Supplementary Information

Methods

Generation of REV1$^{-/-}$ mouse. Zygotes isolated from C57BL/6N mice were co-injected with *in vitro* transcribed Cas9 mRNAs and sgRNAs (target sequences AGAAATCTAATGATGTTGCATGG and TGAAGCACTGATTGACGTCACGG, designed using the crispr.mit.edu tool). The first sgRNA targets Cas9 to exon 4 of the *Rev1* locus (ENSMUSE00000155622) and the second sgRNA targets Cas9 to exon 11 of the *Rev1* locus (ENSMUSE00000155613). Offspring was tested by PCR for *Rev1* inactivating mutations using the primers (FW: GGCAACATGGCCAAGAAGAAC, Rv1: ACTCAGTCAGCAGACACATGC, Rv2: TTATTCAGCTTGGCGAGCGCTTTTG) and ABI sequencing (3730 DNA analyzer, Applied Biosystems). A *Rev1* mutant, carrying a 4800 bp deletion (1514 bp in cDNA) was selected, crossed three times onto C57BL/6J and maintained heterozygous. For genotyping the wild-type and mutant alleles are distinguished by size differences. Experiments were approved by an independent animal ethics committee of the Netherlands Cancer Institute (Amsterdam, Netherlands) (DEC number 13007) and executed according national and European guidelines.

Generation of the inducible DDT-deficient models and tamoxifen injections: Similar procedures were performed to obtain inducible DDT-deficient mice. Briefly, mice were generated that obtained LoxP-flanked sites in the *Rev1* locus in the intron before exon 4 and after exon 13, using CRISPR/Cas9 and LoxP containing HR template to the selected sites. gRNA sequences were: 5'-CCCTAGCCCTTTAATATAACAGG-3', 5'-CAAACGTGCATTCGAGGGACAGG- 3' and LoxP sequences were: 5'-ACCAAAGTTTCCTCTGAGTCAGTGATTGTCAACCCTCTTAATGCCCTAGCCCTTTAATATgacctaATAACTTCGTATAGCATACATTATACGAAGTTATATtaagggttAACAGGTCCTTATGATATGGTGACCCCAACAATAAAATTATTTTTGTTGTACTTCATAAC-3' and 5'-CCACAGGTTGAGATACCCTGCCTCTGTAGCAGTAATGCAAGTGAATTGGTGTTTCCTGTCgacctaATAACTTCGTATAGCATACATTATACGAAGTTATATtaagggttCCTCGAATGCACGTTTGCATTGGGCACCTCTCTGGGATTTAGGGCCATACACTGCACATT-3'. These mice were crossed with Actin-Cre mice to obtain the mice containing a deletion of *Rev1*, termed Rev1-Del mice, after which these mice were crossed with C57BL/6J to remove the Actin- Cre gene. Mice were subsequently crossed with RCM2 mice -which contain a tamoxifen-inducible Cre^{ERT2} under the control of the *Rosa* locus; with *Pcna*$^{K164R/K164-FL}$ or *Pcna*$^{K164/K164-FL}$ to allow for inducible removal of PCNA-ubiquitination; and with previously described Confetti mice -which carry a construct that upon Cre-Lox mediated deletion results in stochastic expression of GFP, RFP, YFP or CFP and is thus used as a marker for cells that have high Cre expression and likely a deletion of the *Pcna*$^{K164-FL}$ allele. These crossings ultimately resulted in the final mouse strain used were tamoxifen could conditionally systemically ablate DDT; The mouse strain used for the experiments with adult mice featured the following genotype: RCM2; *Pcna*$^{K164R/K164-F}$ or *Pcna*$^{K164/K164-FL}$;*Rev1*$^{-/+}$ or *Rev1* $^{-/-}$;Confetti. Actin-cre mice were obtained in-house, Confetti mice were a kind gift from the Van Rheenen lab. PCNA^{fl} mice have been described previously (1). RCM2 mice were purchased from the Jackson laboratory (stock: 008463). Offspring was genotyped via PCR, using primers listed in table S2.

To ablate DDT, mice were weighed, and injected intraperitoneally with tamoxifen that had been dissolved in corn oil (5mg/30gr bodyweight, tamoxifen was purchased from MedChem express, Corn Oil was a kind gift from the Te Riele lab). Mice were injected on day 1 and day 3, and two

weeks after the last injection, mice were sacrificed and bone marrow was analyzed as described in the immunophenotyping section of the materials and methods. Experiments were approved by an independent animal ethics committee of the Netherlands Cancer Institute (Amsterdam, Netherlands) (DEC number 13007) and executed according national and European guidelines.

Timed matings. Timed-matings of PCNA$^{K164R/+}$ Rev1$^{+/-}$ and PCNA$^{K164R/+}$ Rev1$^{-/-}$ females were set up with corresponding males to obtain *Pcna*$^{+/+}$ *Rev1*$^{+/+}$, *Pcna*^{K164R} *Rev1*$^{+/+}$, *PCNA*$^{+/+}$ *Rev1*$^{-/-}$, *PCNA*^{K164R} *Rev1* $^{-/-}$ mutants. *Klf1*^{Nan} mutants were obtained from Harlan UK Ltd, and maintained on a C57BL/6 background. Heterozygous *Klf1*^{Nan} males were set up with wildtype C57BL/6 females. Females were checked for the presence of a vaginal plug the following morning, which

was considered day E0.5. Females were allowed to give birth or euthanized at E14.5–E18.5 to address the embryonic lethality.

Histopathological analysis, immunohistochemistry and *ex vivo* immunophenotyping. For histopathological analysis, embryos were collected and fixed in ethanol-acetic acid-formaldehyde (EAF) for 24 hours after necropsy. Fixed tissues and organs were subsequently dehydrated, embedded in paraffin, and 2 μm sections were prepared and stained with hematoxylin and eosin (H&E). For immunohistochemistry, 4 μm thick sections were prepared and staining for Ter119 was performed according to standard procedure (NKI-AVL Animal Pathology facility). Blood was isolated from E18.5, collected in ETDA microvette tubes and analyzed on a COUNTER Ac·T dif (Beckman Coulter). Blood smears were stained with Wright-Giemsa. For immunophenotyping fetal liver cell suspensions were prepared by passing the tissue gently through a 70 mm filter. The following antibodies were used to identify HSCs, multipotent progenitors, myeloid progenitors, erythroid progenitors, on fetal livers and bone marrow and sort single cells from E18.5 fetal livers: Mouse Lineage Cell Detection Cocktail antibody (1:30 Macs Miltenyi Biotec biotinilated followed by Streptavidin APC-Cy7 cat: 7100-19S Southern Biotech), CD34 (FITC eBioscience, clone:Ram34, cat: 11-0341-82), CD135 (PE, BioLegend, clone A2F10 cat: 135306), Sca1 (PerCpCy5.5, Bio-Legend, clone D7, cat: 108124) CD16/32 (PECy7, eBioscience, clone 93, REF: 25016182), cKit (APC, e Bioscience, clone 2B8, cat: 11-1171-83), DAPI for DNA staining, Ter119 (APC, BD cat: 561033), CD19 (PercpCy5.5, BD cat: 561113), CD71 (1:1000, PE, BD,cat:561937),γH2AX(1:1000,ser139,BioConnect, cloneJBW301),AF488(1:500,Invitrogen Alexa-Fluor 488 Goat anti mouse IgG, A11001), CD24 (PE, 01572D, Pharmigen), NPM1 Monoclonal Antibody (FC-61991,32-5200, Invitrogen), CD93 (BV650, 563807,BD), CD135 (Flt3) Monoclonal Antibody (A2F10), PerCP-eFluor 710 (46-1351-82, e Bioscience), CD150 (PECy-7, 115913, Biolegend). . The following antibodies were used for thymus development studies: CD25 (1:1000, PE, BD, clone PC61), CD3 (FITC, Biolegend, clone 17A2), CD8b (PercpCy5.5, Biolegend, clone YT5156.7.7), CD4 (APC, Biolegend, clone RM4-5), CD44 (APCCy7, Biolegend, clone IM7), DAPI for DNA staining. The following antibodies were used for B cell development: cKit (APC, eBioscience, clone 2B8), CD19 (APCH7, BD, clone 1D3), CD25 (PE, 553070, Pharmigen), DAPI is used for dead cell exclusion. All the antibodies for FACS analysis were used 1:200, unless otherwise specified.

To analyze the bone marrow of adult DDT-deficient mice, tibia and femur were cut at the ends and the bone marrow was flushed out using a 21-gauge syringe with cold PBEA buffer (1× PBS 0.5% BSA, 2 mM EDTA, and 0.02% sodium azide). The samples were kept on ice. 10×10^6 cells

were used per staining. The following antibodies were used: Mouse Lineage Cell Detection Cocktail biotin antibody (1:30; same clone as used for fetal livers) followed by c-kit-BUV395 (clone 2B8, BD horizon), Streptavidin-APC-Cy7 (Southern Biotech), CD135-APC (clone A2F101, BD pharmingen), CD48-BUV737 (clone HM48-1, BD horizon), Zombi-NIR (1:100,

Biolegend), Sca1- BV711 (clone D7, BD horizon), CD34-PE-Cy7 (1:100, clone HM34, Biolegend), CD150-BV650 (clone 11-26c.2a, Biolegend), CD16/32-BV785 (clone 2.4G2, BD biolegend). All the antibodies for FACS analysis were from Biolegend and used 1:200, unless otherwise specified. All measurements were performed with a BD FACSymphony A5 (BD Biosciences). Analyses were performed using FlowJo version 10.0.8r1.

Single cell RNA sequencing. SORT-seq was performed as previously described (24) on FACS sorted LSK cells from the DM, SM and WT embryos. Cells were sorted in Vapor-Lock, Mineral Oil (M5310, Sigma-Aldrich). Mosquito® HTS (TTPlabtech) and the BioNex NanodropTM II (GC biotech) liquid handling platforms were used for the processing of the sorted plates. Mosquito is used to prepare primer plates, Nanodrop is used for dispensing enzymes.

Single cell-RNA sequencing analysis. Mapping was performed according to (24). Briefly, BWA was used for aligning reads the mouse transcriptome and read counts were corrected for UMI composition. Downstream analysis was performed using the Seurat package (version 4.0.1) in R (version 4.0.3). First, the data was split based on genotype. For each split dataset the top 2000 most variable genes were determined. Based on these common variable genes between the split data "anchors" were computed and the data was integrated again based on these anchors. The integrated data was then scaled and PCA was preformed using the default parameters in Seurat. The top 30 PCs were picked based on the jackstraw and elbow functions and a resolution of 0.5 was used for the clustering of the data. Finally, differential gene analysis was preformed using the FindMarkers function of Seurat.

Isolation of genomic DNA and PCR. To obtain DNA for mouse-genotyping, cells were lysed in lysis buffer containing ProtK overnight at 55ºC, followed by a brief inactivation of ProtK at 85ºC for 5 minutes. The lysate was mixed with sterile BRAUN water, and 2 µl of this was used in a reaction containing MyTaq polymerase (1:100), MyTaq buffer (BioLine), and primers (see table 2 for primers). DNA was amplified by PCR as indicated in table 3. DNA was amplified with the following steps: denaturation (95ºC, 3min),75ºC 1,5min, 72ºC 45sec, followed by 30-40 cycles of denaturation (94ºC 30sec), annealing (57-63ºC, 30sec), extension (72ºC, 45sec), followed by a final extension step of 72 ºC for 10min.

Statistical analysis. To assess the statistical significance of our data we used the *t*-test or Mann–Whitney *U* test (*P < 0.05, **P < 0.01, *** P < 0.001, **** P < 0.0001) by Prism 7 (GraphPad). No statistical methods were used to predetermine sample size.

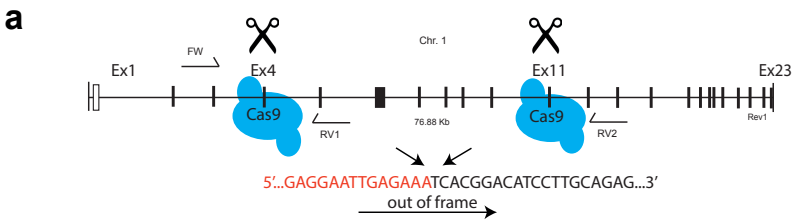
Supplementary figures

Supplementary Fig. 1 CRISPR/Cas9-mediated inactivation of *Rev1* and embryonic lethality of DM embryos at E14.5 and E18.

a Schematic summary of the CRISPR/Cas9-based inactivation of *Rev1* in the C57BL/6N zygotes. REV1 is encoded by 23 exons, the first is non-coding (open box). Two gRNAs (5'-AGAAATCTAATGATGTTGCATGG-3' and 5'-TGAAGCACTGATTGACGTCA-3') targeting exon 4 and 11 of REV1 gene were used to delete the intervening sequence. This deletion renders the *Rev1* transcript out of frame.

b Representative genotyping of a PCR from a $Rev1^{-/-}$ (1), $Rev1^{+/+}$ (2), and $Rev1^{+/-}$ (3) mouse tail DNA. Three primers were designed. The single FW primer was positioned at 5' of exon 4 (5'GGCAACATGGCCAAGAAGAAC3'), the first RV1 primer (5'GCATGTGTCTGCTGACTGAGT3') detects the non-deleted WT allele and hybridize to the 3' region of exon 4, the second RV2 primer (5'-CAAAAGCGCTCGCCAAGCTGAATAA-3') detects the *ko* allele and hybridize to the 3' region of exon 11, as indicated in a.

c Observed and expected frequencies (%) of all possible genotypes at E14.5 (n= 68) and E18.5 (n= 457) obtained from $Pcna^{K164R/+}$ $Rev1^{-/-}$ intercrosses. Fisher exact test, 95% confidence interval was used. **** $P<0.0001$.



E14.5

Genotype	Number of mice	Expected %	Observed %
$Pcna^{+/+}$ $Rev1^{-/-}$	28	25	44,44
$Pcna^{KR/+}$ $Rev1^{-/-}$	37	50	48,15
$Pcna^{KR/KR}$ $Rev1^{-/-}$	3	25	7,41
TOTAL	68		

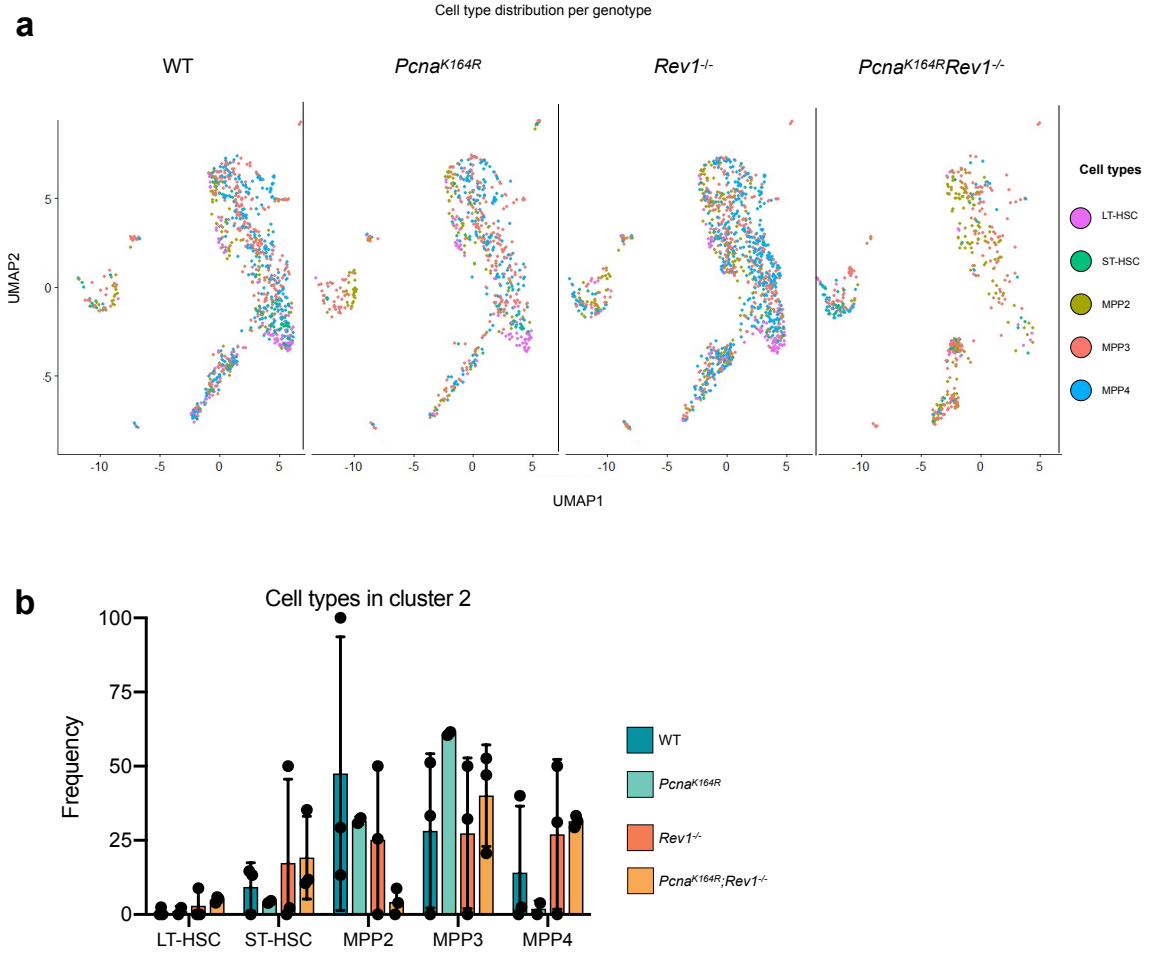
E18.5

Genotype	Number of mice	Expected %	Observed %	
$Pcna^{+/+}$ $Rev1^{-/-}$	152	25	33,26	
$Pcna^{KR/+}$ $Rev1^{-/-}$	270	50	59,08	
$Pcna^{KR/KR}$ $Rev1^{-/-}$	35	25	7,66	***
TOTAL	457			

Supplementary Fig. 2 SORT-Seq and the distribution of cells over the UMAP clusters

a Distribution of all cell types over UMAP clusters.

b Contribution of each cell type to cluster 2 in each genotype (WT n=3, *Pcna*^{K164R} n=2, *Rev1*$^{-/-}$ n=3, *Pcna*^{K164R};*Rev1*$^{-/-}$ n=3).



Supplementary Fig 3. Defective thymocyte development and strong reduction of the B cell compartment in E18.5 *Pcna*^{K164R} *Rev1*$^{-/-}$ embryos

a Microphotographs of H&E sections of thymus from E18.5 *WT, Pcna*^{K164R}*, Rev1*$^{-/-}$*, and Pcna*^{K164R} *Rev1*$^{-/-}$ embryos. Absence of small dark blue cells in the thymus of DM embryos indicates absence of mature thymocytes. Scale bars, 20 mm.

b Representation of FSC-A and SSC-A of thymocytes of the indicated genotypes. The median cell size is increased in DMs.

c Development stages of double negative thymocytes.

d Development stages of single positive thymocytes.

e Statistical analysis of the above-mentioned gating strategies by FlowJo analysis. *p* values were calculated using Mann-Whitney *U* test.

f Gating of E18.5 fetal liver cells on the base of cKit and CD19 markers.

g Representative sub-gates on the base of cKit lineage and CD25 marker. In *Pcna*^{K164R} *Rev1*$^{-/-}$ embryos the percentage of B cells was highly decreased, indicating a defect in B cell maturation compared to the control embryos.
h Statistical analysis of the above-mentioned gating strategies by FlowJo analysis. The graphs indicate cell counts per million of live cells. *p* values were calculated using Mann-Whitney *U* test.