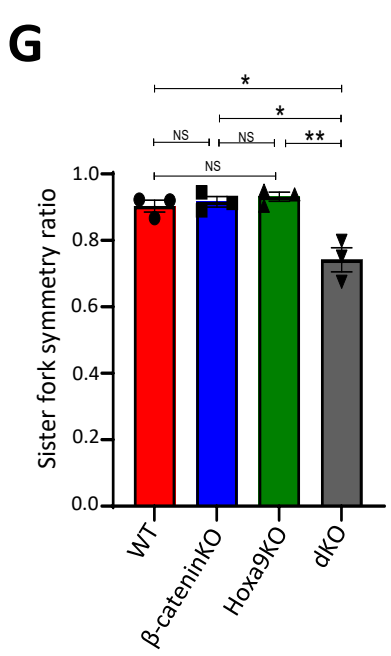
Supplemental Figure 2

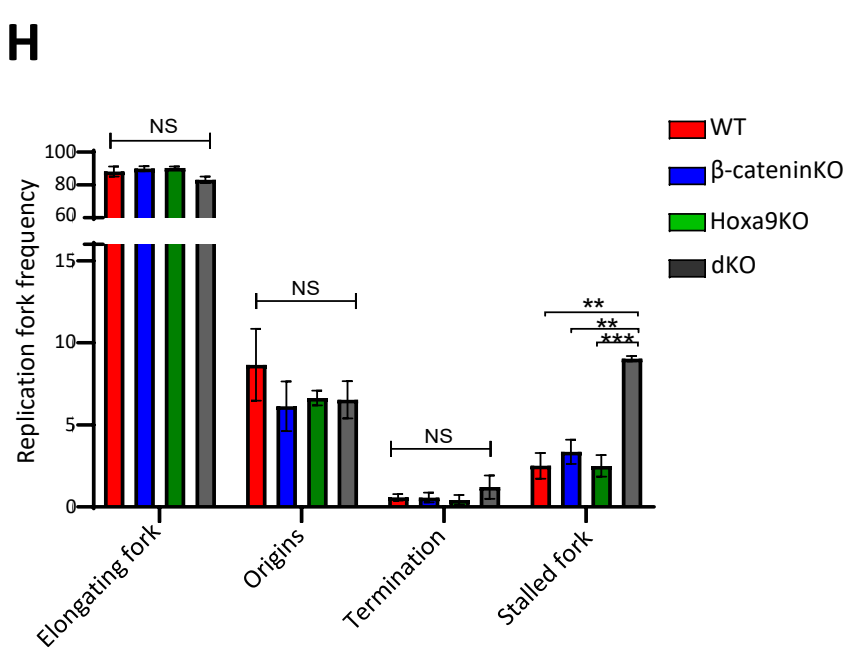


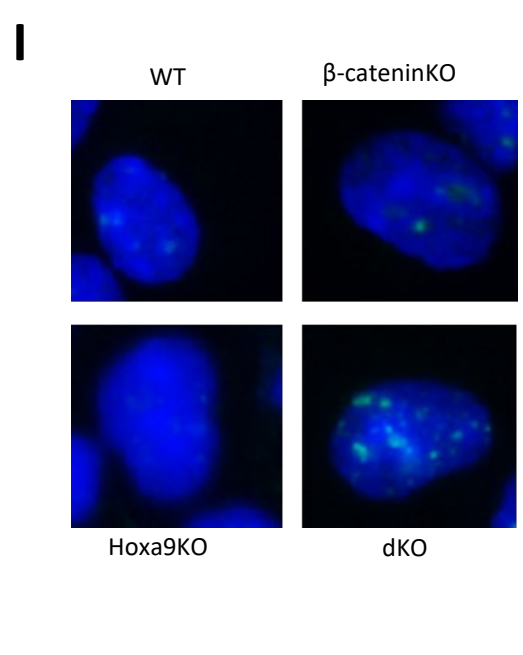






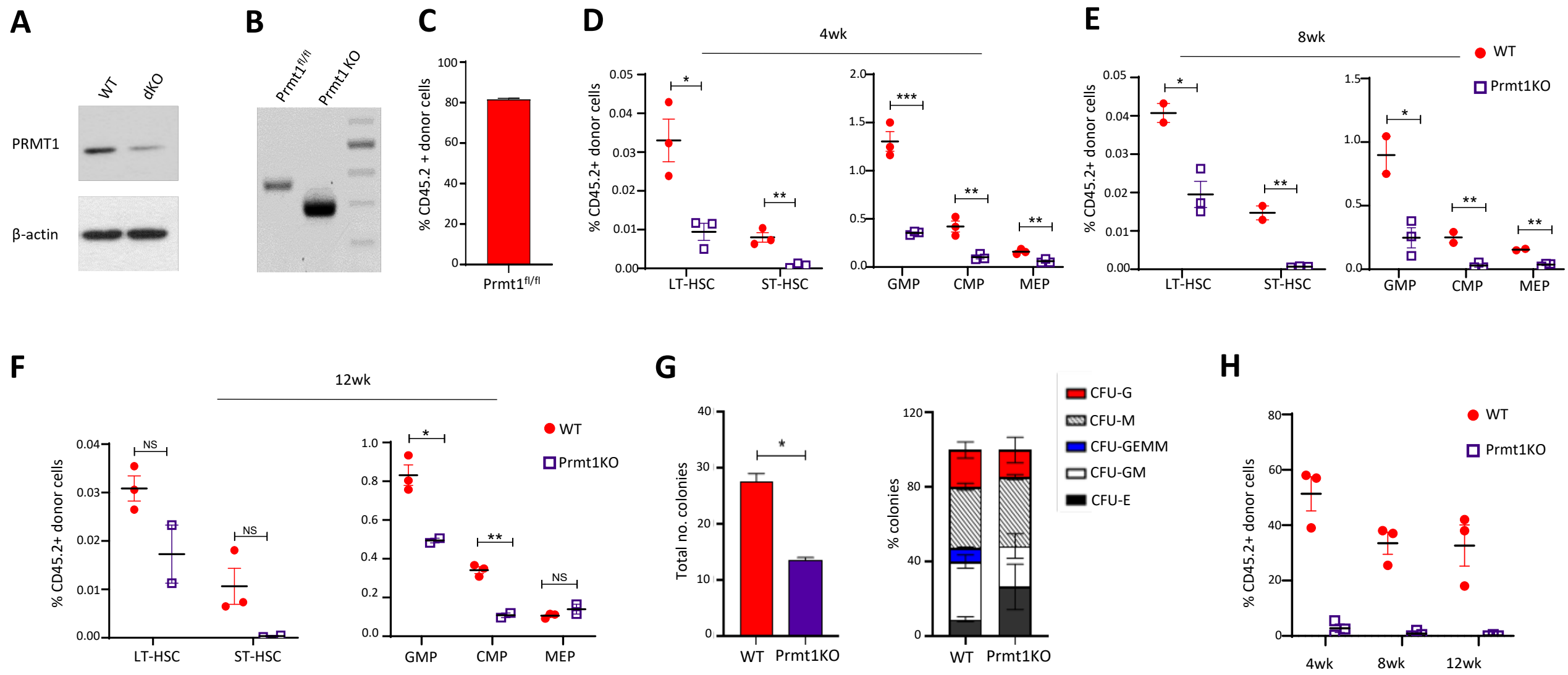






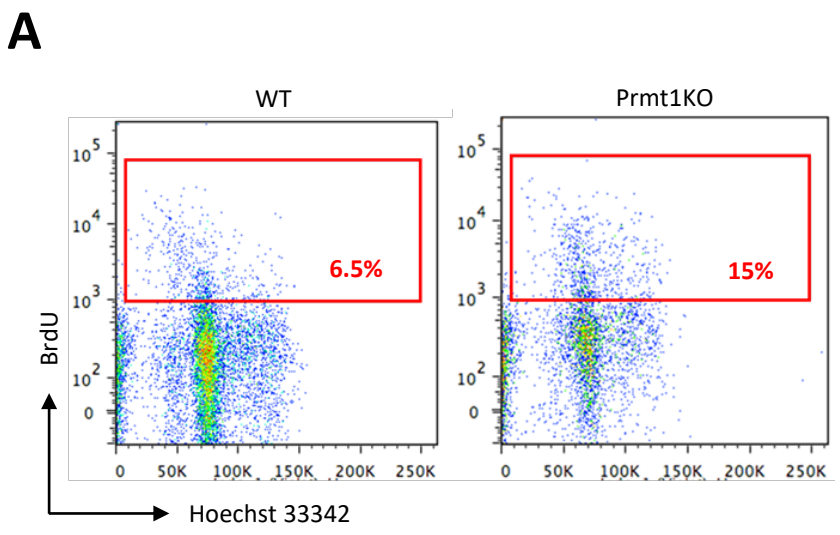
(A) Representation FACS plot showing percentage of LSKs in G_0 cell cycle phase and $Ki67^+$ fraction from 5-FU treated mice. (B) Representation FACS plots of BrdU and Hoechst 33342 immunostained LSKs isolated from primary transplanted mice (C) Schematic representation of DNA fiber staining technique with CldU (green) and IdU (red) nucleotide analogues. (D) Representative images of replication structures produced by DNA fiber technique. (E) Average CldU track length (μm) of $CD45.2^+$ $ckit^+$ cells isolated from primary transplanted mice, n=3 independent experiments. (F) Representative images of symmetric and asymmetric sister forks. Scale bar, 10 μm (G) Sister fork symmetry ratios of n=3 experiments. (H) Frequency of all replication fork structures from bone marrow of indicated mice, n=3 experiments. (I) Representative images of YH2AX foci.

Supplemental Figure 3



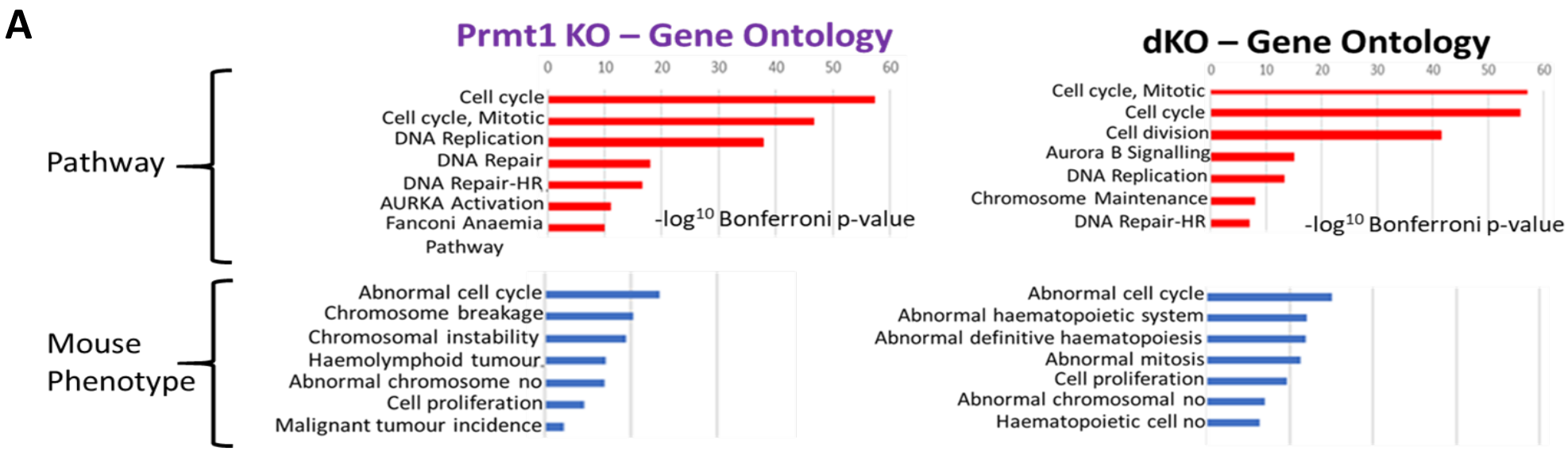
(A) Western blot of PRMT1 expression in HSPCs treated with 4-OHT *in vitro* for 72 h. (B) Representative gel electrophoresis of genotyping PCR from CD45.2$^+$ bone marrow isolated from primary transplanted mice. (C) Percentage CD45.2$^+$ donor cells present in the peripheral blood of *Prmt1*$^{fl/fl}$ transplanted mice three weeks post transplantation (pre-Tamoxifen treatment). Percentage of indicated stem and progenitor populations present in bone marrow of transplanted mice at 4 weeks (D), 8 weeks (E) and 12 weeks (F) post Tamoxifen treatment. n=3 mice per group per time point. (G) Total number and composition of colonies formed from whole bone marrow of *Prmt1*$^{fl/fl}$ mice treated with Tamoxifen or vehicle control in *in vitro* clonogenic assay. Data from three independent experiments are shown. (H) Percentage CD45.2$^+$ donor cells in peripheral blood of secondary recipient mice at 4, 8 and 12 weeks post transplantation. n=3 mice per group per time point.

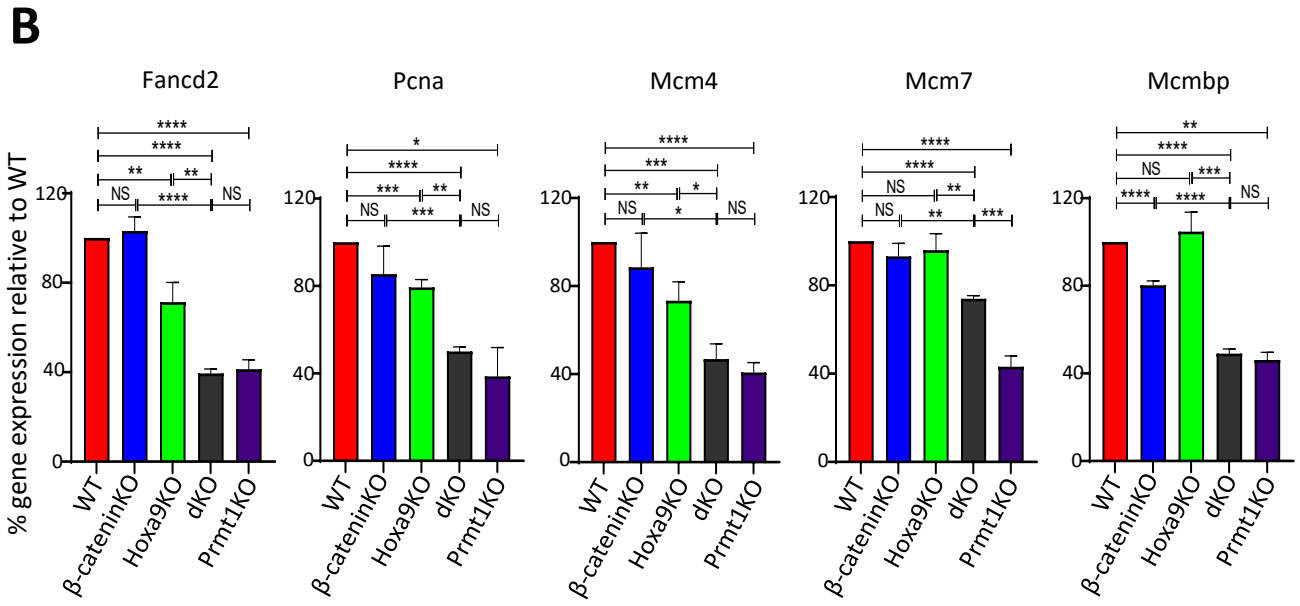
Supplemental Figure 4

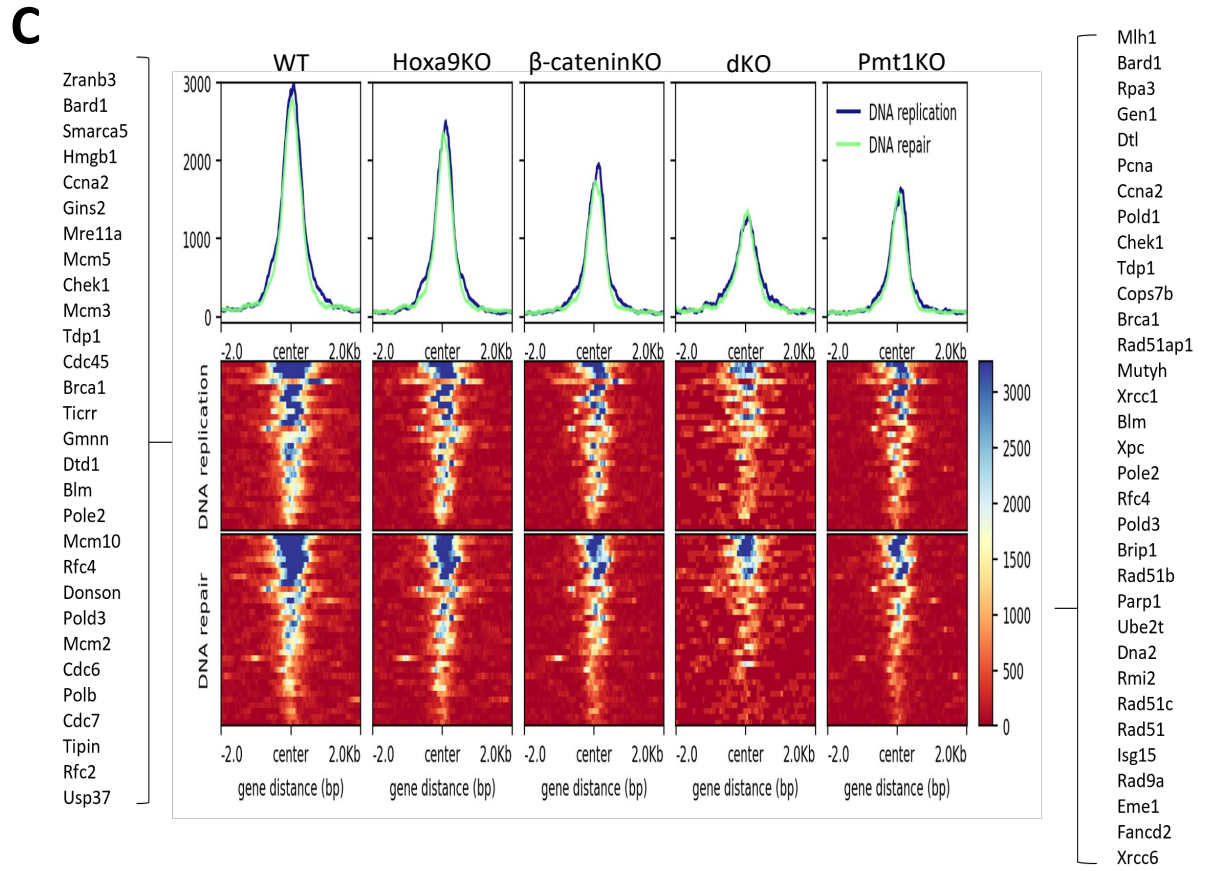


(A) Representation FACS plots of BrdU and Hoechst immunostained LSKs isolated from primary transplanted mice. (B) Average CldU track length (μm) of CD45.2^{+} ckit^{+} cells isolated from primary transplanted mice, n=3 independent experiments. (C) Sister fork symmetry ratio from n=3 independent experiments (D) Frequency of all replication fork structures from bone marrow of indicated mice, n=3 experiments. (E) Representative images of YH2AX foci.

Supplemental Figure 5

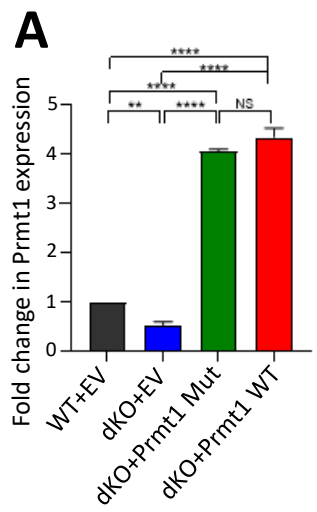


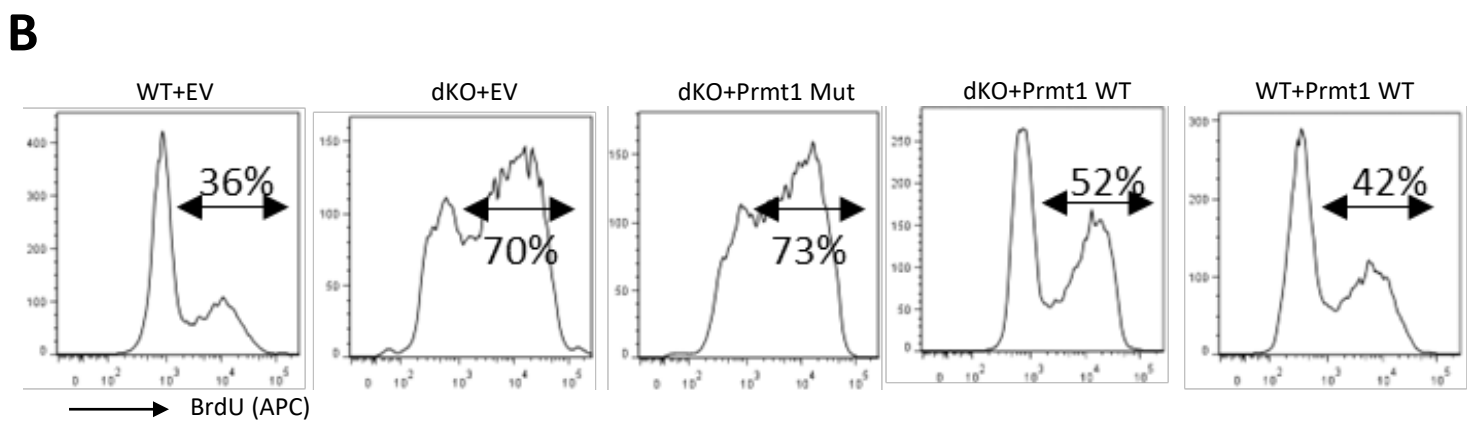


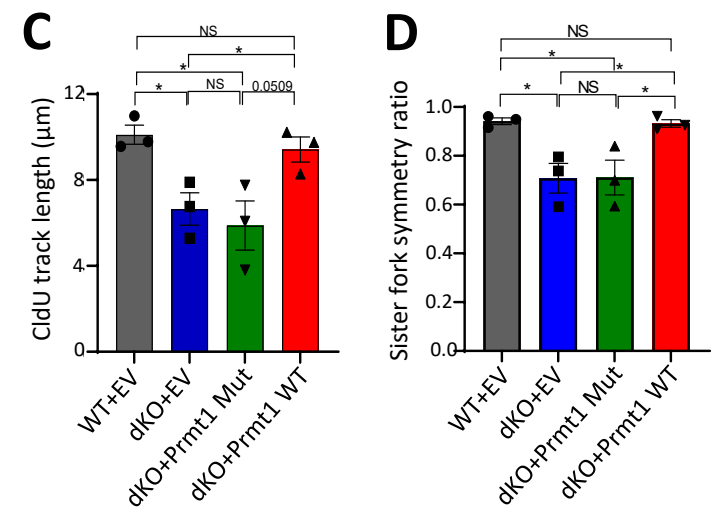


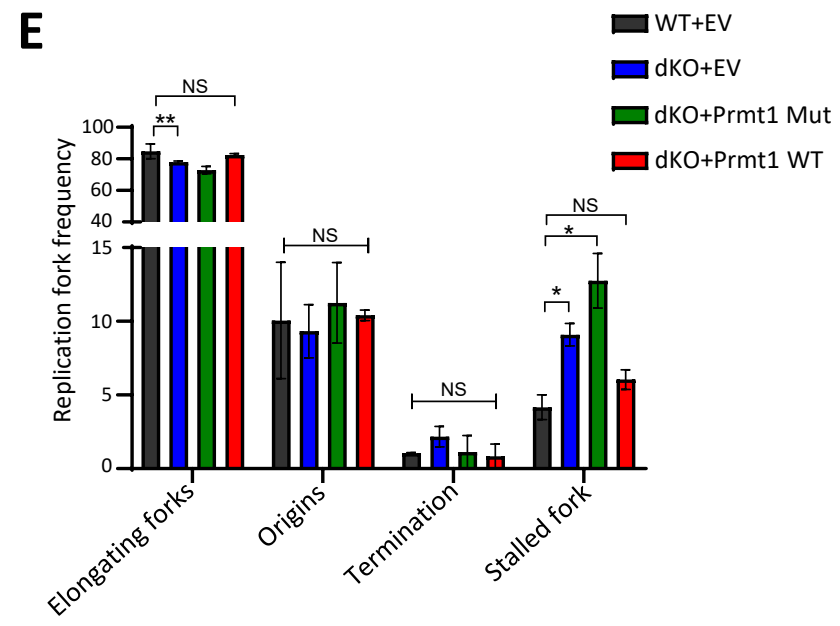
(A) Gene ontology signatures generated from Prmt1 KO and dKO LSKs plotted according to $-\log_{10}$ Bonferroni-corrected p-values. (B) Independent qPCR validation of target gene suppression from indicated HSPCs following *in vitro* 4-OHT treatment. (C) Heatmap of chromatin accessibility of DNA replication and repair genes as listed in WT, β-catenin/Hoxa9 KO and Prmt1 KO LSKs from ATAC-seq analysis.

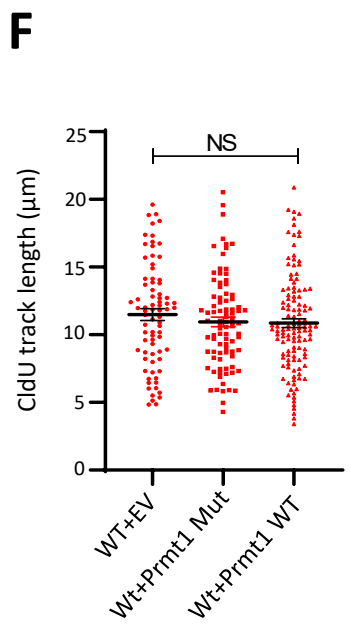
Supplemental Figure 6

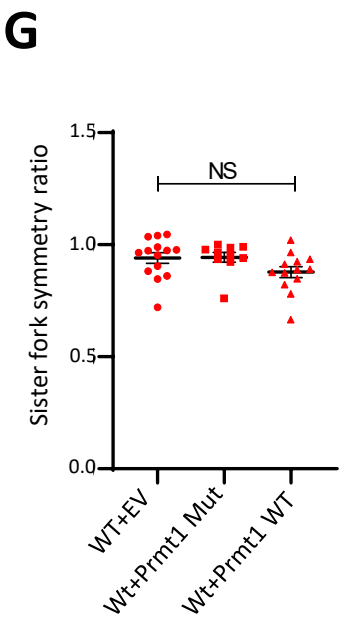


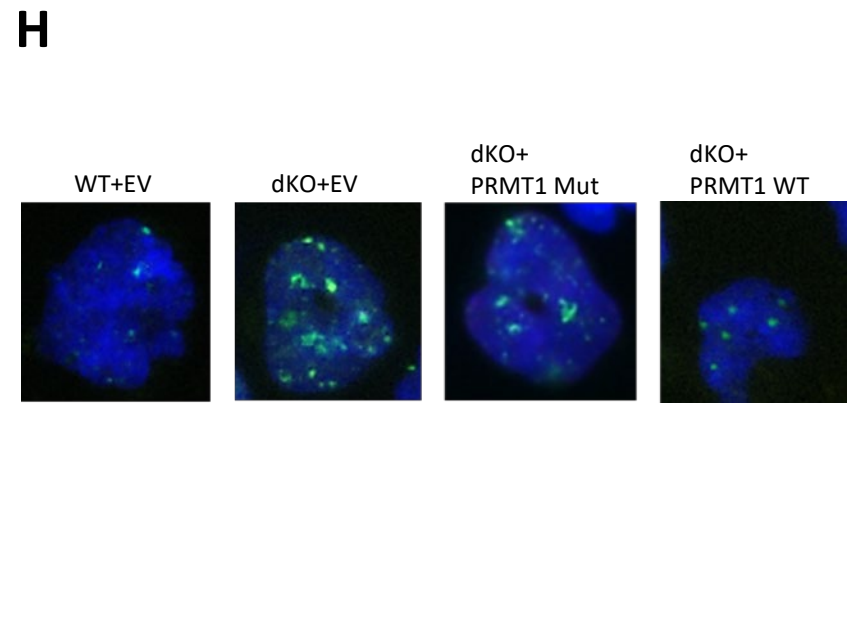


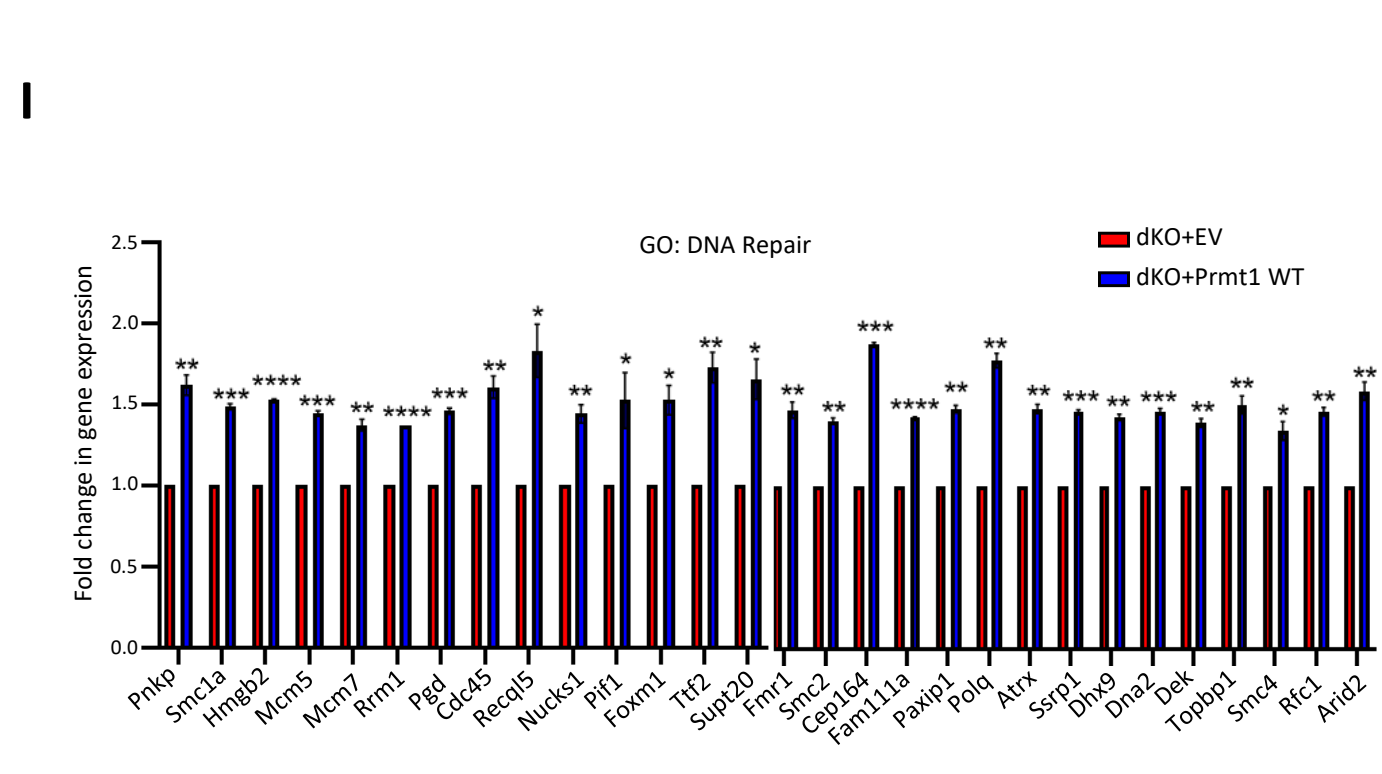


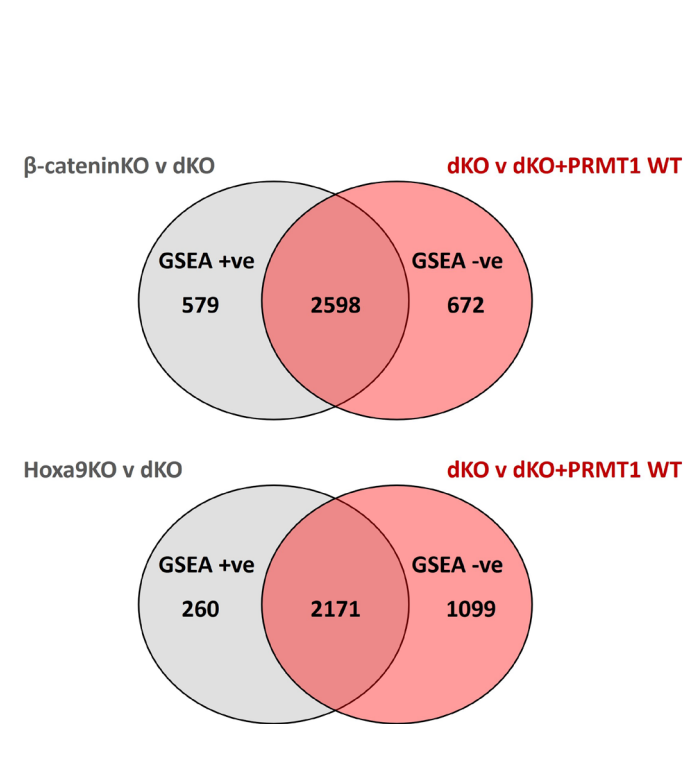


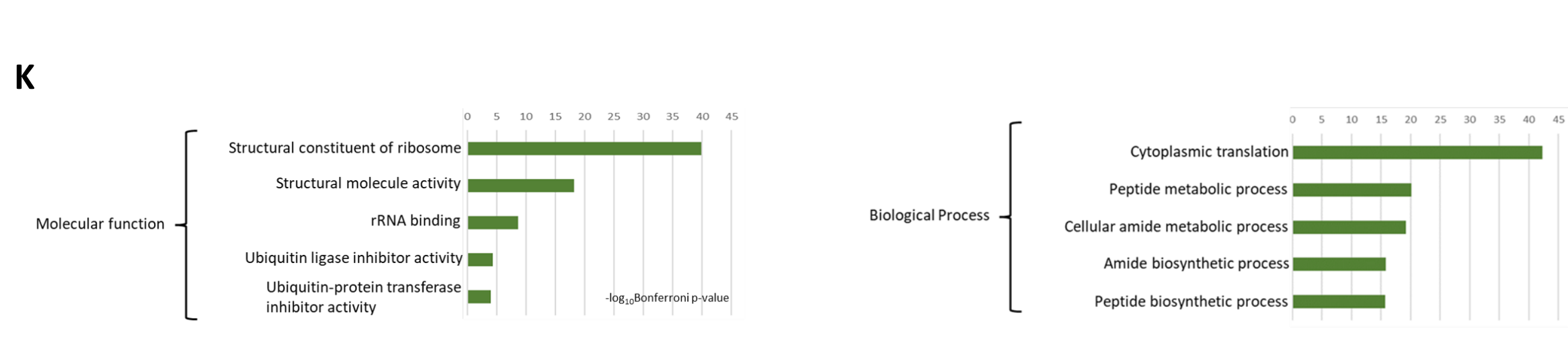












(A) qPCR validation of ectopic expression of Prmt1 in HSPCs. n=3 independent experiments. (B) Representative histogram plots from *in vitro* BrdU labeling of HSPCs from indicated samples. (C) Average CldU track length from n=3 independent experiments. (D) Ratio of sister fork symmetry from n=3 experiments. (E) Frequency of replication fork structures present in indicated samples. n=3 experiments. (F) CldU track length and (G) sister fork ratio of WT HSPCs transduced with PRMT1 WT or Mut. (H) Representative images of YH2AX foci. (I) Fold change in gene expression of transcripts associated with DNA repair. (J) Overlap of all GSEA pathways positively enriched in β-cateninKO v dKO or Hoxa9KO v dKO comparisons with all GSEA pathways negatively enriched in dKO v dKO+PRMT1 comparison (K) GO analysis of significantly upregulated genes from HSPCs isolated from dKO primary transplanted mice and transduced with PRMT1 Mut compared to EV transduction (n=288).

Supplemental Material

Supplemental Methods

Mouse models and transplantation studies

All experimental procedures were approved by King's College London ethics committees and conform to the UK Home Office regulations. *β-catenin*$^{fl/fl}$ mice[1] were crossed with Rosa26-CreER mice (Jackson Lab, JAX:008463) to generate *β-catenin*$^{fl/fl}$ Rosa-CreER/+ mice. These mice were crossed with *Hoxa9*$^{-/-}$ knockout mice[2,3] to generate *Hoxa9*$^{-/-}$ *β-catenin*$^{fl/fl}$ Rosa-CreER mice. Conditional *Prmt1* knockout mouse models were generated using targeted ES cell clones of C57BL/6 background provided by European Conditional Mouse Mutagenesis program (EUCOMM), as described previously[4]. Microinjections of the ES clones and PCR confirmation of germline transmission were conducted by the Mammalian Genetics Unit at the Medical Research Council (UK). The Prmt1 allele is specifically targeted with two loxP sites flanking exon 5 and 6, which encode part of the methyltransferase domain. Homozygous *Prmt1*$^{fl/fl}$ mice were mated with heterozygous Rosa26-Cre-ER mice (Jackson Lab) to generate the conditional *Prmt1*$^{fl/fl}$ Rosa-Cre-ER/+ mice. All knockout mice express pan-leukocyte marker CD45.2. Ctnnb1-Biotin-3xFLAG knockin mice express a CTNNB1 protein with a C terminus biotin/FLAG tag and were purchased from The Jackson Laboratory (JAX: 029511). All genotypes were confirmed by PCR.

1x10^6 CD45.2$^+$ donor mononuclear bone marrow cells were transplanted intravenously into lethally irradiated (13Gy total body Y-irradiation) C57BL/6 congenic strain expressing pan-leukocyte marker CD45.1 (Jackson Lab; JAX:002014) recipient mice along with 2x10^5 CD45.1$^+$ helper bone marrow cells. Tail vein bleeding was conducted 3 weeks post transplantation to analyze level of donor cell engraftment. Deletion of floxed alleles were achieved via daily intraperitoneal injection of Tamoxifen (60 µl at 20 mg/ml) for five consecutive days. β-catenin$^{fl/fl}$ mice treated corn oil (vehicle) served as control mice for transplantation studies and are indicated as "WT" in figures. For secondary transplantation studies, 0.5x10^6 CD45.2$^+$ whole bone marrow cells from primary recipient mice were first purified by FACS and then transplanted into sublethally irradiated (11Gy) secondary recipient CD45.1$^+$ mice.

Immunophenotype analysis and sorting

To analyze the level of donor cell engraftment, approximately 20ul of peripheral blood from tail vein were red cell lyzed using erythrocyte lysis buffer for 10 min at room temperature (RT), and then resuspended in staining medium (Phosphate buffered saline (PBS) + 2% fetal bovine serum (FBS)), stained with CD45.2 APC, CD45.1 FITC, cKit PE, Mac1 PE-Cy7, Gr1 PerCP-Cy5.5, B220 Pacific Blue, CD4 AlexaFluor700, CD8 APC Cy7 (Biolegend) antibodies on ice for 30 min. Cells were then washed, resuspended in staining medium and subjected to flow cytometry.

Immunophenotype analysis of hematopoietic and progenitor populations was carried out by flow cytometric analysis. Femora and tibiae were isolated from transplanted mice and gently crushed in staining medium. Red blood cells were lyzed using erythrocyte lysis buffer for 10 min at RT. Bone marrow cells were incubated with antibodies against mature lineage markers (purified unconjugated anti-CD11b (clone M1/70), anti-Gr-1 (RB6-8C5), anti-CD3ϵ (145-2C11), anti-Ter119 (Ter119), anti-CD4 (RM4-5), anti-B220 RA3-6B2) and anti-CD8a (53-6.7)) (Biolegend). These antibodies were subsequently labeled with goat F(ab')2 anti-rat IgG (H+L). For stem populations, cells were stained with CD45.2 PE-Cy7, CD45.1 FITC, Sca1 Pacific Blue (PB), c-kit APC-Cy7, CD150 PE, CD48 APC, Flk2 Biotin and Straptavadin QDot605. For progenitor populations, cells were stained with CD45.2 APC, CD45.1 PE-Cy7, Sca1 PB, c-Kit APC-Cy7, CD16/CD32 PerCP-Cy5.5 and CD34 FITC. Propidium Iodide (ThermoFisher Scientific) was used for negative selection of dead cells. Stained cells were analyzed on a thirteen parameter BD LSRII flow cytometer using the gating strategy illustrated in supplemental Figure 1F and described previously[5].

For isolation of LSK ($Lin^{-}c\text{-}Kit^{+}Sca1^{+}$) population, cells were stained with lineage cocktail as above and lineage depletion was performed using Invitrogen™ Dynabeads™ Sheep Anti-Rat IgG according to manufacturer's instructions, prior to staining with CD45.2 FITC, Sca1 PB and c-Kit APC-Cy7 and sorting using BD FACS ARIAII cell sorter.

Ki67 and BrdU immunostaining

For *in vivo* BrdU incorporation analysis, primary transplanted mice were intraperitoneally injected with 150 µl (10 mg/ml solution) BrdU (BD Biosciences) two weeks post induction of target gene deletion. After 48 h, mice were sacrificed and femora, tibiae and pelvis were isolated and LSK population FACS sorted as described previously. Isolated LSK cells were fixed and permeabilized using BD Cytofix/Cytoperm Buffer and stored at 4 °C overnight. LSK cells were subsequently re-fixed, DNase I treated at 37 °C for 1 h and stained with anti-BrdU APC (BD Biosciences) and Hoechst 33342 (20 µg/ml, Sigma) before cytometric analysis. For *in vitro* BrdU labeling, HSPCs (ckit-enriched stem and progenitor cells) were incubated overnight with 10 µM BrdU solution and processed as described above.

For Ki67 staining, LSK cells were isolated from primary transplanted mice two week post targeted gene deletion, fixed and permeabilized using BD Cytofix/Cytoperm Buffer and stored at 4 °C overnight prior to RNase treatment. Finally, LSK cells were stained with Ki67 PE antibody (Biolegend) and Hoechst 33342 (20 µg/ml, Sigma for 1 h at 4°C prior to cytometric analysis. For control purposes to force quiescent LSK cells into cell cycle, WT C57BL/6 mice were treated with 150 mg per kg body weight Fluorouracil for 2 days via intraperitoneal injection.

Immunofluorescent staining

Bone marrow isolated from knockout mouse models were enriched for cKit$^+$ cells using CD117 Micro-Beads (Miltenyi Biotec) by positive selection according to manufacturer instructions and maintained in StemSpan (StemCell Technologies) supplemented with 50 ng/ml murine SCF, TPO and FLT3-ligand (Peprotech). HSPCs were treated with either 4-hydroxytamoxifen (4-OHT, 25 ng/ml, Sigma) or EtOH (vehicle) for 48 h to induce target gene deletion. 1x10^5 HSPCs were centrifuged onto a glass slide using a Cytospin (300 rpm for 5 min), fixed using 4 % paraformaldehyde for 20 min at RT. After washing in 1 X PBS, cells were permeabilized and blocked in 10% FBS, 1 % bovine serum albumin (BSA), 0.8 % TritonX-100 in 1 X PBS for 15 min. Cells were incubated with mouse anti-mouse YH2AX (Merck, 05-636, clone JBW301) or anti-active-β-catenin antibody (Merck, 05-665, clone 8E7) overnight at 4 °C. Cells were subsequently washed in PBS+0.8% TritonX-100 and incubated with donkey anti-mouse Alexa Fluor 488 (Abcam, ab150105) and 0.2 ug/ml DAPI (Abcam) at RT for 1 h prior to washing and mounting with Vectashield Antifade mounting medium (Vector lab, H-1000). Images were acquired using Leica DM 4000B microscope under 40X magnification. Cells were scored as YH2AX-positive by the presence of at least 10 YH2AX foci in a cell. Mean fluorescent intensity for active β-catenin staining was calculated using ImageJ software.

Western Blotting

Western blotting was performed on HSPCs treated with 4-OHT or EtOH (vehicle) for 72 h. Anti-PRMT1 (A33, Cell Signaling Technology, #2449) was used at 1:1000 dilution followed by incubation with Actin-HRP (I-19, Santa Cruz) secondary antibody and proteins detected using West Pico Plus Chemiluminescent Substrate (Thermo Scientific).

Viral transduction

cKit$^+$ HSPCs from WT or KO mice were transduced with GFP$^+$ retroviral constructs encoding either human wild type Prmt1 or deletion mutant PRMT1Δ (GSGTG, amino acids 86–90 (MSCV_Prmt1 WT, MSCV_Prmt1 Mut)[6], as described previously[7]. Prmt1 constructs were kindly provided by Quan Zhao. Briefly, retroviral supernatants were collected 48 h after transfection of HEK293T cells. Spinoculation was performed by centrifugation at 800 x g for 2 h at 32 °C. Viral supernatant was removed and replaced with StemSpan medium supplemented with cytokines. GFP$^+$ HSPCs were isolated by FACS sorting 48 h post transduction.

***In vitro* clonogenic assays**

cKit$^+$ HSPCs were isolated from bone marrow and treated with 4-OHT in StemSpan medium as described above. To determine the colony forming capability of WT and KO cells, 5000 whole bone marrow cells or isolated LSKs were seeded into 1 mL of MethoCult GF M3434 (StemCell Technologies). Colonies were scored after 10-14 days according to manufacturer's instruction.

For long term culture- initiating cells (LTC-IC) assay, CD45.2$^+$ ckit$^+$ HSPCs were isolated from transplanted mice and seeded into 96-well plates with MS5 stroma cells in 200 µl of medium (StemSpan with 50 ng/mL murine TPO, SCF, and FLT3 ligands). Different cell doses were seeded for 10 wells per cell dose. Cells were maintained for 3 weeks with media replenished every week. Cell clusters (cobblestone colonies) were scored from each well, and the initiating frequency was calculated using online resources—WEHI Extreme Limiting Dilution Analysis http://bioinf.wehi.edu.au/software/elda/

DNA fiber assay

CD45.2$^+$ ckit$^+$ HSPCs were isolated from transplanted mice, 5000 cells per sample were incubated at 37 °C for 30 min in StemSpan medium. Subsequently, 19mM 5-Chloro-20 -deoxyuridine (CldU, Sigma) was added to the medium for 30 min. Medium was then exchanged and cells incubated with 28mM 5-iodo-20 -deoxyuridine (IdU, Sigma) for 30 min. DNA fibers were then spread on glass slides as described previously[8]. Briefly, 3 µL of cell suspension was mixed with 7 µL lysis buffer (200 mM Tris-HCl, pH 7.5, 50 mM EDTA, and 0.5 % (w/v) SDS) on a glass slide. After 5 min, the slides were tilted at 25–40°, and the resulting spread DNA fibers were air dried, fixed in 3:1 methanol/acetic acid overnight at 4 °C. Fibers were denatured with 2.5 M HCl for 1 h, washed with PBS and blocked with 0.2% Tween 20 in 1% BSA/PBS for 40 min. Anti-BrdU antibodies recognizing CldU (1:400, Abcam, ab6326) and IdU (1:20, BD Biosciences, 347580) were incubated for 2.5 h in the dark at RT, followed by 1 h incubation with secondary antibodies anti-rat Alexa Fluor 488 (LifeTechnologies, A110060) and anti-mouse Cy3 (Sigma-Aldrich, C2181) at RT in the dark. Fibers were visualized using the Nikon inverted A1R confocal microscope (60X, oil) and analyzed using ImageJ software. At least 100 fibers were measured per sample per experiment for total n=3 experiments.

Quantitative real time RT-PCR

Total RNA was extracted using RNeasy Mini kit (Qiagen). cDNA was prepared using High Capacity cDNA Reverse Transcription Kit (Applied Biosystems). Quantitative real time PCR was prepared with using FAST SYBR-green based chemistry (Applied Biosystems) with StepOnePlus Real-Time PCR system (Applied Biosystems). RT-qPCR experiments were run in triplicate by triplicate experimental samples and analyzed by ΔΔCT method. Primer sequences are listed in supplemental Table 2.

RNA-sequencing

Total RNA was isolated from LSK cells harvested six weeks post Tamoxifen induction using the RNeasy Plus Micro Kit (Qiagen) according to manufacturer's instructions. Library preparation was performed using Ultra II Directional RNA Library Prep Kit for Illumina (New England BioLabs, E7760L). The quality of RNA and library was examined by Agilent Bioanalyzer 2100 and quantity measured using Qubit Fluorometer. Sequencing was carried out by Novogene, UK.

The quality of the data was inspected with FastQC. The raw reads were trimmed using trim galore (version 0.6.6) (Kreuger F. Babraham Bioinformatics - Trim Galore) and aligned to the mouse reference genome GRCm38 using STAR (version 2.7.6a)[9]. The featureCounts tool was used to summarize read counts across the genes[10]. Differential gene expression analysis was performed using R package DESeq2[11]. Genes with less than 1 read per million in two or more samples were excluded from analysis. To examine and identify the expression changes between the two conditions, the Wald test was used and the genes with FDR ≤0.05 were considered as significantly differentially expressed. Heatmaps were generated using R package ComplexHeatmap[12].

The desktop client of gene set enrichment analysis (GSEA, v4.0.2)[13] was downloaded from Broad Institute website. The weighted GSEA analysis was performed on pre-ranked gene list with the reference c2.all.v7.0/v2023.symbols.gmt [Curated] gene list and 10000 permutations. FDR q-values ≤ 0.25 were considered as statistically significant[13]. Gene Ontology (GO) analysis was performed on significant differentially expressed genes (fold change ≥1.5, padj ≤0.05) using ToppGene Suite. GO graphs were plotted according to -$\log_{10}$Bonferroni corrected p-values.

ATAC-sequencing

ATAC-seq was performed as described previously with minor modifications[14]. Briefly, 50,000 LSK cells from WT or KO mouse models, harvested immediately following four days Tamoxifen treatment, were used for each experiment. Cells were washed in PBS and then resuspended in lysis buffer containing 10 mM Tris-HCL (pH 7.4), 10 mM NaCl, 3 mM MgCl2 and 0.1% NP-40. Immediately after lysis, samples were treated with 2.5µl of Tn5 transposase (Illumina, #20034197) and tagmentation reactions were carried out at 37 °C for 30 min. Tagmented DNA was purified using the MinElute PCR Purification Kit (Qiagen, #28004) and amplified using NEBNext High-Fidelity 2x PCR Master Mix (New England Labs, #M0541) with the following PCR conditions: 72 °C for 5 min, 98 °C for 30 s, followed by 10 cycles of 98 °C for 10 s, 63 °C for 30 s and 72 °C for 1 min. PCR-amplified ATAC-seq libraries were purified and size-selected using AMPure beads (Beckman Coulter, #A63881) with ratios of 0.55x-1.7x, enriching for DNA fragments of 170-800 bp. Quality and size distribution of the libraries were examined by Agilent Bioanalyzer 2100 prior to paired-end sequencing (2x125 bp) using Illumina NovaSeq 6000 (Novogene, UK).

Trimmed reads were aligned to the mouse reference genome GRCm38 using bowtie2 (version 2.4.2) with the options '-X 1000 --very-sensitive'[15]. Any reads mapping to non-canonical chromosomes, and mitochondria were removed, as well as the PCR duplicates, which were marked using the Picard tool (version 2.27.4) (http://broadinstitute.github.io/picard/). To visualize the data as tracks in Integrative Genomic Viewer (IGV), BAM files were converted into BigWig files using bamCoverage of deepTools (RPKM normalised and with '--extendReads' flag) (version 3.5.1)[16]. Peaks in ATAC-seq data were

detected using function callpeak from the MACS2 tool with '--format BAMPE' option[17]. Profile plots were generated with computeMatrix and plotProfile from deepTools[16].

Cleavage Under Targets & Release Using Nuclease (CUT&RUN) sequencing

CUT&RUN was performed according to Epicypher CUT&RUN Protocol V2.0. Briefly, 0.5 million ckit$^+$ HSPCs from WT BL6 mice or Ctnnb1-Biotin-3xFLAG knockin mice (JAX:029511) were rinsed with wash buffer (20 mM HEPES, pH 7.5, 150 mM NaCl, 0.5 mM Spermidine, and 1× Complete Protease Inhibitor Cocktail) and incubated with the activated Concanavalin A (ConA) beads (Bangs Laboratories BP531) at RT for 10 min. 0.5 µl antibody (HOXA9 Atlas HPA061982, FLAG Genscript A00170, IgG Cell Signaling Technology 3900S DA1E) was added and incubated at 4 °C overnight. After permeabilization with digitonin buffer (wash buffer plus 0.01% digitonin), 20 ng pAG-MNase (kindly provided by Dr Paul Lavender, King's College London) was added and bound to antibody-labelled chromatin. MNase was subsequently activated by addition of $CaCl_2$ to cleave and release antibody-bound chromatin. DNA purification was performed using Monarch PCR cleanup kit (NEB, Monarch PCR & DNA Cleanup Kit, T1030), DNA libraries were prepared using the NEB Ultra II DNA Library Prep Kit (NEB #E7103) and sequenced on Illumina NovaSeq 6000 by Novogene UK.

Cut&Run analysis was conducted using the CUT&RUNTools pipeline with default parameters, including read trimming, alignment, and peak calling. Reads were aligned to the reference mouse genome GRCm38. Peak calling was carried out utilizing MACS2, and the identified peaks were used to generate heatmaps and profile plots. To visualize the distribution of signal intensity around peak regions, computeMatrix and plotProfile functions from deepTools were employed.

Statistical analysis

All experiments were analyzed using unpaired student's t-test. p values of less than 0.05 were considered statistically significant. In the figures, asterisks indicate $*p \leq 0.05$, $**p \leq 0.01$, $***p \leq 0.001$, $****p \leq 0.0001$, ns $p > 0.05$

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Supplemental Table

qPCR Primer	Forward	Reverse
Hoxa9	GCCTTCTCCGAAAACAATGCCG	TTCCGAGTGGAGCGAGCATGTA
β-catenin	ATGGAGCCGGACAGAAAAGC	CTTGCCACTCAGGGAAGGA
Prmt1	ACCCTCACATACCGCAACTC	CAGCAAACATGCAGAGGATG
Mcmbp	GGGATCGTGCAAGGCTTCTT	GGCACCCATTTAGGAGCATTAT
Fancd2	CAAAATCAGCTAGGTGTGGATCA	CCAGGCCATTAACAAACTCTTCT
Mcm4	ACAGGAATGAGTGCCACTTCTCGT	AAAGCTCGCAGGGCTTCTTCAAAC
Mcm7	CCCTGCCCAATTTGAACCTTTGGA	TCTCCACATATGCTGCGGTGATGT
Pcna	TAAAGAAGAGGAGGCGGTAA	TAAGTGTCCCATGTCAGCAA

Supplemental Table 2. RT-qPCR primer sequences.