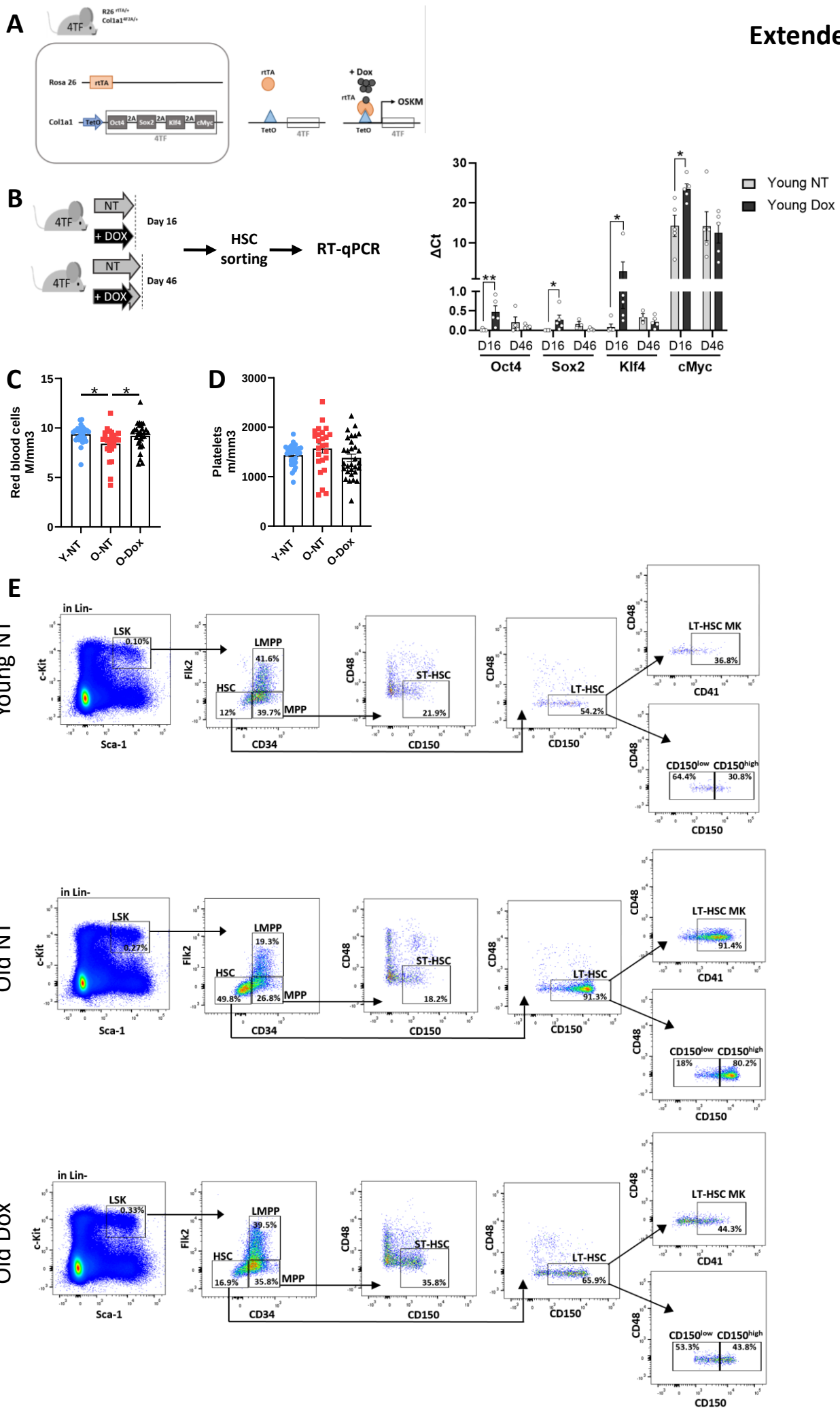




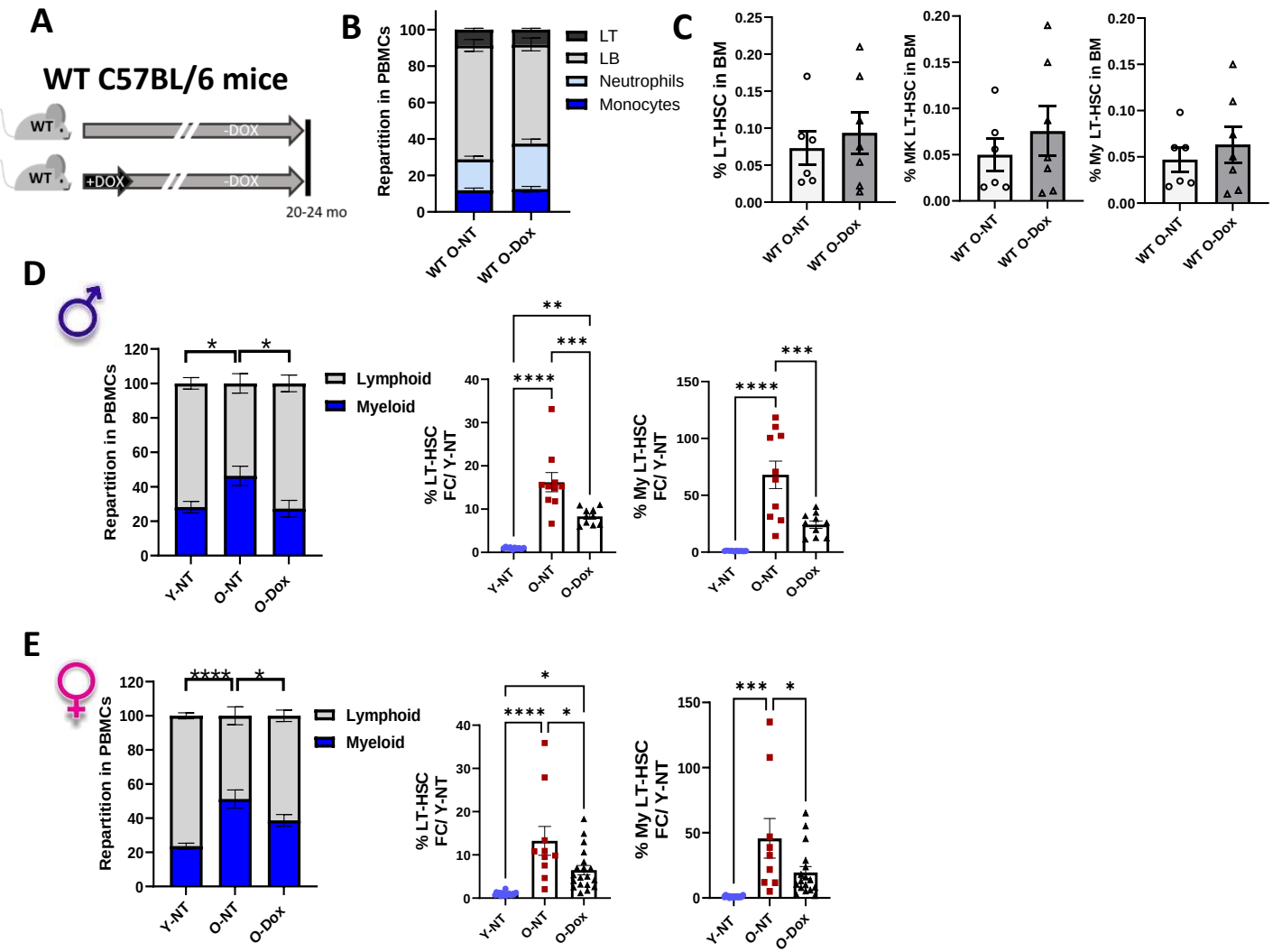
Extended Figure 1 (related to Fig. 1)

(A) Rosa26^{rtTA/+}; Cola1^{4TF2A/+} heterozygous 4TF mouse model expressing a polycistronic cassette containing OSKM sequences into the ubiquitous Collagen 1 locus and carrying the rtTA trans-activator into the Rosa26 locus. In the presence of Dox, rtTA is ubiquitously expressed and able to bind TetO promotor allowing OSKM expression.

(B) *Oct4*, *Sox2*, *Klf4* and *Myc* mRNA expression levels measured by RT-qPCR at the end of the Dox treatment (Day 16) and 1 month after Dox treatment arrest (Day 46), compared to NT mice. DCt values normalized to *Hprt* levels. Means +/- SEM. Unpaired t-test. * p-val <0.05.

(C, D) Red blood cell (C) and platelet counts (D) in the peripheral blood of Y-NT, O-NT and O-Dox mice. Each dot represents a mouse. Means +/- SEM. One-way ANOVA with multiple comparison test. Adjusted p-values: * p<0.05.

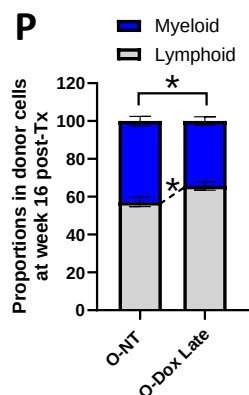
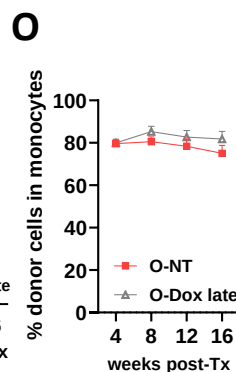
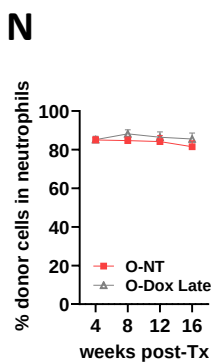
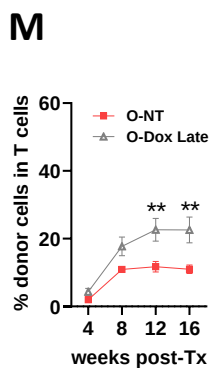
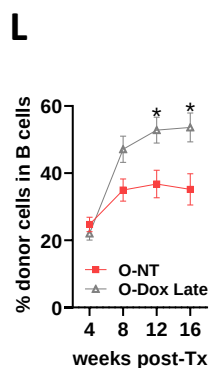
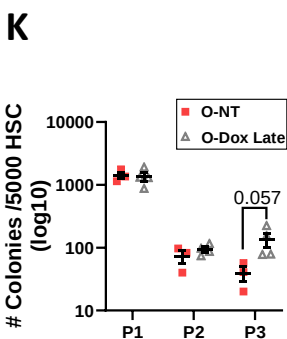
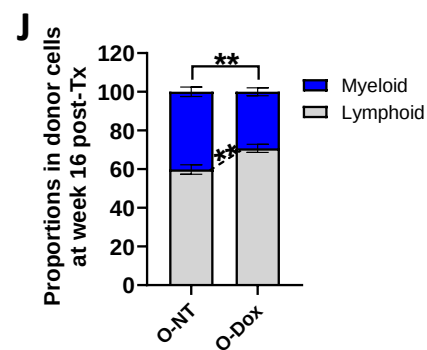
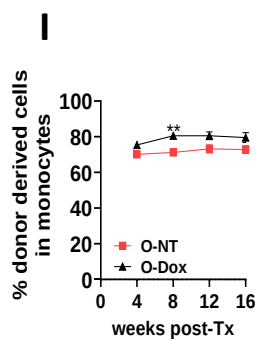
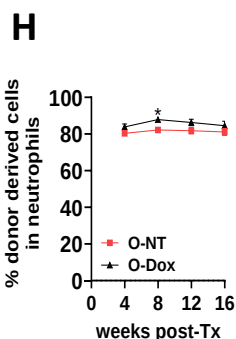
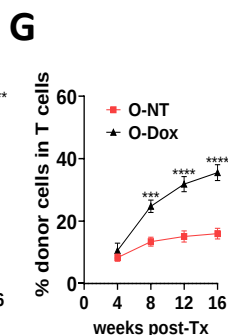
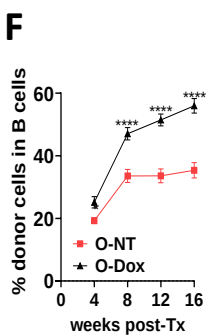
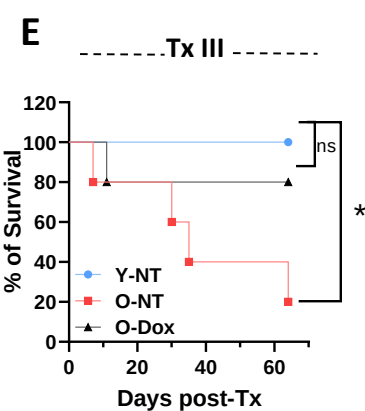
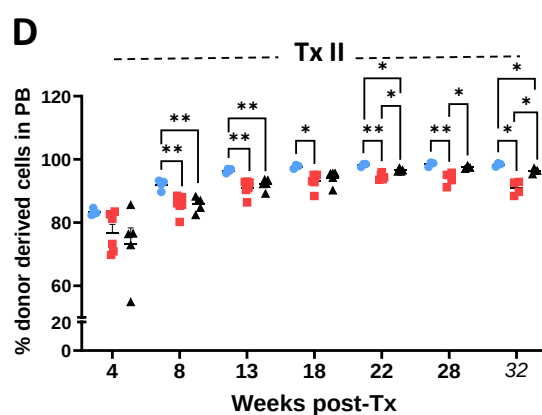
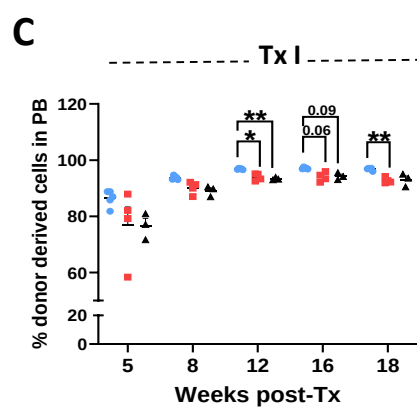
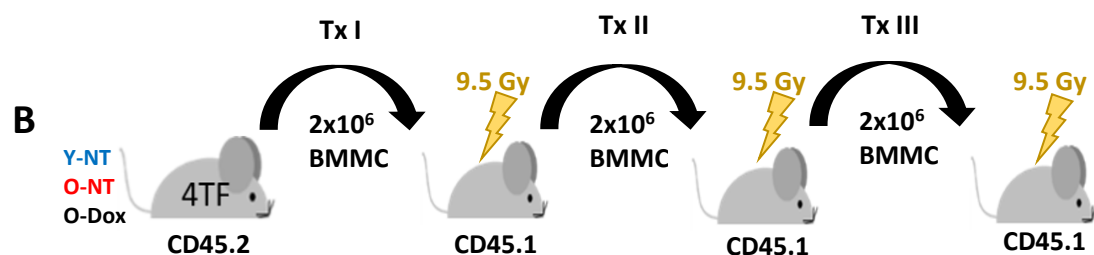
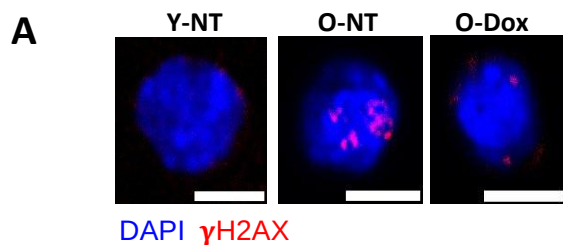
(E) Representative FACS gating strategy for the analysis of HSCs (LSK CD34⁻ Flk2⁻), LT-HSCs (LSK CD34⁻ Flk2⁻ CD150⁺ CD48⁻), myeloid-biased LT-HSCs (LSK CD34⁻ Flk2⁻ CD150^{high} CD48⁻), lymphoid-biased LT-HSCs (LSK CD34⁻ Flk2⁻ CD150^{low} CD48⁻), megakaryocytic-biased LT-HSCs (LSK CD34⁻ Flk2⁻ CD150⁺ CD48⁻ CD41⁺), lymphoid multipotent progenitor (LMPP) (LSK CD34⁺ Flk2⁺), multipotent progenitors (MPP) (LSK CD34⁺ Flk2⁻) and ST-HSCs (LSK CD34⁺ Flk2⁻ CD150⁺ CD48⁻) for Y-NT, O-NT and O-Dox mice.



Extended Figure 2 (related to Fig. 1)

(A-C) Young (2-3 months) non-transgenic (WT) B6 mice were treated with Dox or left untreated. Blood (B) and BM LT-HSC (C) cell composition was analyzed by FACS at 20-24 months of age. NT (n=6), Dox (n=7) mice. Means +/- SEM. Two-way ANOVA with multiple comparison test (B) or unpaired t-test (C).

(D, E) Blood and BM analysis in Y-NT, O-NT and O-Dox 4TF males (D) and females (E) separately. One dot represents a mouse. Data are normalized to Y-NT group. Means +/- SEM. One-way ANOVA with multiple comparison test, adjusted p-values are reported as: * p<0.05; ** p<0.01; *** p<0.001; **** p<0.0001



Extended Figure 3 (related to Fig. 2)

(A) Representative pictures of γ H2AX IF presented in Fig. 2F. Scale bars: 5 μ m.

(B-E) Non-competitive serial transplantations of CD45.2⁺ Y-NT, O-NT and O-Dox BM cells into young lethally irradiated CD45.1⁺ recipients. (B) Experimental design. (C, D) Percentages of donor-derived cells in the blood of primary (C) and secondary recipients (D) over time. Each dot represents an individual mouse. Two-way ANOVA with multiple comparison test. Adjusted p-values are reported as: * $p < 0.05$; ** $p < 0.01$. (E) Survival of the third recipients during the first 60 days following transplantation. Log-rank Mantel Cox test.

(F-I) Percentage of donor-derived cells in B (F) and T (G) lymphocytes, neutrophils (H) and monocytes (I) of recipients in the BM competitive transplantation presented in Fig. 2G. Means $\pm$ SEM.

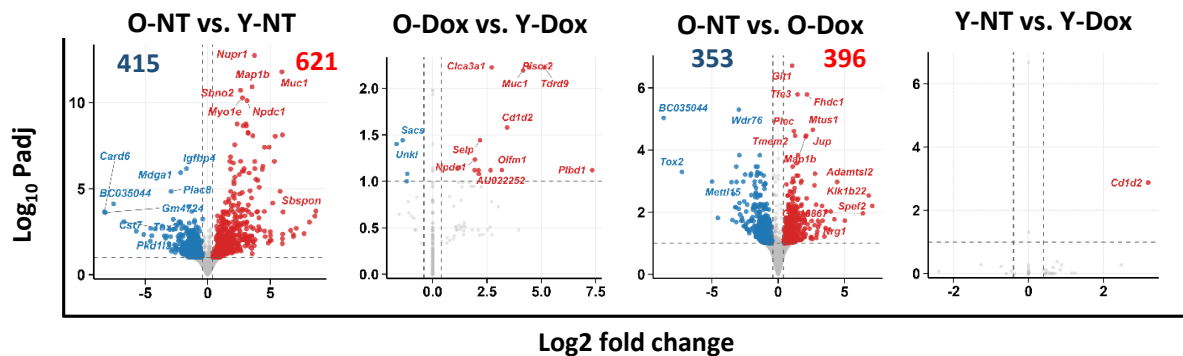
(J) Reconstitution bias illustrated as the percentages of myeloid (monocytes and neutrophils) and lymphoid (B and T cells) cells within donor derived cells in the blood of recipient 4 months following competitive transplantation with O-NT or O-Dox BM cells. Two-way ANOVA, adjusted p-values are reported as: * $p < 0.01$.

(K) Number of colonies generated from 5000 HSCs from O-NT and O-Dox late mice after plating in methylcellulose at passages 1, 2 and 3. Each dot represents an individual mouse. Means $\pm$ SEM. Multiple t tests. * $p < 0.05$; ** $p < 0.01$.

(L-O) Percentages of donor derived cells in B (K) and T (L) lymphocytes, neutrophils (M) and monocytes (N) of recipients after competitive transplantation with O-NT or O-Dox late BM cells (as presented in Fig2 L).

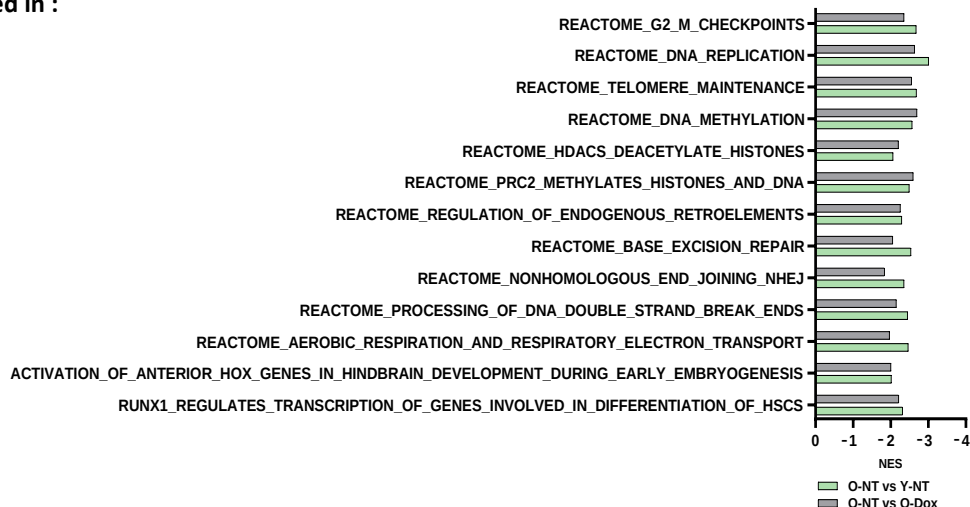
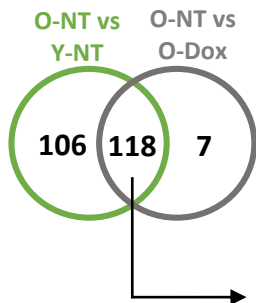
(P) Reconstitution bias illustrated as the percentages of myeloid (monocytes and neutrophils) and lymphoid (B and T cells) cells within donor derived cells in the blood of recipient 4 months following competitive transplantation with O-NT or O-Dox late BM cells. Two-way ANOVA, adjusted p-values are reported as: * $p < 0.05$.

A



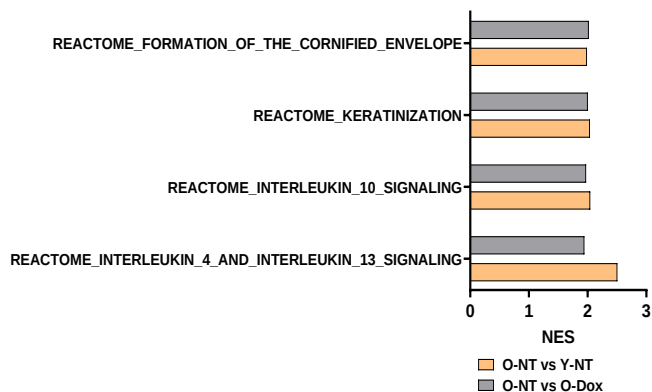
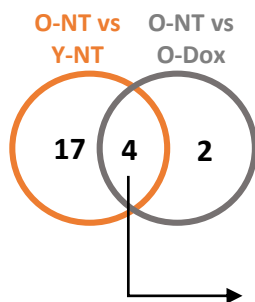
Pathways downregulated in :

B

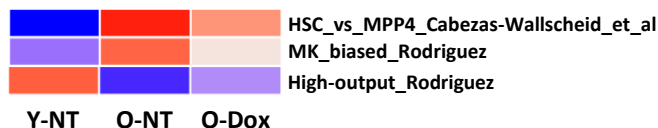


C

Pathways enriched in :

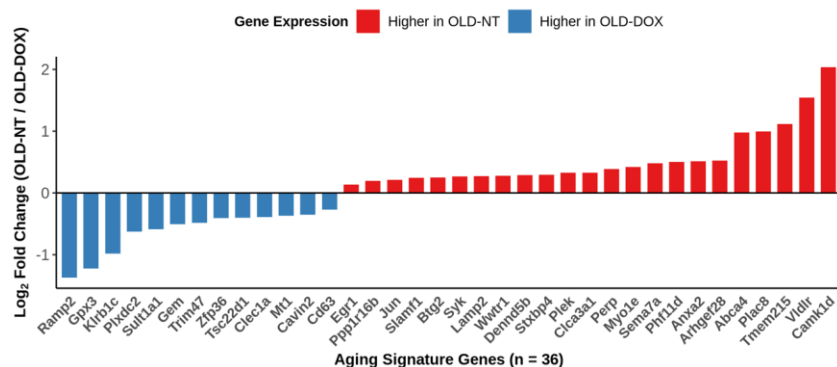


D



E

Differentially Expressed Aging Genes between OLD-NT and OLD-DOX



Extended Figure 4 (related to Fig. 3)

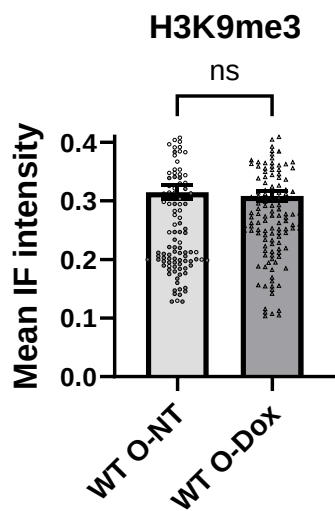
(A) Volcano plots showing up- (red) and down- (blue) regulated ($\text{FDR} < 0.1$; $\log_2\text{FC} \geq 0.4$) and non-significant DEGs (grey) identified by bulk RNA-Seq for the indicated comparisons.

(B, C) Venn diagram and normalized enrichment scores (NES) of representative REACTOME gene sets ($\text{FDR} < 0.05$) commonly downregulated (B) or up-regulated (C) in O-NT samples compared to Y-NT and O-Dox. O-NT vs Y-NT: green and orange circles; O-NT vs O-Dox (grey circles).

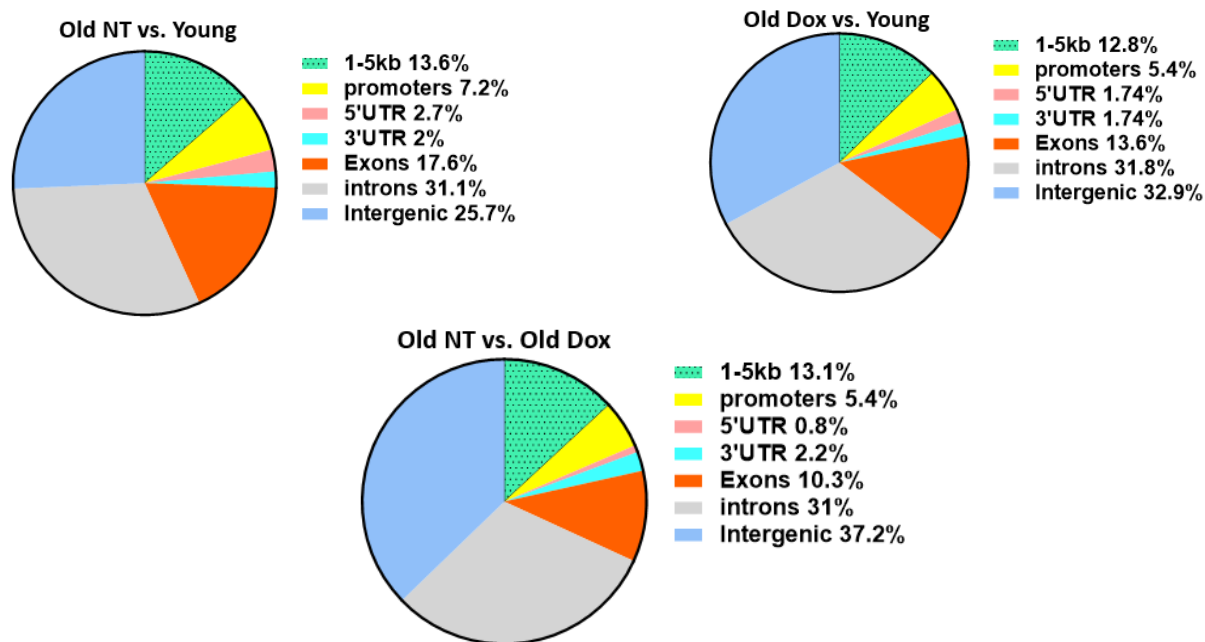
(D) Gene set variation analysis (GSVA) measuring pathway activity using published RNA-Seq data sets in Y-NT, O-NT, O-Dox samples.

(E) Comparison of differentially expressed aging genes between O-NT and O-Dox LT-HSCs in single cell RNA-Seq. For each DEG, the Log2 Fold Change in gene expression between O-NT vs O-Dox was calculated using expression values from individual LT-HSCs.

A



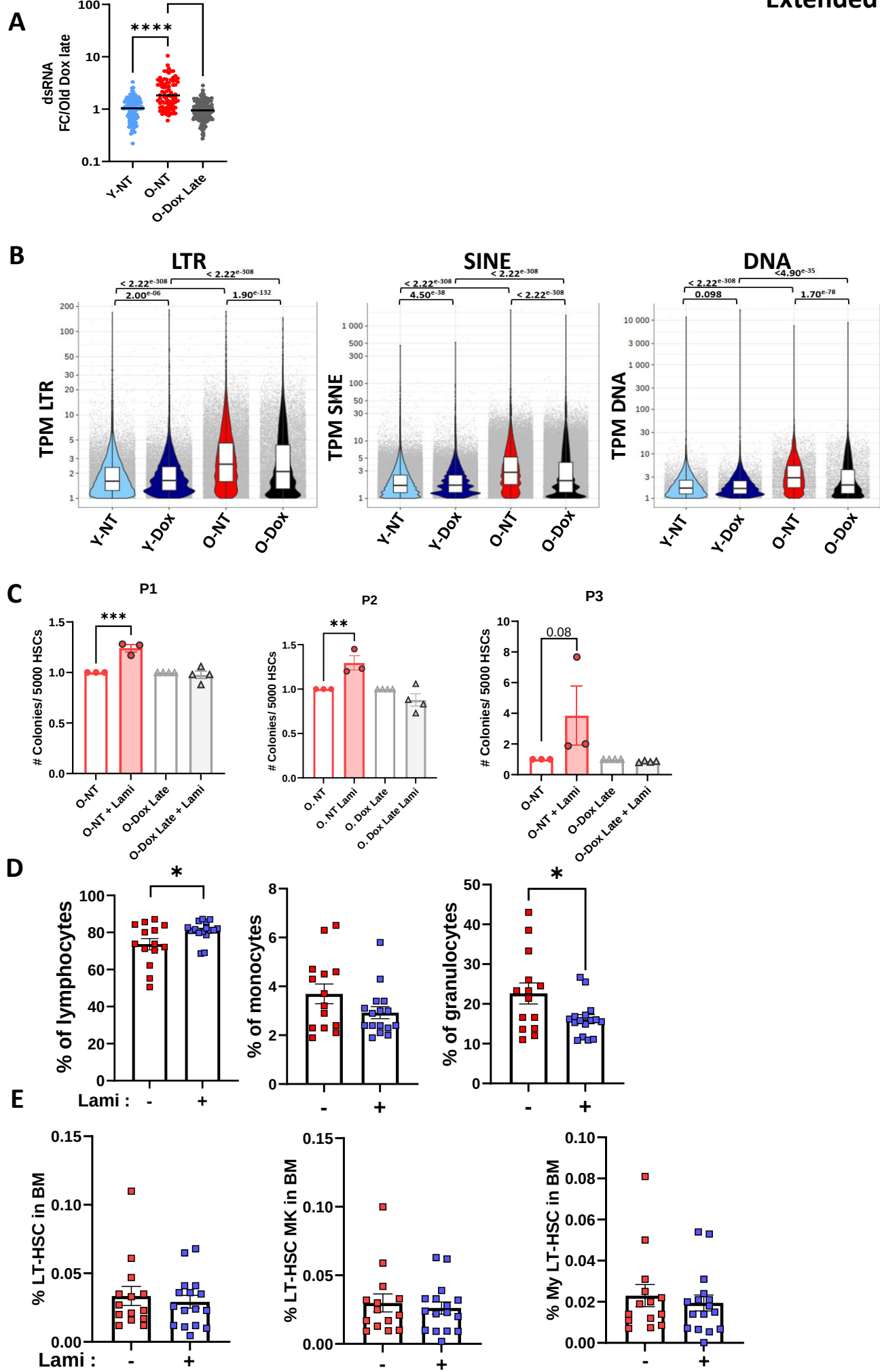
B



Extended Figure 5 (related to Fig. 4)

(A) IF analysis of H3K9me3 signal in HSCs from O-NT and O-Dox WT B6 mice. Results from at least 50 HSCs per mouse. 2 mouse per group.

(B) Distribution of H3K9me3 differential peaks genomic repartition for the indicated comparisons.



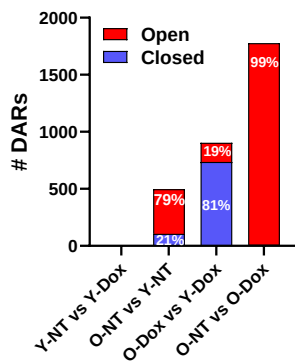
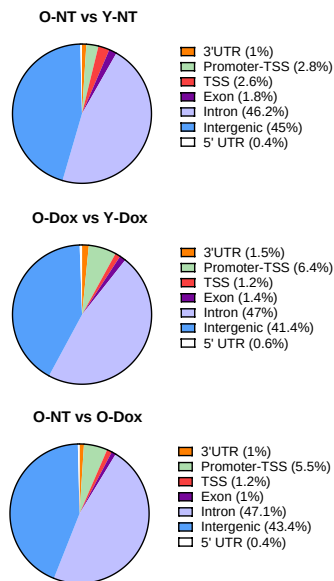
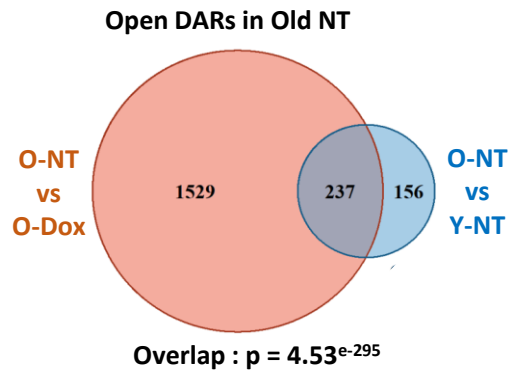
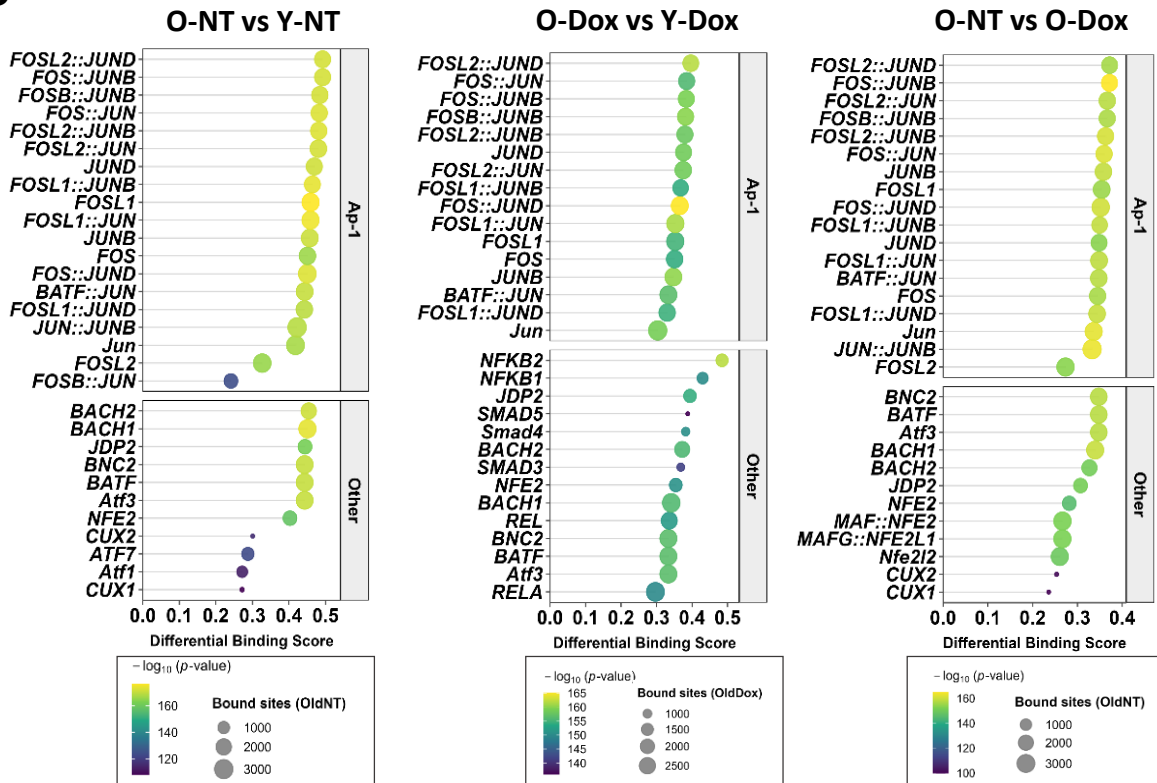
Extended Figure 6 (related to Fig. 5)

(A) Representative images and quantification of dsRNA IF in HSCs from Y-NT (n=2), O-NT (n=2) and O-Dox Late (n=5) mice. Results from at least 50 HSCs per mouse. Each dot represents one HSC. Means $\pm$ SEM. One-way ANOVA. Adjusted p-values reported as **** $p > 0.0001$. Scale bars represent 5 μ m.

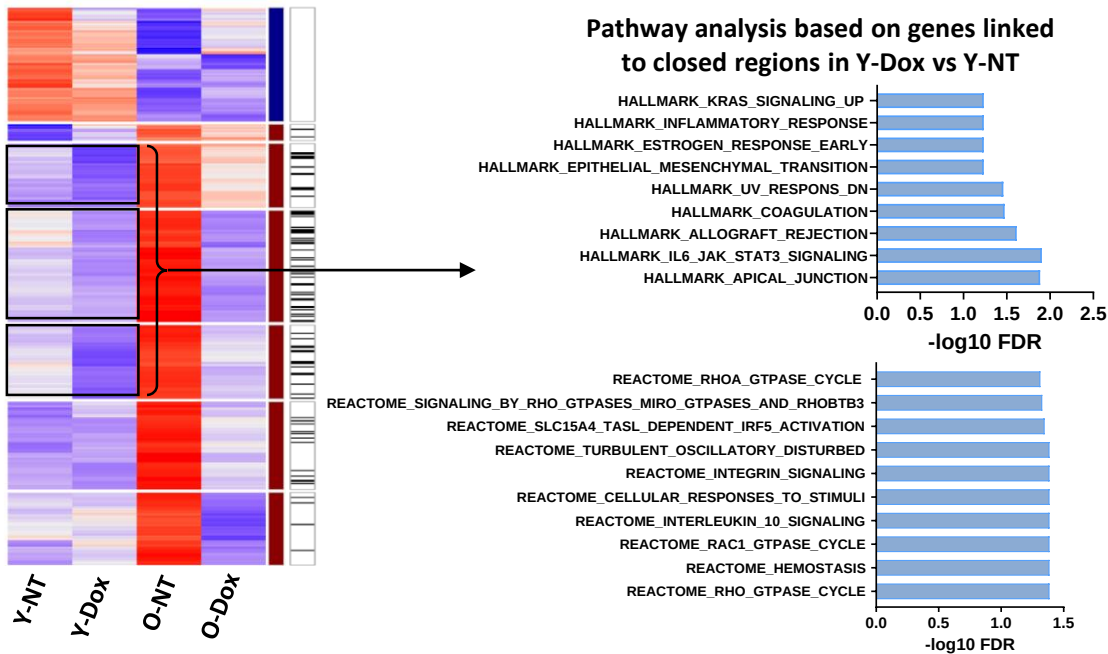
(B) Violin plots showing the expression of LTR, DNA and SINE family TEs copies in bulk RNA-Seq data from HSCs from the indicated groups. One-way ANOVA, adjusted p-values are shown.

(C) Clonogenic activity of HSCs from O-NT and O-Dox late mice treated or not for 48h in vitro with lamivudine (lami, 10 μ M) at passages 1, 2 and 3. Paired t-test. ** p-val < 0.01 and *** p-val < 0.001 .

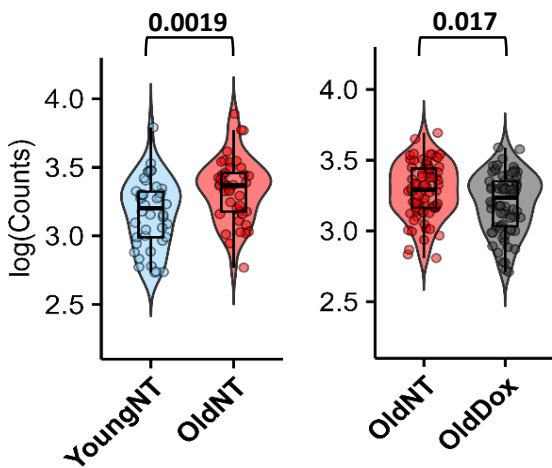
(D, E) Blood cells counts (D) and FACS analysis of LT-HSCs (E) in the BM from aged WT mice treated or not with Lami *in vivo* for 4 months, as in Fig. 5E. Each dot represents an individual mouse. Means $\pm$ SEM. Unpaired t- test. * p-val < 0.05 .

A**B****C****D****Extended Figure 7 (related to Fig. 6)****(A)** Number of ATAC-seq open DARS (red) and closed DARS (blue) for the indicated comparisons.**(B)** Genomic distribution of DARS for the indicated comparisons.**(C)** Overlap of open DARS identified in the O-NT vs Y-NT and O-NT vs O-Dox comparisons. All peaks (significant and non-significant) are used as background and the statistical value of overlap is computed with a hypergeometric test.**(D)** Top 30 HOMER TF binding site motifs identified in O-NT vs Y-NT (left), O-Dox vs Y-Dox (middle) and O-NT vs O-Dox (right) DARS. TFs are ranked by binding score. AP-1 TFs are grouped on the top panels.

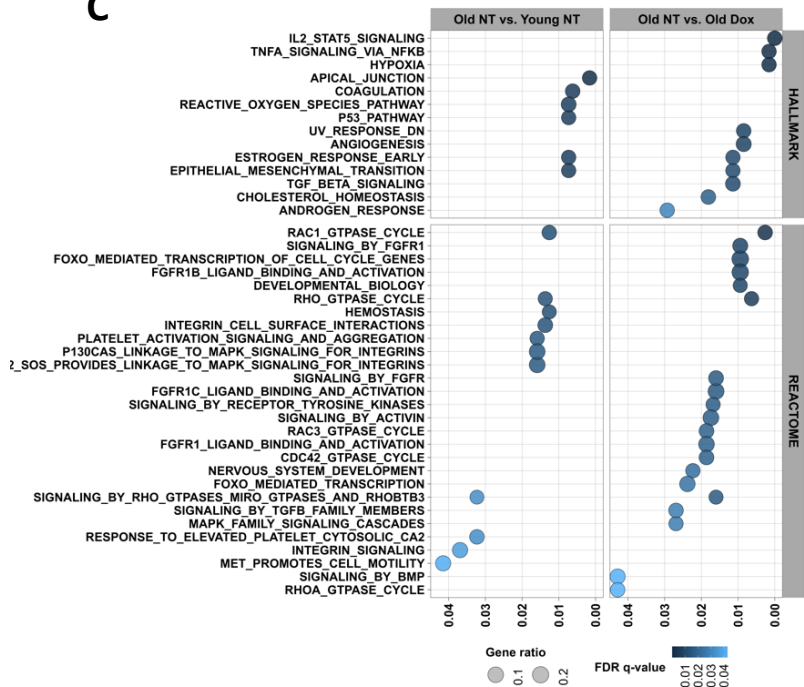
A



B



C

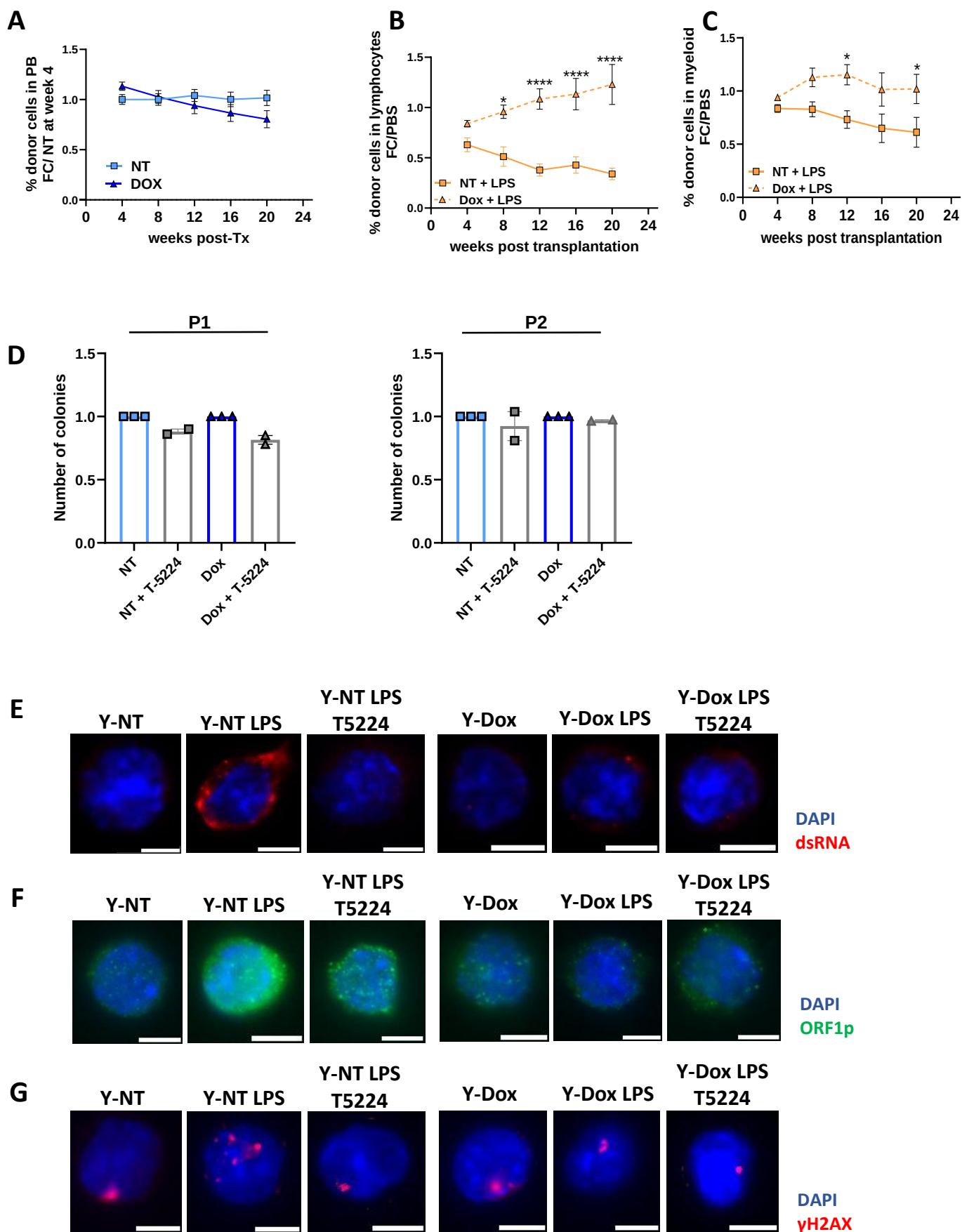


Extended Figure 8 (related to Fig. 7)

(A) GSEA pathway analysis performed with the list of genes associated to the ATAC-seq regions closed in Y-Dox vs Y-NT and open in Y-NT vs O-NT (black boxes).

(B) Violin plots showing the log-transformed RNA-seq read counts at DEGs associated with O-NT vs Y-NT (left) and O-NT vs O-Dox (right) ATAC-seq open DARs. One dot represents a single DEG. Unpaired t-test.

(C) Bubble plots presenting the pathways linked to DAR-closest DEGs in B for the indicated comparisons.



Extended Figure 9 (related to Fig. 8)

(A) Percentages of donor-derived cells in the peripheral blood of recipient mice engrafted with BM from Y-NT and Y-Dox PBS controls (Fig. 8A). Results presented as a fold change compared to NT group at week 4 post-Tx.

(B, C) Percentages of donor-derived cells in the lymphoid (B) and myeloid (C) compartments of mice engrafted with Y-NT or Y-Dox mice treated with PBS or LPS. Results are shown as the fold-change of LPS over PBS control group. Means $\pm$ SEM, n=10 mice per group from 2 independent experiments. Two-way ANOVA with multiple comparisons. Adjusted p-values are reported as: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

(D) Number of colonies generated from 5000 HSCs from Y-NT and Y-Dox mice in the presence or absence of AP-inhibitor alone (10 μ M). (E-G) Representative pictures of dsRNA (E) ORF1p (F) and γ H2AX (G) IF presented in Fig. 8H, 8I and 8J, respectively. Scale bars: 5 μ m.