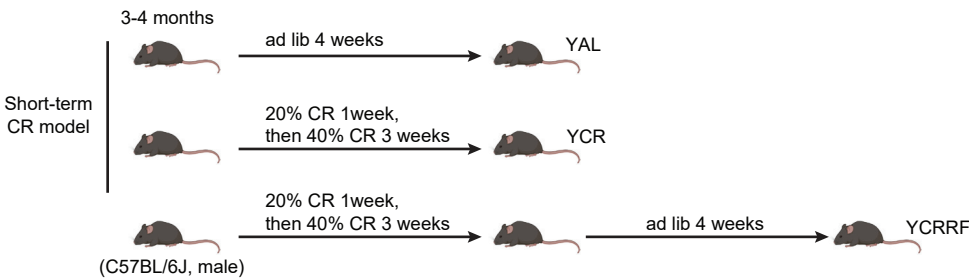
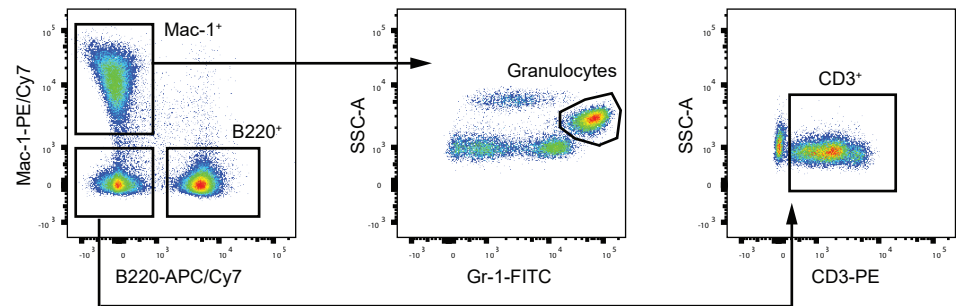


Supplementary Fig. 1

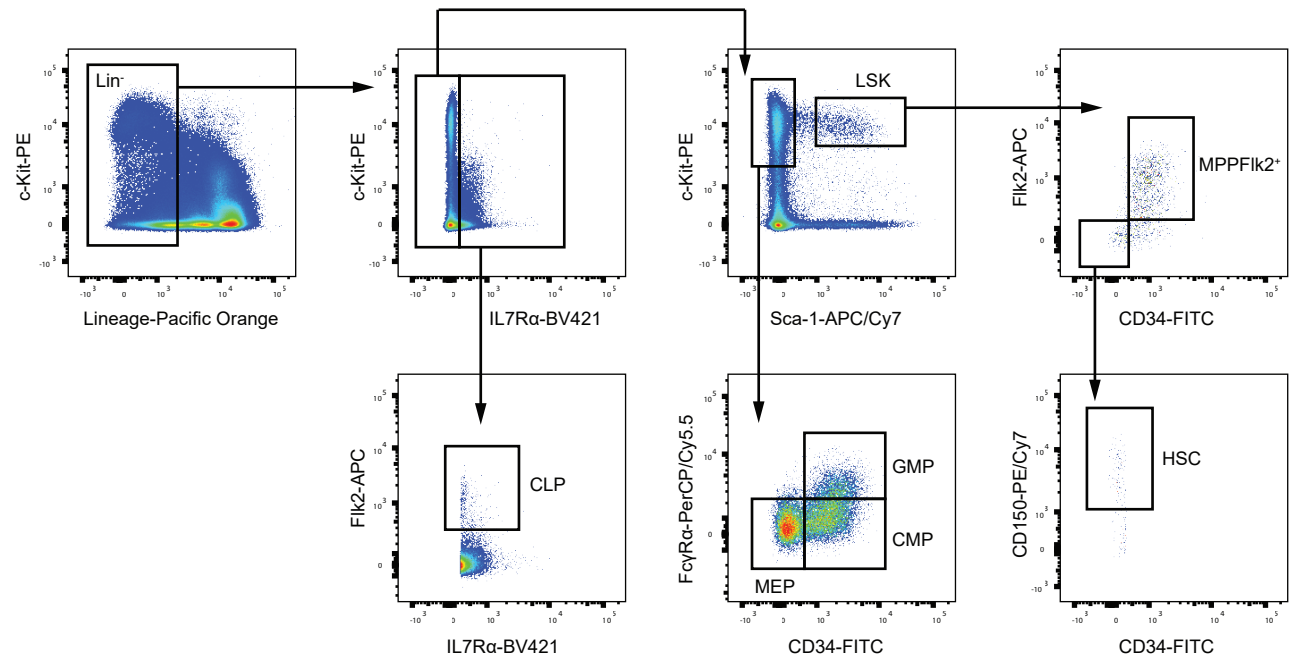
a



b



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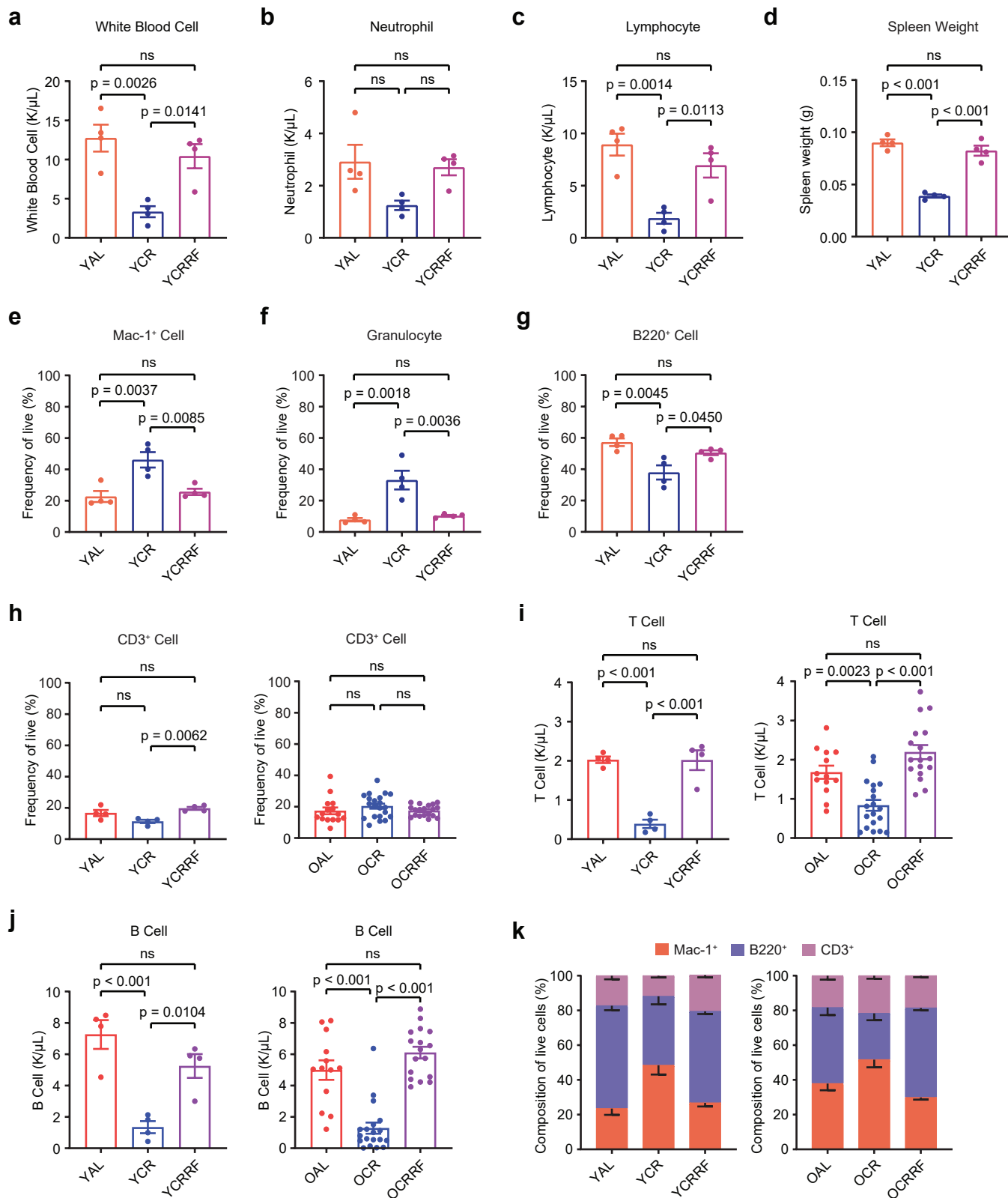
Supplementary Fig. 1: Short-term CR paradigms, and the gating strategy.

(a) Short-term CR paradigms. YAL (Young Ad Libitum), 4 weeks ad libitum feeding; YCR (Young Caloric Restricted), 4 weeks CR; YCRRF (Young Caloric Restricted Re-Fed), 4 weeks ad libitum access to food after 4 weeks CR. Created in BioRender. Ma, F. (2025) <https://BioRender.com/fdntz9h>.

(b) Gating strategy of peripheral blood (PB) analysis.

(c) Gating strategy of whole bone marrow (WBM) analysis.

Supplementary Fig. 2



Supplementary Fig. 2: Complete blood count and FACS analysis of peripheral blood in short-term and lifelong CR mice.

(a-c) Complete blood count of white blood cells (a), neutrophils (b), and lymphocytes (c) in YAL, YCR, and YCRRF mice. Data are represented as mean $\pm$ SEM, n = 4, one-way ANOVA. Source data are provided as a Source Data file.

(d) Spleen weight of YAL, YCR and YCRRF mice. Data are represented as mean $\pm$ SEM, n = 4, one-way ANOVA. Source data are provided as a Source Data file.

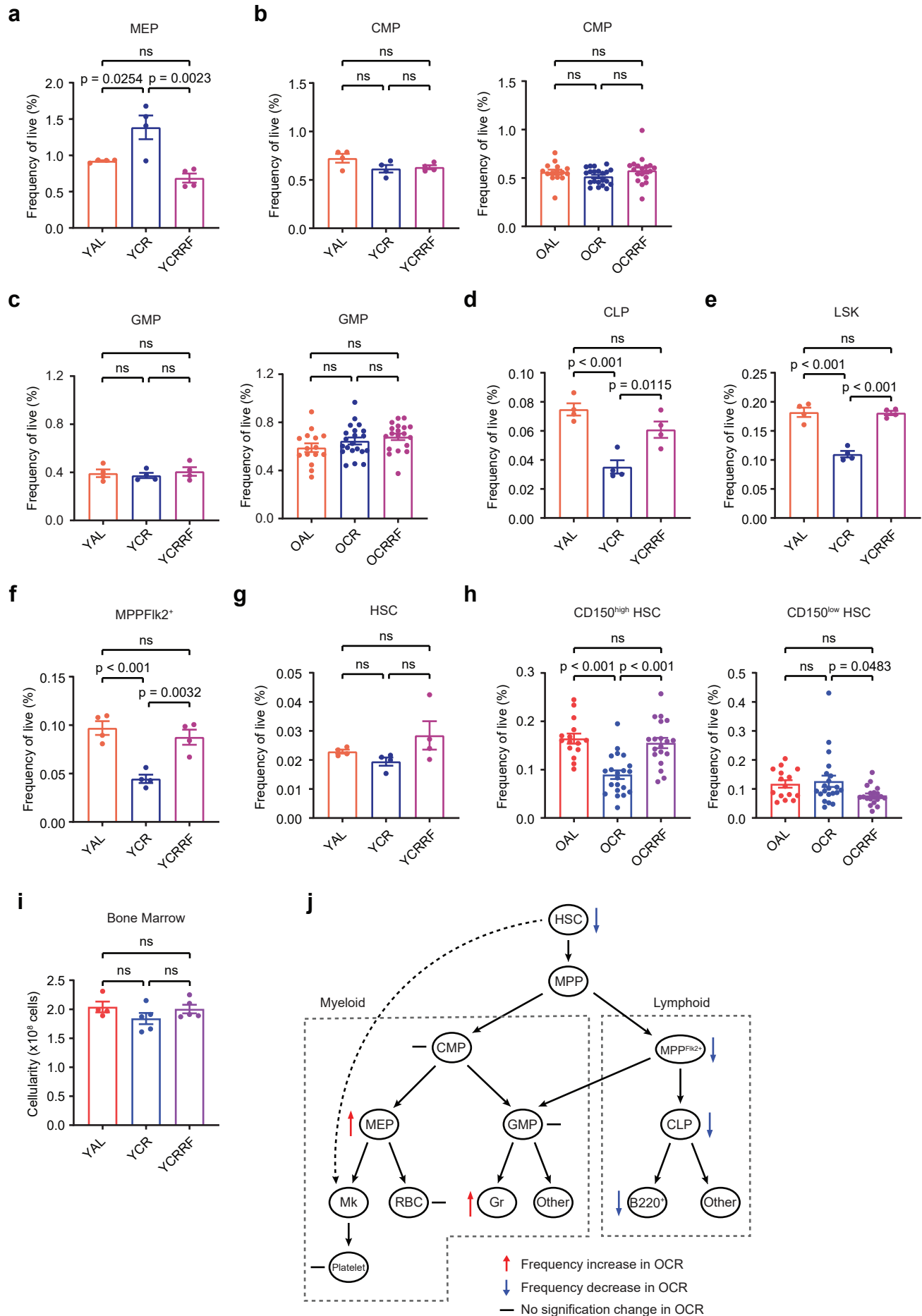
(e-g) Frequency of Mac-1⁺ cells (e), granulocytes (f), and B220⁺ cells (g) in the PB of YAL, YCR, and YCRRF mice. Data are represented as mean $\pm$ SEM, n = 4, one-way ANOVA. Source data are provided as a Source Data file.

(h) Frequency of CD3⁺ cells in the PB of young and old CR mice. Data are represented as mean $\pm$ SEM, young (n = 4), OAL (n = 15), OCR (n = 21), OCRRF (n = 19), one-way ANOVA. Source data are provided as a Source Data file.

(i,j) Absolute count of T cells (i) and B cells (j) in young and old CR mice. Data are represented as mean $\pm$ SEM, young (n = 4), OAL (n = 13), OCR (n = 19), OCRRF (n = 17), one-way ANOVA. Source data are provided as a Source Data file.

(k) Lineage composition of the PB in young and old CR mice. Data are represented as mean $\pm$ SEM, young mice (n = 4), OAL (n = 15), OCR (n = 21), OCRRF (n = 19). Source data are provided as a Source Data file.

Supplementary Fig. 3



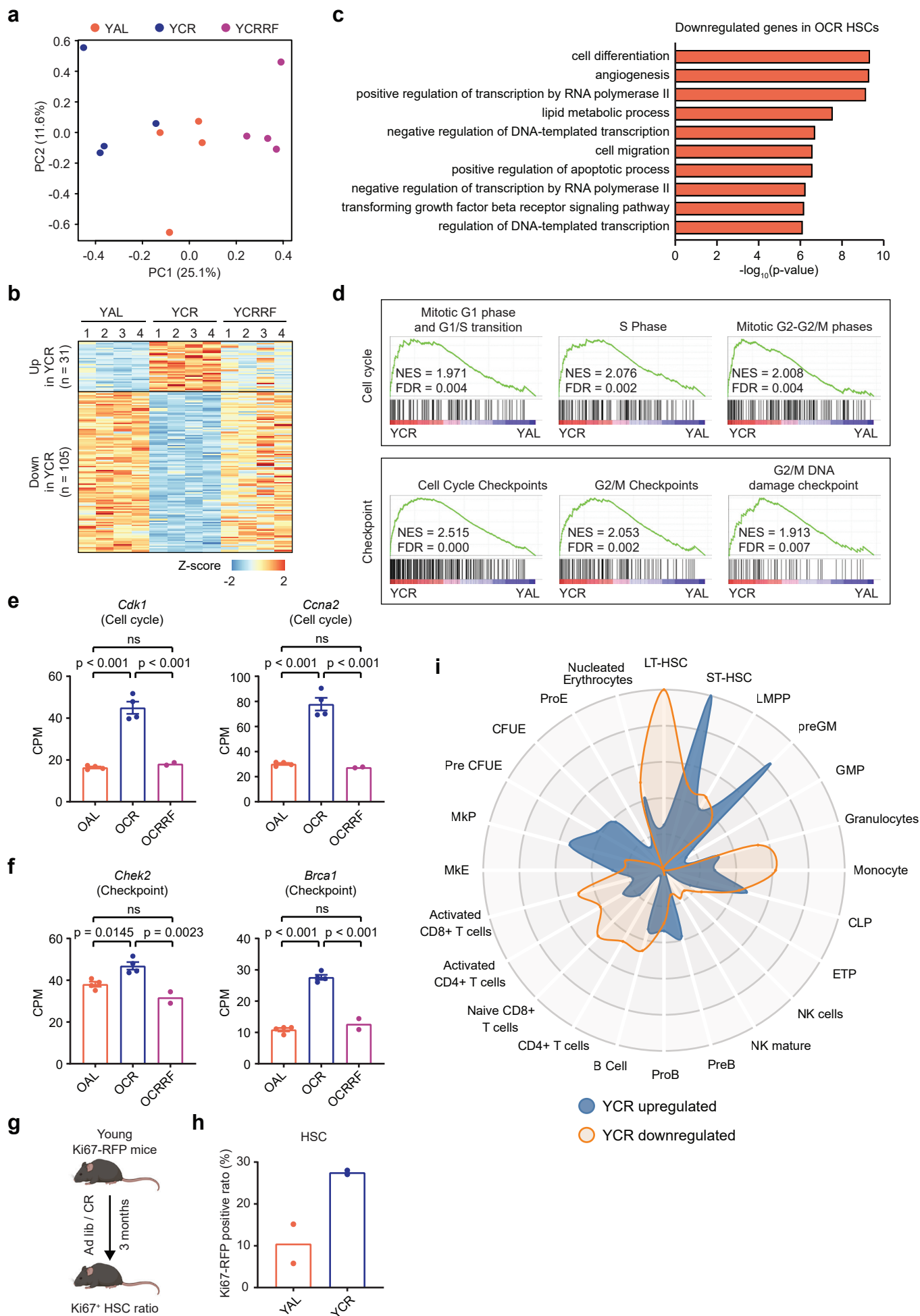
Supplementary Fig. 3: FACS analysis of the WBM in short-term and lifelong CR mice.

(a-h) Frequency of megakaryocyte-erythroid progenitors (MEP) (a), common myeloid progenitors (CMP) (b), granulocyte-monocyte progenitors (GMP) (c), common lymphoid progenitors (CLP) (d), Lin-Sca-1⁺c-Kit⁺ cells (LSK) (e), lymphoid-biased multipotent progenitors (MPPFlk2⁺) (f), and hematopoietic stem cells (HSCs) (g), CD150^{high} and CD150^{low} HSCs (h) in the bone marrow of young and/or old CR mice. Data are represented as mean $\pm$ SEM, young (n = 4), OAL (n = 15), OCR (n = 21), OCRRF (n = 19), one-way ANOVA. Source data are provided as a Source Data file.

(i) Bone marrow cellularity in YAL, YCR, and YCRRF mice. Data are represented as mean $\pm$ SEM, one-way ANOVA. YAL (n = 4), YCR (n = 5), YCRRF (n = 5). Source data are provided as a Source Data file.

(j) Model of hematopoiesis under CR stress showing prioritized myeloid differentiation and repressed lymphoid differentiation.

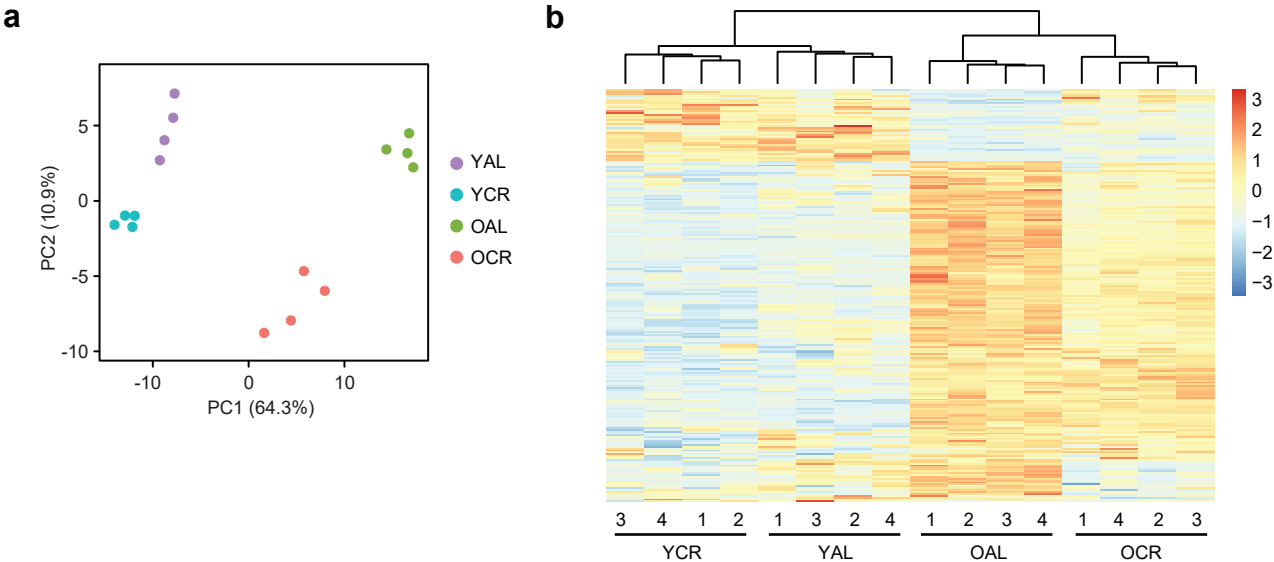
Supplementary Fig. 4



Supplementary Fig. 4: RNA-seq analysis of HSCs from short-term and lifelong CR mice.

- (a) PCA plot of RNA-seq datasets from YAL (n = 4), YCR (n = 4), and YCRRF (n = 4) HSC samples.
- (b) Heatmap of differentially expressed genes (DEGs) in YAL versus YCR comparison ($FC > 1.2$, $FDR < 0.05$). RNA-seq data of HSCs purified from YAL (n = 4), YCR (n = 4), and YCRRF (n = 4) mice were used.
- (c) Pathway analysis of downregulated DEGs in OCR HSCs. Gene Ontology enrichment was performed using DAVID. Enrichment p-values correspond to the DAVID EASE Score (modified one-sided Fisher's exact test) without correction for multiple testing.
- (d) GSEA analysis of cell cycle and checkpoint related pathways using RNA-seq data from YAL and YCR HSCs.
- (e-f) Expression levels of *Cdk1* and *Ccna2* (E), *Chek2* and *Brca1* (F) in OAL (n = 4), OCR (n = 4), and OCRRF (n = 2) HSCs. Data are represented as mean $\pm$ SEM, one-way ANOVA.
- (g) Short-term CR treatment in young Ki67-RFP mice. Ki67-RFP mice (3-4 months old) were subjected to 20% CR for the first week, followed by 40% CR for a total of 3 months. Ad lib fed mice were used as controls. Created in BioRender. Ma, F. (2025) <https://BioRender.com/fdntz9h>.
- (h) Ratio of Ki67-RFP positive, cycling HSCs in ad lib and CR-treated Ki67-RFP mice. YAL (n = 2), YCR (n = 2). Source data are provided as a Source Data file.
- (i) CellRadar plot derived from DEGs of YAL versus YCR comparison ($FC > 1.2$, $FDR < 0.05$). RNA-seq data of HSCs purified from YAL (n = 4), YCR (n = 4) mice were used.

Supplementary Fig. 5

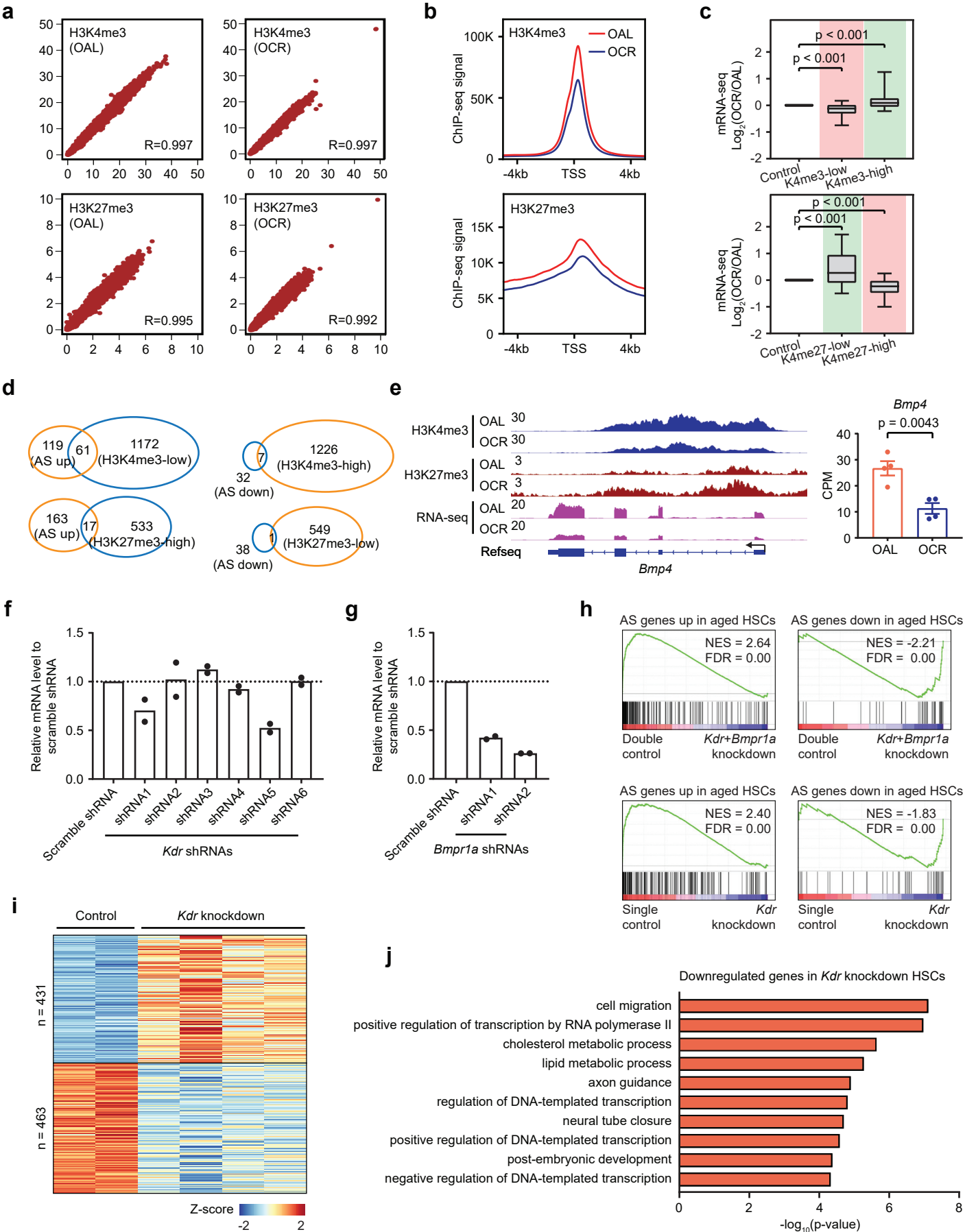


Supplementary Fig. 5: PCA and unsupervised clustering of RNA-seq data from HSCs of short-term and lifelong CR mice.

(a) PCA plot of RNA-seq data from HSCs of short-term and lifelong CR mice (n = 4).

(b) Unsupervised clustering of RNA-seq data from HSCs of short-term and lifelong CR mice (n = 4).

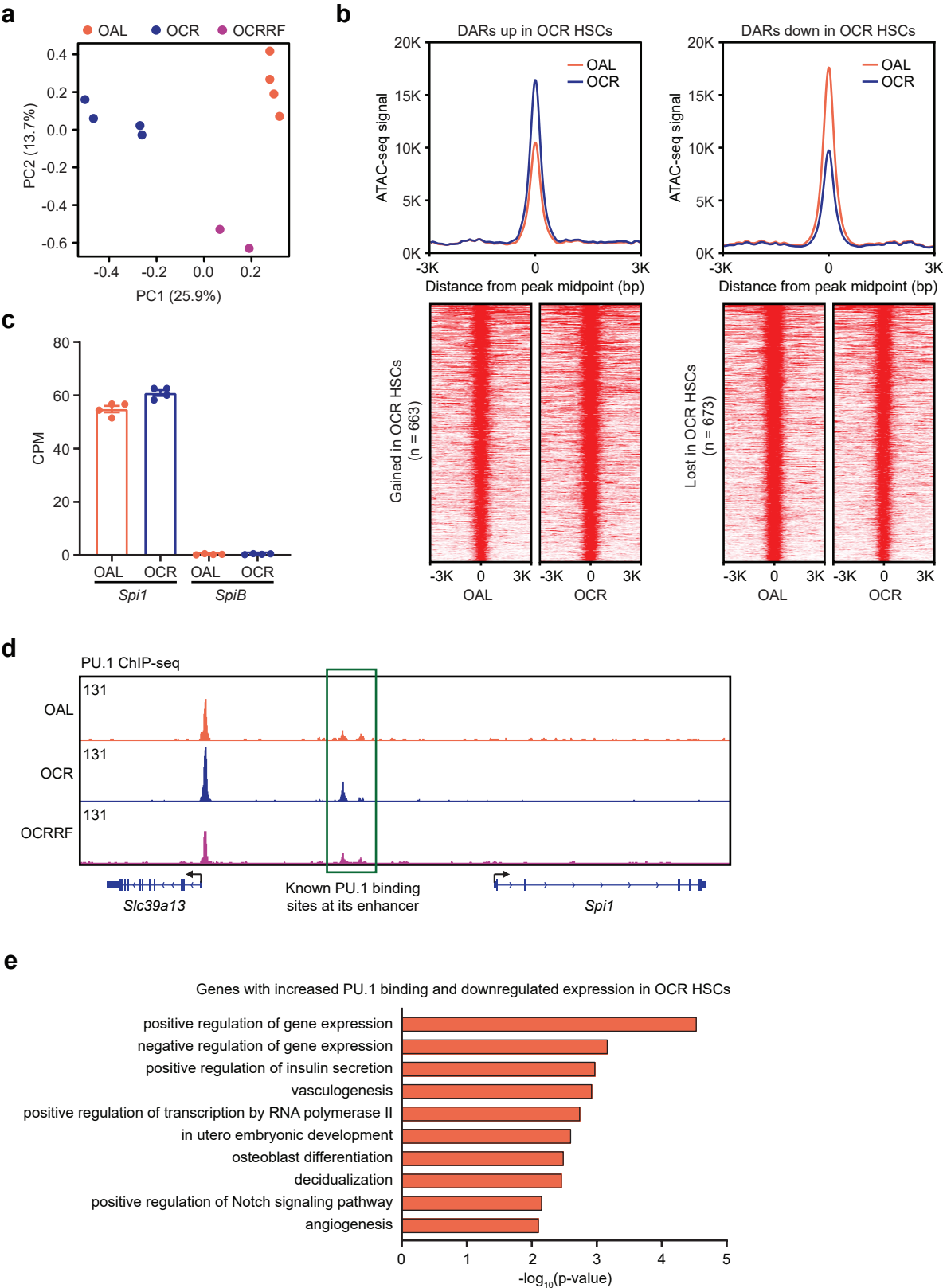
Supplementary Fig. 6



Supplementary Fig. 6: ChIP-seq analysis of H3K4me3 and H3K27me3 in OAL and OCR HSCs, along with the analysis of *Kdr* and/or *Bmpr1a* knockdown in aged HSCs.

- (a) Scatter plots visualizing the correlation between two biological replicates of indicated histone modification ChIP-seqs generated with OAL or OCR HSCs. Pearson correlations are noted.
- (b) Composite plots of H3K4me3 and H3K27me3 levels at the promoters of all genes. TSS, transcription start site.
- (c) Box plots showing gene expression changes for genes categorized as H3K4me3-low and H3K4me3-high (upper) and H3K27me3-low and H3K27me3-high (lower) in OCR HSCs. RNA-seq data from OAL and OCR HSCs were used. Two-tailed t test. Box plots show the median (centre line), the 25th and 75th percentiles (box), and the 5th and 95th percentiles (whiskers).
- (d) Venn diagram illustrating the overlap between AS genes and genes categorized as H3K4me3-high/low or H3K27me3-high/low in OCR HSCs.
- (e) IGV tracks displaying H3K4me3, H3K27me3, and gene expression levels at the *Bmp4* loci. Expression levels of replicates were shown on the right. Data are represented as mean $\pm$ SEM, n = 4, two-tailed t test, p-value: < 0.01 **.
- (f-g) Testing the knockdown efficiency of various shRNAs in vitro using HSCs purified from aged mice. HSCs were sorted into PVA-based media⁴⁰, then infected with shRNA viruses targeting *Kdr* (f) or *Bmpr1a* (g). GFP positive cells were sorted for RNA-seq 3.5 days post virus transduction.
- (h) GSEA plots generated using AS genes. RNA-seq data from *Kdr* and *Bmpr1a* double knockdown (upper two panels) and *Kdr* single knockdown (lower two panels) HSCs were used. Double knockdown control, n = 2; double knockdown, n = 4; single knockdown control, n = 2; single knockdown, n = 4.
- (i) Heatmap of DEGs in control versus *Kdr* knockdown comparison (FC > 1.2, p < 0.05). RNA-seq data of purified control (n = 2) or *Kdr* knockdown (n = 4) HSCs from recipient mice were used.
- (j) Pathway analysis of downregulated DEGs in *Kdr* knockdown HSCs. Gene Ontology enrichment was performed using DAVID. Enrichment p-values correspond to the DAVID EASE Score (modified one-sided Fisher's exact test) without correction for multiple testing.

Supplementary Fig. 7



Supplementary Fig. 7: Chromatin accessibility and PU.1 ChIP-seq analysis in OAL, OCR and OCRRF HSCs.

(a) PCA plot of ATAC-seq datasets from OAL (n = 4), OCR (n = 4), and OCRRF (n = 2) HSC samples.

(b) Heatmap and composite plots of differentially accessible regions (DARs) between OAL and OCR HSCs (FC > 1.5, FDR < 0.01).

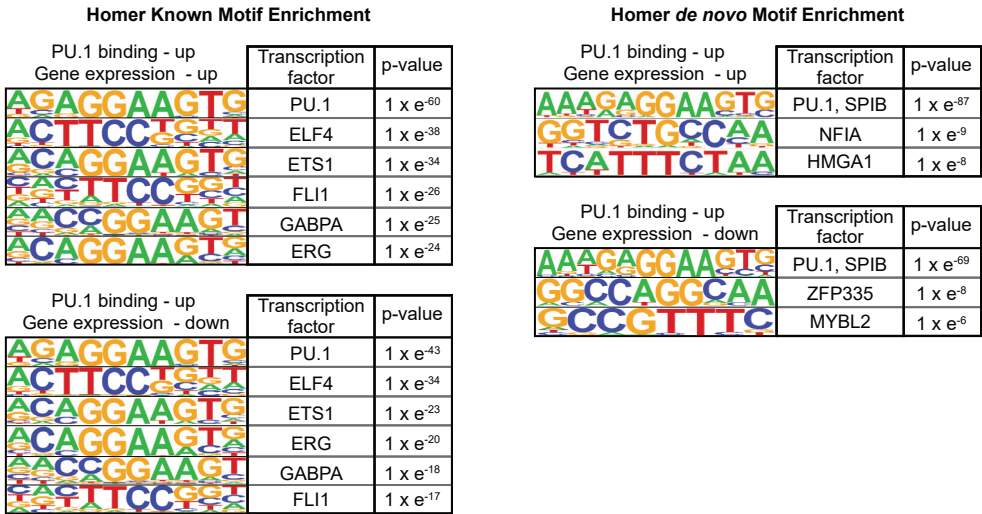
(c) Expression levels of *Spi1* and *SpiB* in OAL (n = 4) and OCR (n = 4) HSCs. Data are represented as mean ± SEM.

(d) IGV tracks displaying PU.1 signals at the *Spi1* loci.

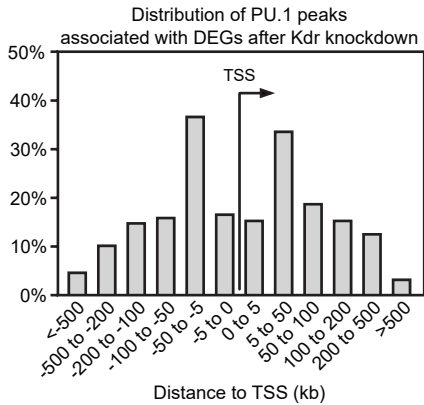
(e) Pathway analysis of genes with increased PU.1 binding and downregulated expression in OCR HSCs. Gene Ontology enrichment was performed using DAVID. Enrichment p-values correspond to the DAVID EASE Score (modified one-sided Fisher's exact test) without correction for multiple testing.

Supplementary Fig. 8

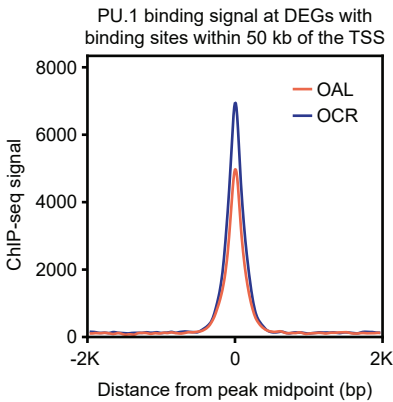
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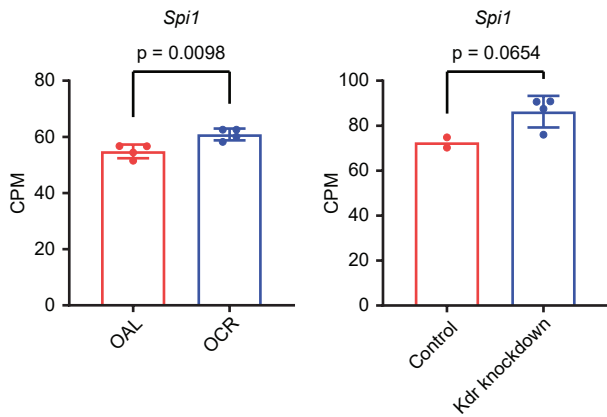
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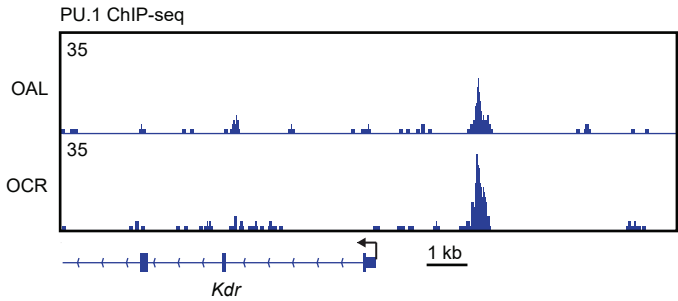
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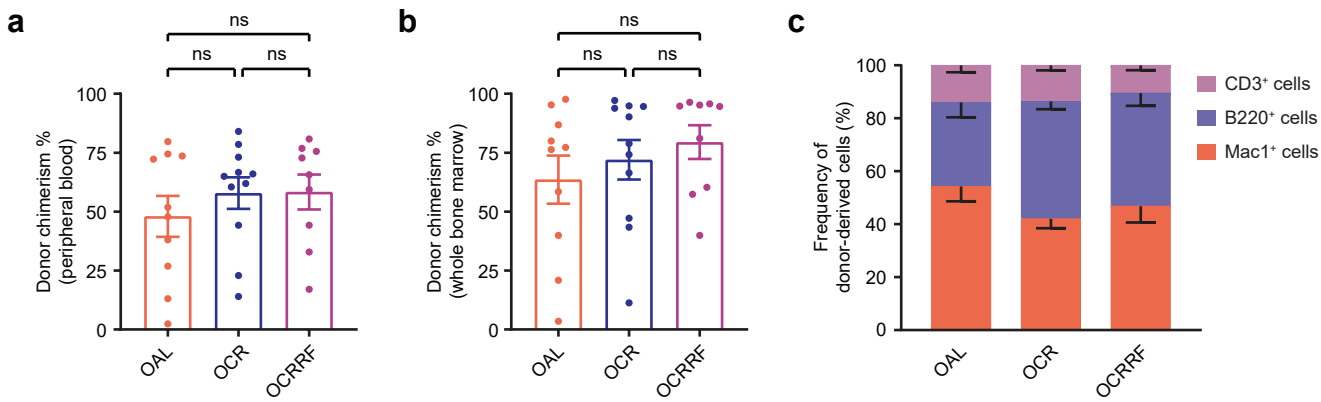
e



Supplementary Fig. 8: PU.1 motif enrichment and link to KDR signaling under CR.

- (a) Transcription factor motif enrichment analysis of increased PU.1 peaks associated with genes upregulated or downregulated after CR. Both known (left) and *de novo* (right) motif analyses were performed using HOMER, examining sequences within ± 200 bp of PU.1 peak centers.
- (b) Distribution of increased PU.1 peaks associated with DEGs after *Kdr* knockdown.
- (c) Composite plot of PU.1 ChIP-seq signals in OAL and OCR HSCs at *Kdr* knockdown associated DEGs with PU.1 binding sites within 50 kb of the TSS.
- (d) *Spi1* expression in HSCs from lifelong CR, and *Kdr* knockdown mice. Data are represented as mean $\pm$ SEM, OAL and OCR (n = 4), control (n = 2), knockdown (n = 4), two-tailed t test.
- (e) IGV tracks showing PU.1 occupancy near the *Kdr* promoter region.

Supplementary Fig. 9



Supplementary Fig. 9: Donor chimerism and lineage output analysis of transplants with HSCs from OAL, OCR, or OCRRF mice.

(a-b) PB (a, 16 weeks) or WBM (b, 21 weeks) donor chimerism of recipient mice transplanted with HSCs purified from OAL, OCR, or OCRRF mice. 200 HSCs (CD45.2) were transplanted into lethally irradiated (9.56 Gy) recipients (CD45.1) together with 2×10^5 WBM cells (CD45.1) via retro-orbital injection. Data are represented as mean $\pm$ SEM, OAL (n = 10), OCR (n = 11), OCRRF (n = 9), one-way ANOVA. Source data are provided as a Source Data file.

(c) Lineage composition of the PB (16 weeks) in recipient mice transplanted with HSCs purified from OAL, OCR, or OCRRF mice. Data are represented as mean $\pm$ SEM, OAL (n = 10), OCR (n = 11), OCRRF (n = 9). Source data are provided as a Source Data file.