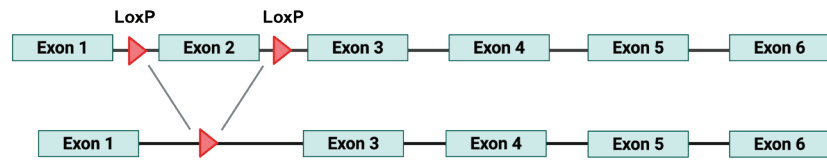
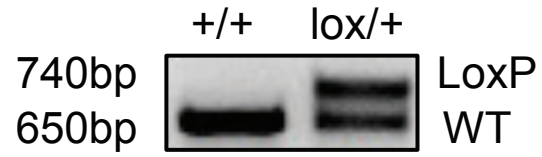
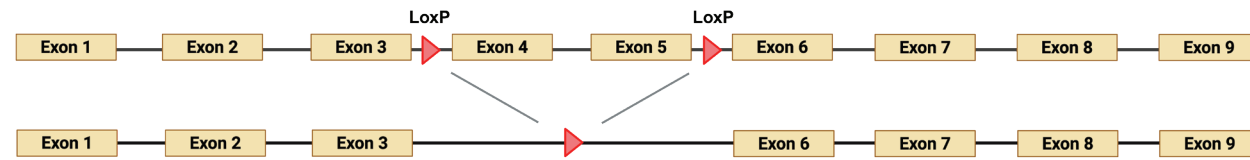
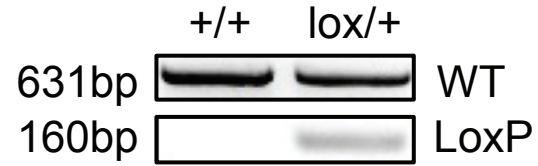
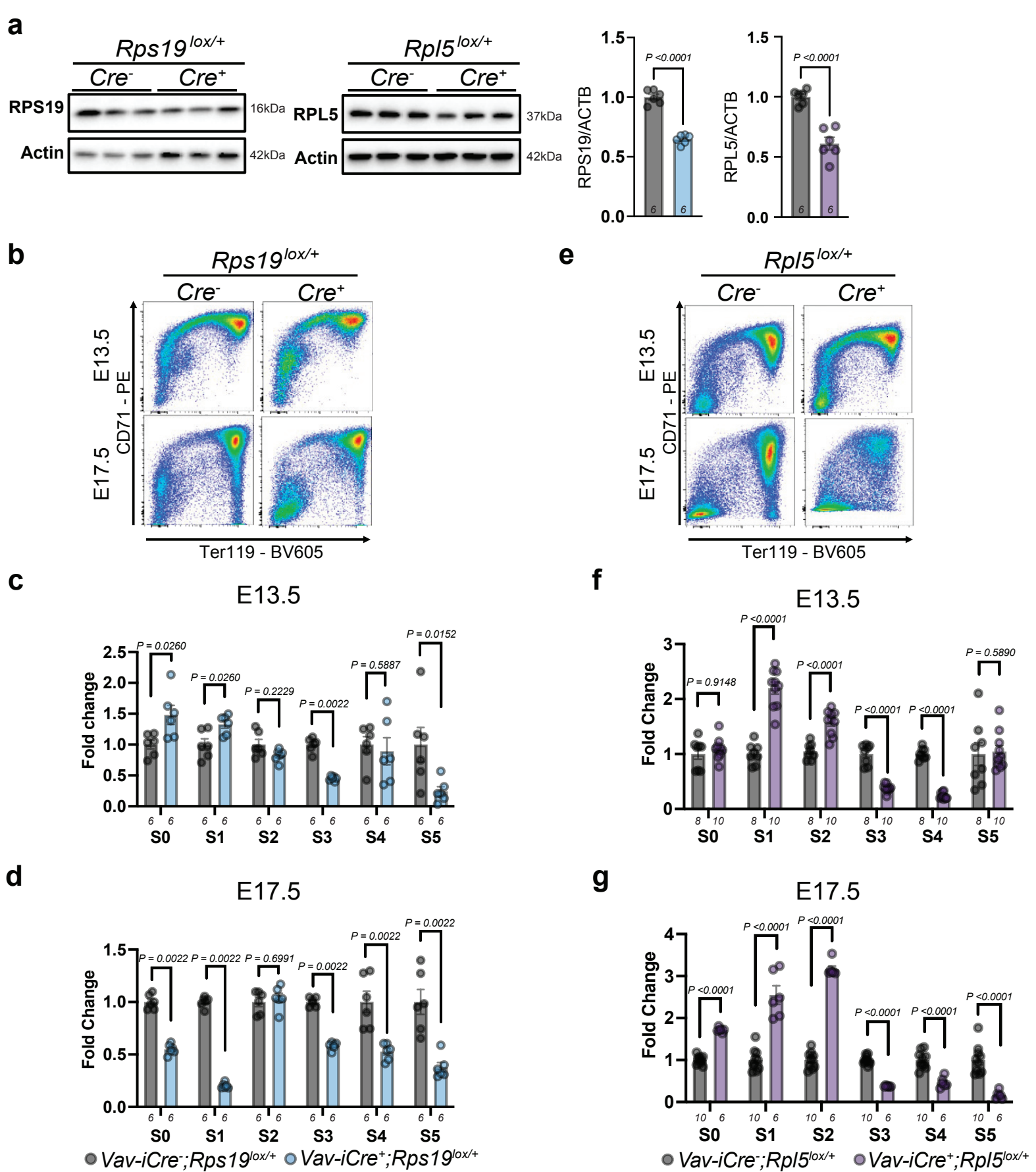


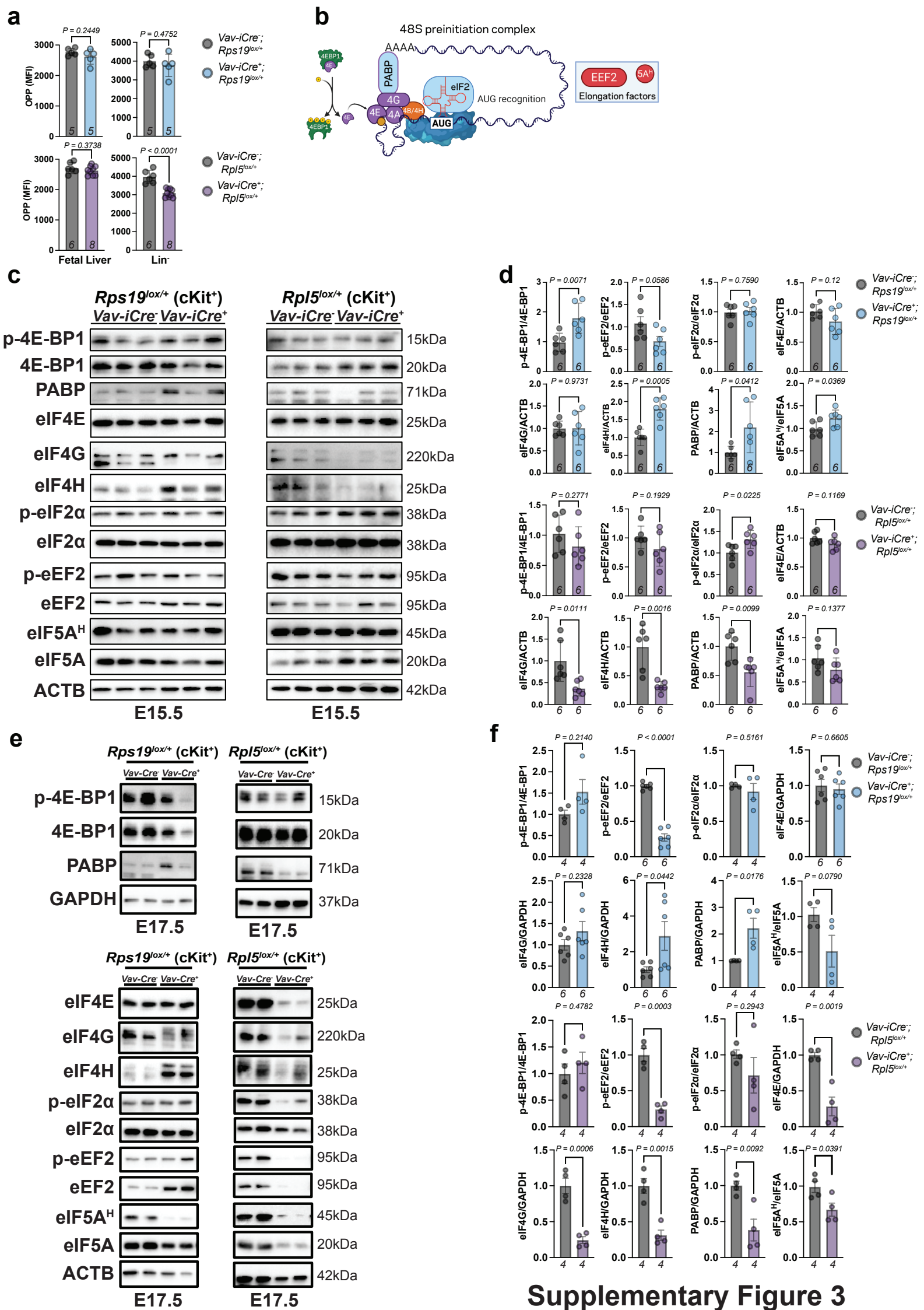
a*Rps19***b***Rpl5***Supplementary Figure 1**

Supplementary Fig. 1 CRISPR/Cas9 generation of *Rps19*lox/lox and *Rpl5*lox/lox mice. (a) Upper panel: sequence of mouse *Rps19* along with the 2 loxP sites flanking exon 2, before and after deletion. Bottom panel: genotyping after *Vav-cre* excision. (b) Upper panel: sequence of mouse *Rpl5* along with the 2 loxP sites flanking exons 4 and 5, before and after deletion. Bottom panel: genotyping after *Vav-cre* excision. Upper panels were created in BioRender. Blanc, L. (2026) <https://BioRender.com/rayglh1>.



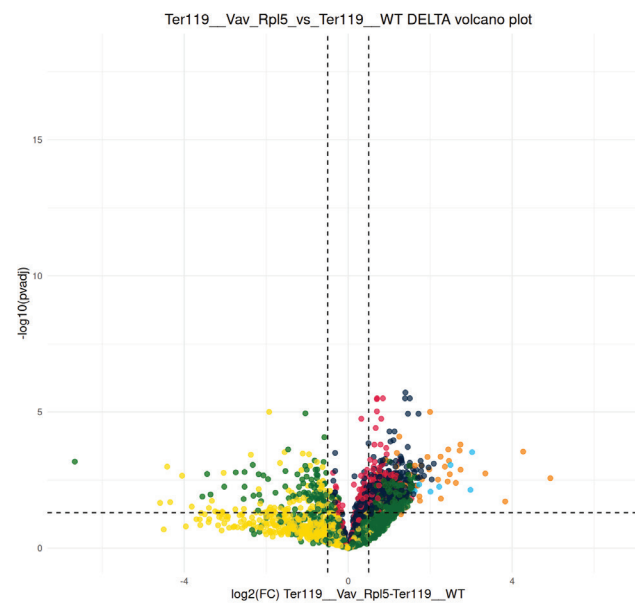
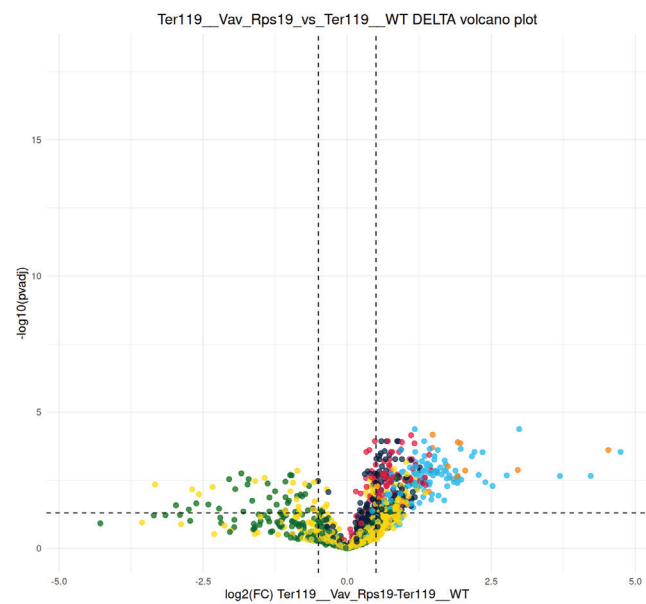
Supplementary Figure 2

Supplementary Fig. 2 Hematopoiesis in *Rps19*^{lox/+} and *Rpl5*^{lox/+} models. (a) Western blot analysis and quantification of RPS19, RPL5 or β -actin in fetal liver (FL) cells from E15.5 control, *Rps19*^{lox/+} or *Rpl5*^{lox/+} embryos. Statistical analysis was performed using two-tailed unpaired t-test. (b) Representative flow cytogram of erythropoiesis in E13.5 and E17.5 control and *Rps19*^{lox/+} embryos. (c) Erythroid populations from S0 to S5 in E13.5 control and *Rps19*^{lox/+} embryos expressed as a fold change relative to control. Statistical analysis was performed using multiple Mann-Whitney test. (d) Erythroid populations from S0 to S5 in E17.5 control and *Rps19*^{lox/+} embryos expressed as a fold change relative to control. Statistical analysis was performed using multiple Mann-Whitney test. (e) Representative flow cytogram of erythropoiesis in E13.5 and E17.5 control and *Rpl5*^{lox/+} embryos. (f) Erythroid populations from S0 to S5 in E13.5 control and *Rpl5*^{lox/+} embryos expressed as a fold change relative to control. Statistical analysis was performed using multiple Mann-Whitney test. (g) Erythroid populations from S0 to S5 in E17.5 control and *Rpl5*^{lox/+} embryos expressed as a fold change relative to control. Statistical analysis was performed using multiple Mann-Whitney test. The number of biological replicates (*n*) is indicated in italics under each histogram. All data are presented as mean \pm standard deviation. Source data are presented in Source Data file.



Supplementary Figure 3

Supplementary Fig. 3 Translational regulation at E17.5 in *Rps19*^{lox/+} and *Rpl5*^{lox/+} mice. (a) Quantification of global translation in total fetal livers and-Lin⁻ populations as measured by OPP in E13.5 control, *Rps19*^{lox/+} or *Rpl5*^{lox/+} embryos. Statistical analysis was performed using two-tailed unpaired t-test. **(b)** Schematics of the main initiation and elongation factors involved in eukaryotic translation. Created in BioRender. Created in BioRender. Papoin, J. (2026) <https://BioRender.com/e7ln1zl>. **(c)** Western blot analysis of regulators of translation in FL-derived ckit⁺ cells from either E15.5 control versus *Rps19*^{lox/+} embryos or E15.5 control versus *Rpl5*^{lox/+} embryos. **(d)** Quantification of the western blots for the regulators of translation in FL-derived ckit⁺ cells from either E15.5 control versus *Rps19*^{lox/+} embryos or E15.5 control versus *Rpl5*^{lox/+} embryos. Statistical analysis was performed using two-tailed unpaired t-test. **(e)** Western blot analysis of regulators of translation in FL-derived ckit⁺ cells either from E17.5 control versus *Rps19*^{lox/+} embryos or E17.5 control versus *Rpl5*^{lox/+} embryos. **(f)** Quantification of the western blots for the regulators of translation in FL-derived ckit⁺ cells from E17.5 control versus *Rps19*^{lox/+} embryos or E17.5 control versus *Rpl5*^{lox/+} embryos. Statistical analysis was performed using two-tailed unpaired t-test. The number of biological replicates (*n*) is indicated in italics under each histogram. All data are presented as mean ± standard deviation. Source data are presented in Source Data file.



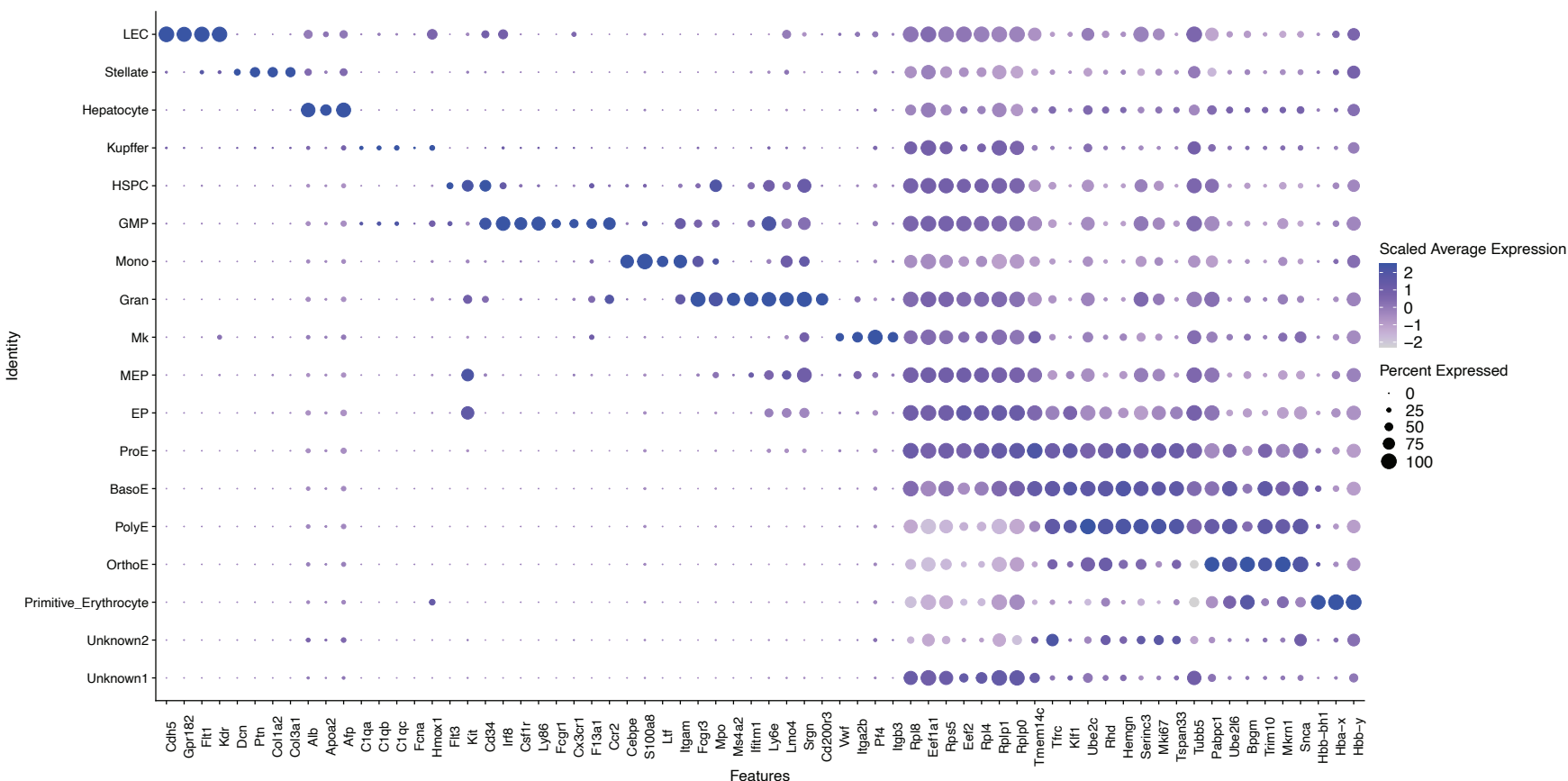
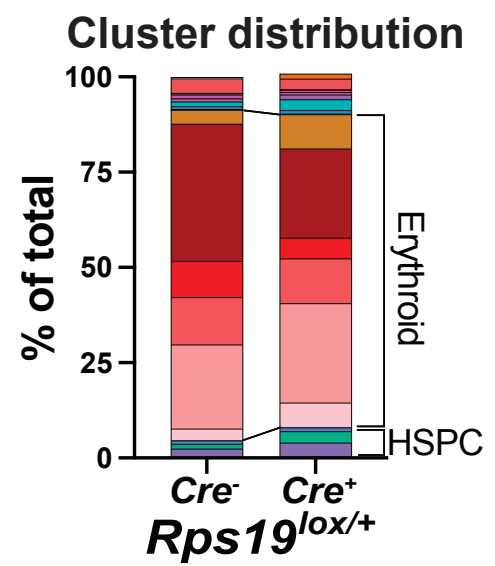
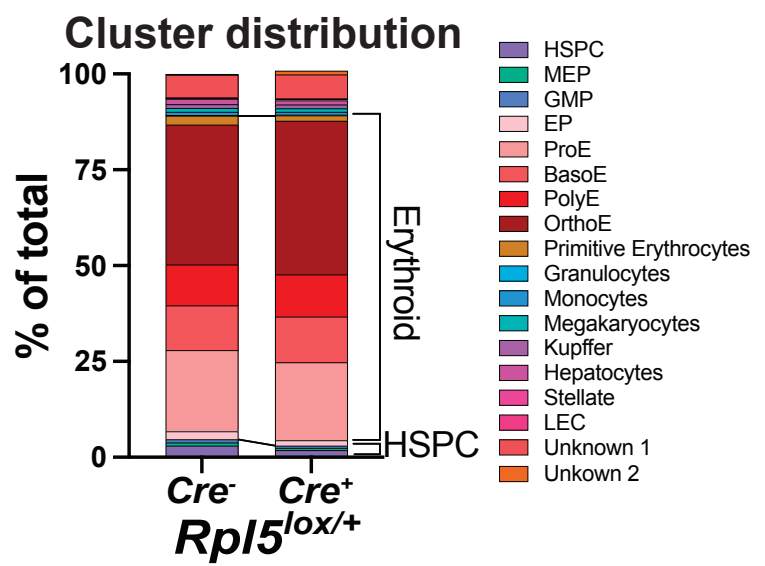
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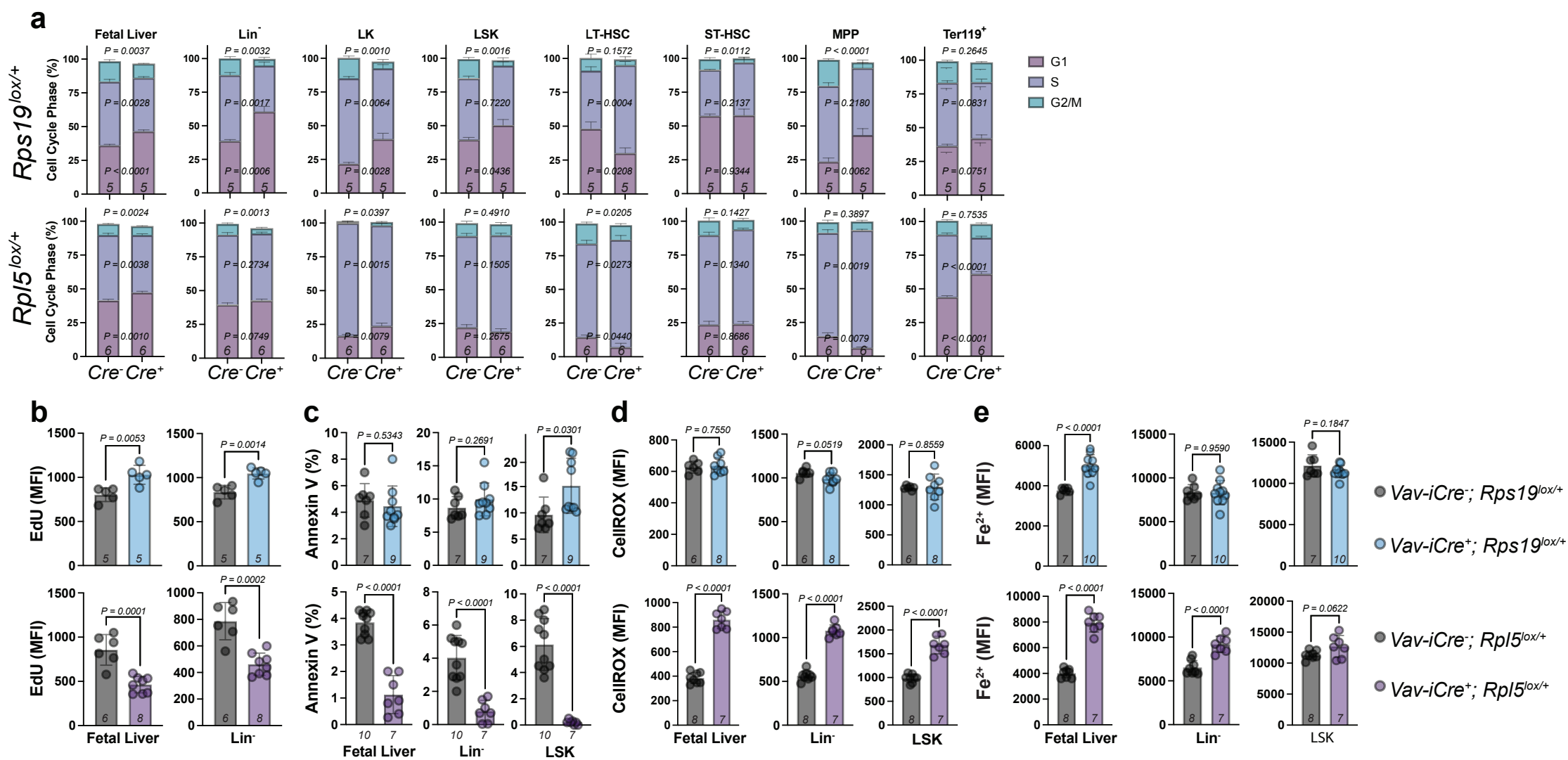


Supplementary Figure 4

Supplementary Fig. 4 Statistical evaluation of translational regulation. Variation in translational expression (Δ -poly) and transcriptional expression (Δ -cyto) in Ter119⁺ cell populations in E15.5 control, *Rps19*^{lox/+} or *Rpl5*^{lox/+} and in cKit⁺ cell population from *Rps19*^{lox/+} embryos. Source data are presented in Source Data file.

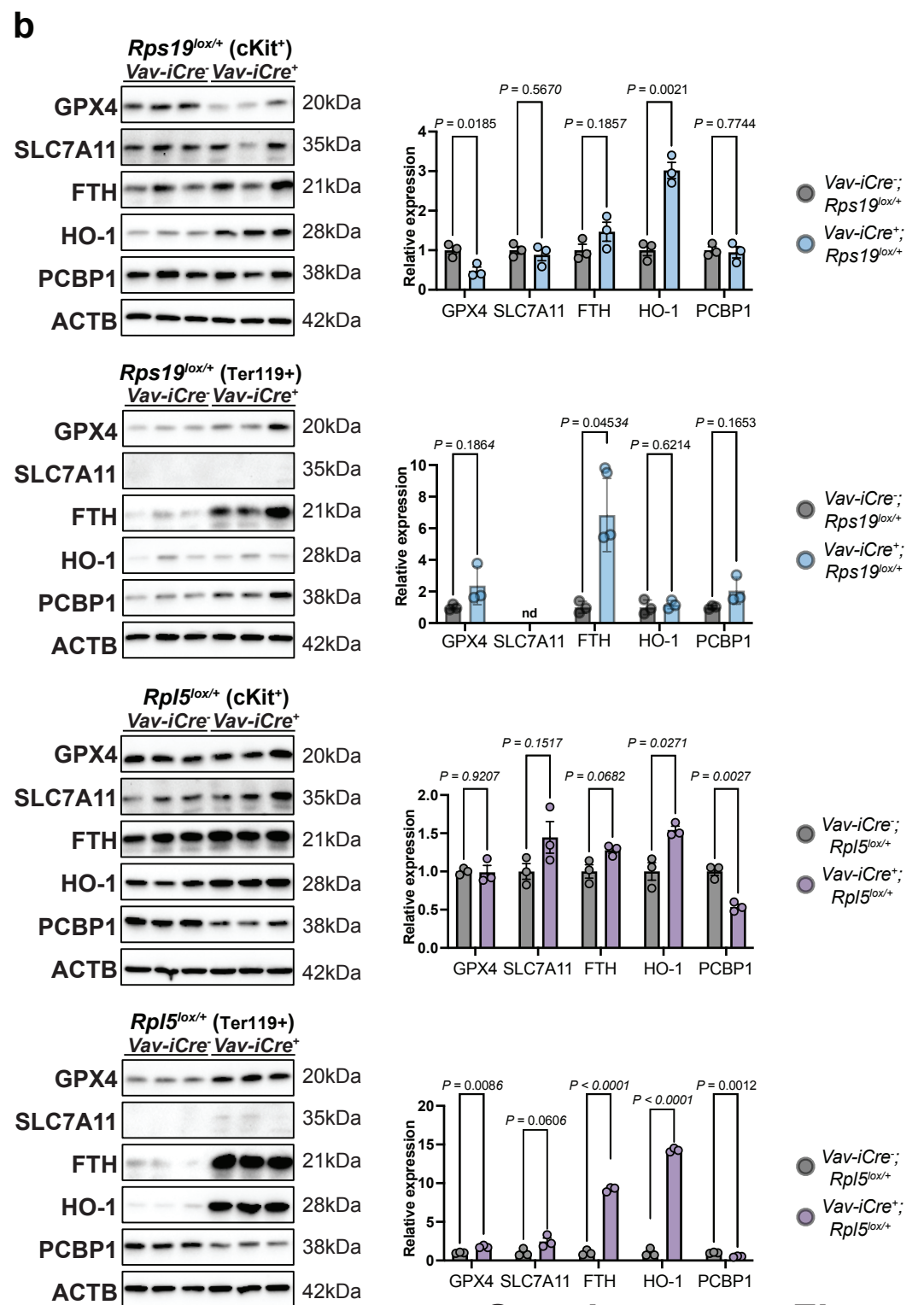
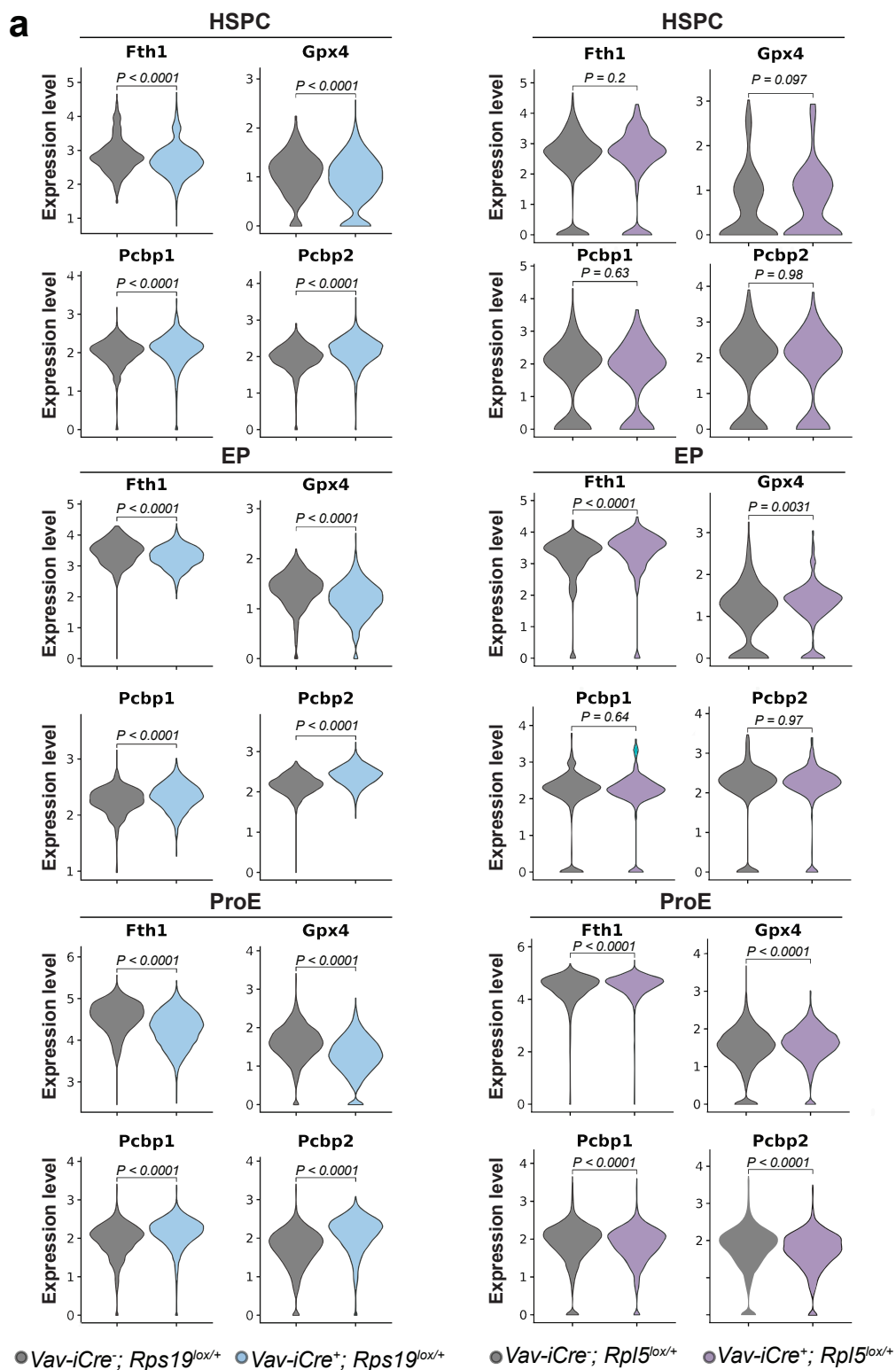
a**b****c****Supplementary Figure 5**

Supplementary Fig. 5 Identification of cell populations. (a) Bubble plots showing the expression of marker genes for cell annotation in each cell type. (b) Distribution of clusters from *Rps19*^{lox/+} FL hematopoietic cells. (c) Distribution of clusters from *Rpl5*^{lox/+} FL hematopoietic cells. (d) pseudobulk gene-expression profiles (Pearson $r > 0.98$) and cluster abundances (Spearman $r = 0.92\text{--}0.98$) for control, *Rps19*^{lox/+} and *Rpl5*^{lox/+} replicates. Source data are presented in Source Data file.



Supplementary Figure 6

Supplementary Fig. 6 Cell cycle phase distribution and quantification of apoptosis and oxidative stress in HSPC populations. **(a)** G1, S and G2/M phase distribution in total FL cells and all different HSPC populations in E13.5 control, *Rps19*^{lox/+} or *Rpl5*^{lox/+} embryos. **(b)** Quantification of the cell cycle speed by measuring the S phase (EdU⁺) MFI in the total FL cells and among the indicated HSPC populations in E13.5 control, *Rps19*^{lox/+} or *Rpl5*^{lox/+} embryos. Statistical analysis was performed using welch t-test with Holm-Šidák correction. **(c)** Percentage of Annexin V⁺ cells as marker of apoptosis in E15.5 control, *Rps19*^{lox/+} or *Rpl5*^{lox/+} embryos. Statistical analysis was performed using two-tailed unpaired t-test. **(d)** Quantification of cellular ROS levels of FL cells and HSPC populations as measured by CellROX dye in E15.5 control, *Rps19*^{lox/+} or *Rpl5*^{lox/+} embryos. Statistical analysis was performed using two-tailed unpaired t-test. **(e)** Quantification of ferrous iron levels of FL cells and HSPC populations measured as by Fe²⁺ biotracker dye in E15.5 control, *Rps19*^{lox/+} or *Rpl5*^{lox/+} embryos. Statistical analysis was performed using two-tailed unpaired t-test. The number of biological replicates (*n*) is indicated in italics under each histogram. All data are presented as mean ± standard deviation. Source data are presented in Source Data file.



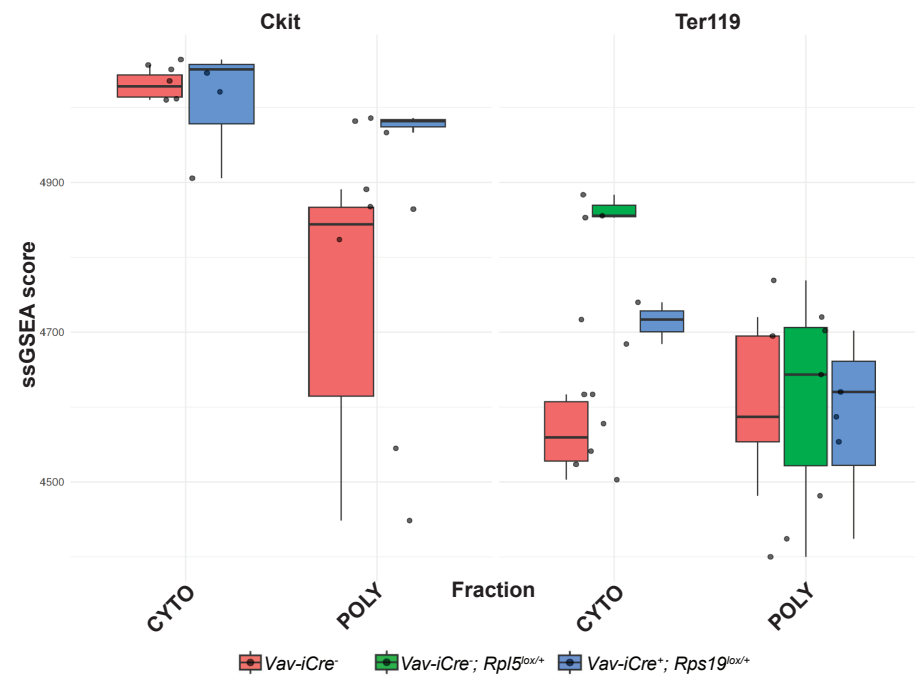
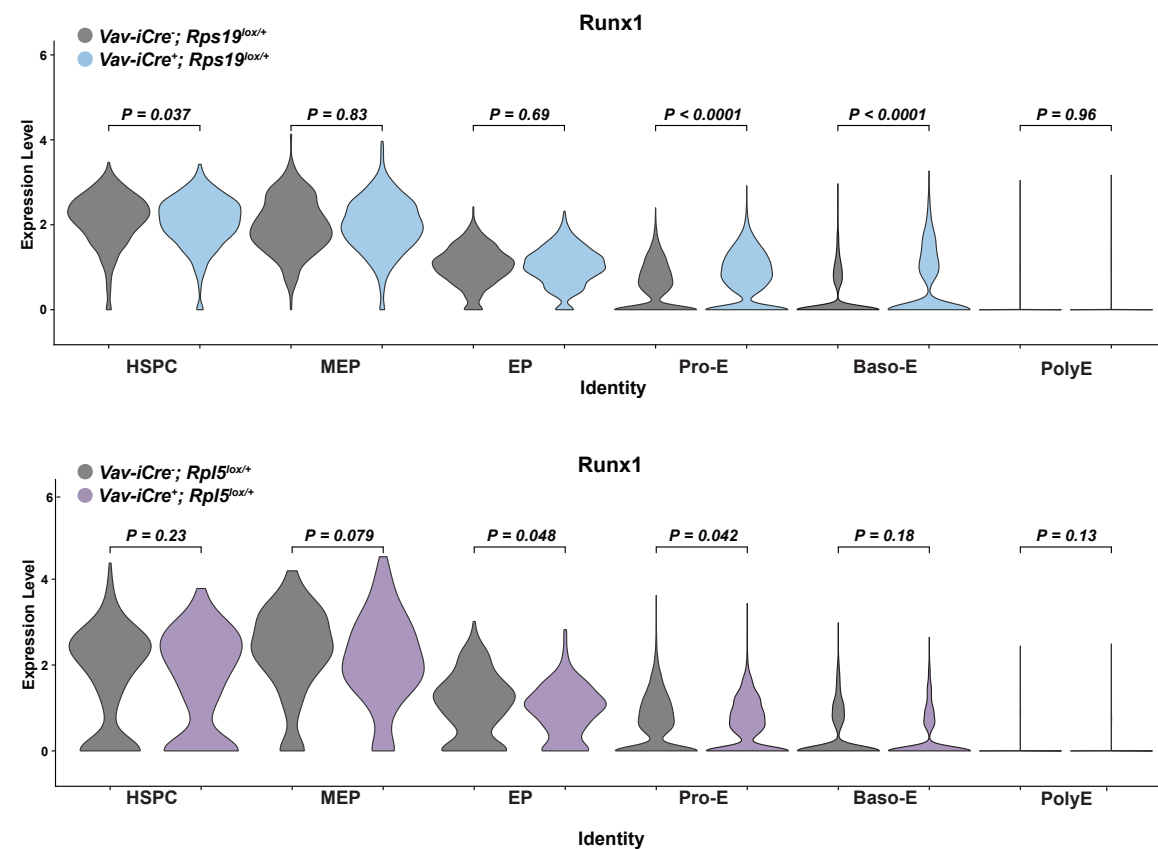
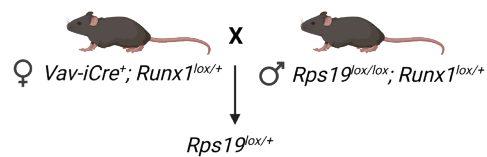
Supplementary Figure 7

Supplementary Fig. 7 Expression of genes related to ferroptosis in RPS19 and RPL5 haploinsufficient HSPCs, erythroid progenitors (EP) and ProErythroblasts (ProE).

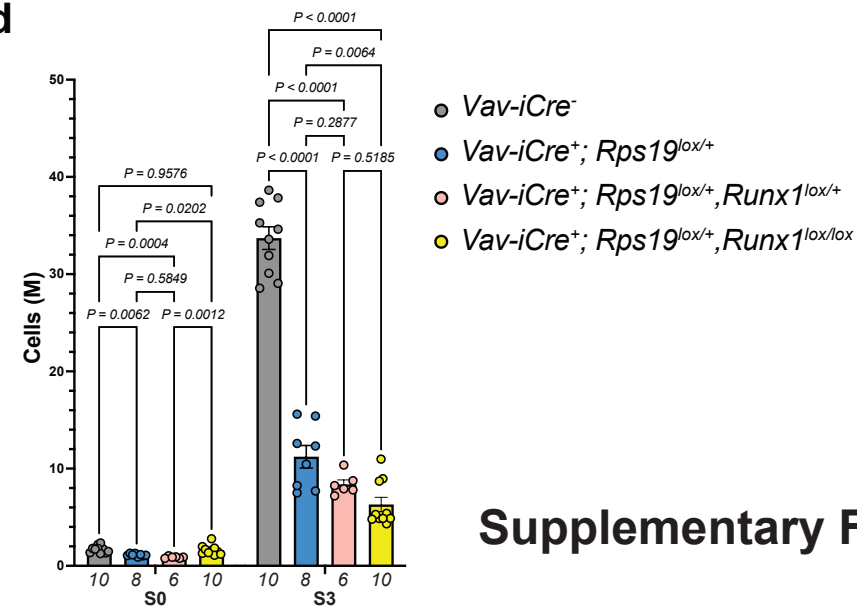
(a) Violin plot from scRNA-seq presented in Fig. 4 plots showing the expression of ferroptosis markers *Fth1*, *Gpx4*, *Pcbp1* and *Pcbp2* in HSPC, EP and ProE cell type from *Rps19*^{lox/+} and *Rpl5*^{lox/+} embryos. Statistical analysis was performed using Wilcoxon rank test (non parametric). **(b)** Western blot analyses and quantification of ferroptosis regulators in FL-derived ckit⁺ cells from either E15.5 control versus *Rps19*^{lox/+} embryos or E15.5 control versus *Rpl5*^{lox/+} embryos. n = 3. Statistical analysis was performed using two-tailed unpaired t-test. All data are presented as mean ± standard deviation. Source data are presented in Source Data file.

a

REACTOME_RUNX1_REGULATES_TRANSCRIPTION_OF_GENES_INVOLVED_IN_DIFFERENTIATION_OF_HSCS Pathway

**b****c**

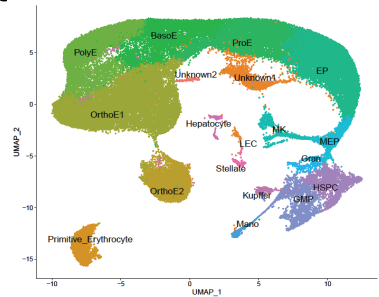
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P28	32	0	0	0	32

d

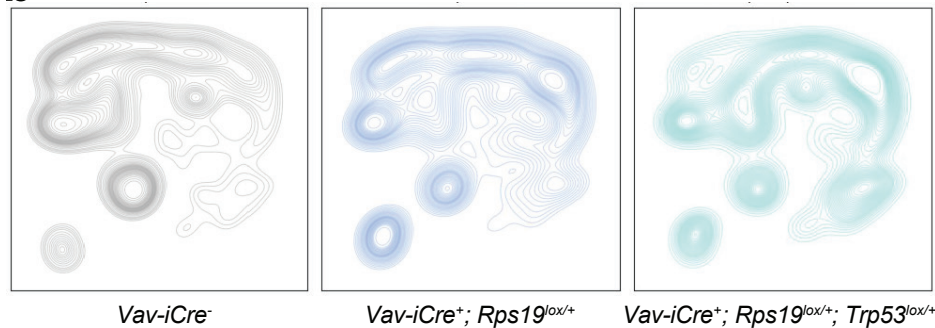
Supplementary Figure 8

Supplementary Fig. 8 Runx1 expression in RPS19 and RPL5 haploinsufficient mice and knockout in *Rps19*^{lox/+} mice. (a) pathM2 ssGSEA analysis highlighting RUNX1 transcriptional regulation of genes involved in differentiation of HSCs in polysome sequencing performed on ckit+ population from E15.5 control and *Rps19*^{lox/+} embryos. (b) Violin plot from scRNA-seq presented in Fig. 4 plots showing the expression of *Runx1* in each cell type from *Rps19*^{lox/+} and *Rpl5*^{lox/+} embryos. Statistical analysis was performed using Wilcoxon rank test (non parametric). (c) Genotype from *Rps19*^{lox/+}; *Runx1*^{lox/lox} intercrossing. Created in BioRender. Blanc, L. (2026) <https://BioRender.com/ql4svuv>. (d) Quantification of cells in S0 and S3 in E17.5 control, *Rps19*^{lox/+}, *Rps19*^{lox/+}; *Runx1*^{lox/+} and *Rps19*^{lox/+}; *Runx1*^{lox/lox} embryos. Statistical analysis was performed using ordinary one-way ANOVA with Tukey correction. The number of biological replicates (*n*) is indicated in italics under each histogram. All data are presented as mean ± standard deviation. Source data are presented in Source Data file.

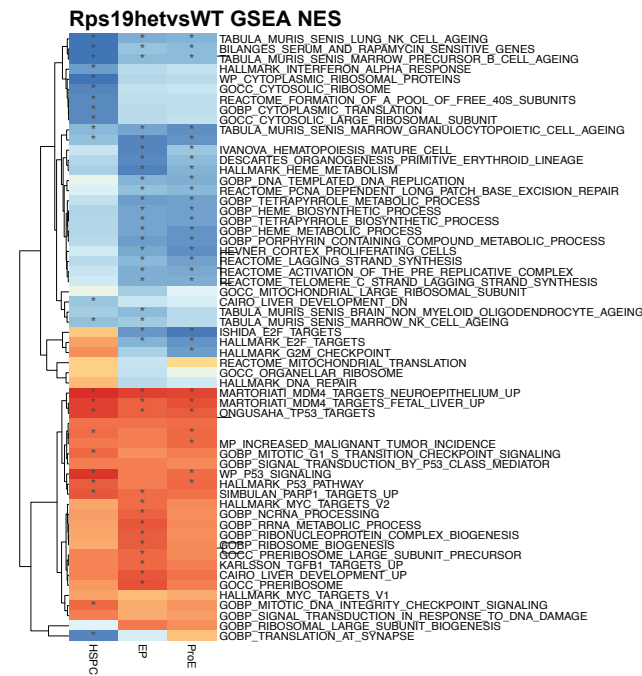
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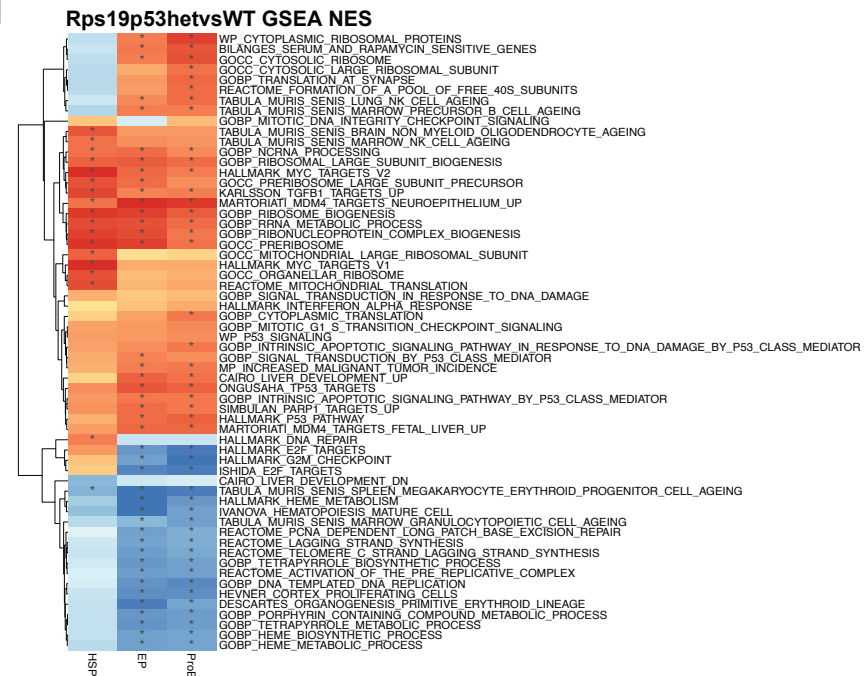
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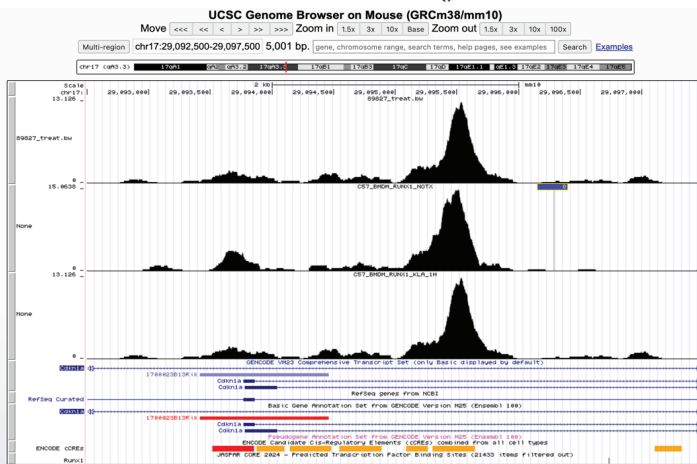


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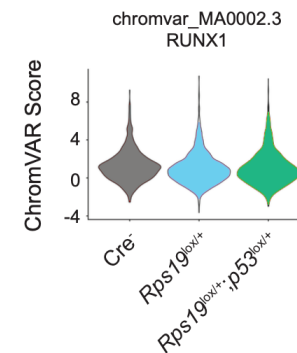


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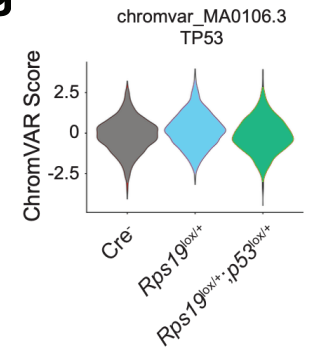
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f



g



Supplementary Figure 9

Supplementary Fig. 9 scRNAseq analyses from control, *Rps19*^{lox/+} and *Rps19*^{lox/+}; *p53*^{lox/+} embryos at E13.5. (a) scRNA-seq UMAP of integrated FL from control, *Rps19*^{lox/+} and *Rps19*^{lox/+}; *p53*^{lox/+} cells with clusters identified by marker genes. **(b)** Density projection of cells on UMAP from control, *Rps19*^{lox/+} and *Rps19*^{lox/+}; *p53*^{lox/+} FL cells. **(c)** Heatmap showing gene set enrichment analysis (GSEA) normalized enrichment scores (NES) for selected modules significantly enriched in hematopoietic stem and progenitor cell (HSPC), erythroid progenitor (EP) or proerythroblast (ProE) populations from control and *Rps19*^{lox/+} embryos. Asterisk (*) indicates FDR < 0.05. **(d)** Heatmap showing gene set enrichment analysis (GSEA) normalized enrichment scores (NES) for selected modules significantly enriched in hematopoietic stem and progenitor cell (HSPC), erythroid progenitor (EP) or proerythroblast (ProE) populations from control and *Rps19*^{lox/+}; *p53*^{lox/+} embryos. Asterisk (*) indicates FDR < 0.05. **(e)** Data from the ENCODE project showing RUNX1 binding region on *Cdkn1a* detected by ChIP- seq. **(f)** ChromVAR score for the RUNX1 (MA0002.3) motif in control, *Rps19*^{lox/+} and *Rps19*^{lox/+}; *p53*^{lox/+} FL cells. **(g)** ChromVAR score for the TP53 (MA0106.3) motif in control, *Rps19*^{lox/+} and *Rps19*^{lox/+}; *p53*^{lox/+} FL cells. Source data are presented in Source Data file.

Rps19 Effects:

Covariate	Cell Type	Final Parameter
Condition[T.LioRps19het]	BasoE	0
Condition[T.LioRps19het]	Basophil	0
Condition[T.LioRps19het]	EP	0.568684
Condition[T.LioRps19het]	GMP	0
Condition[T.LioRps19het]	Gran	0
Condition[T.LioRps19het]	HSPC	0
Condition[T.LioRps19het]	Hepatocyte	0
Condition[T.LioRps19het]	Kupffer	0
Condition[T.LioRps19het]	LEC	0
Condition[T.LioRps19het]	MEP	0.662706
Condition[T.LioRps19het]	Mk	0.571598
Condition[T.LioRps19het]	OrthoE	-0.578451
Condition[T.LioRps19het]	PolyE	-0.705538
Condition[T.LioRps19het]	Primitive Erythrocyte	0.72023
Condition[T.LioRps19het]	ProE	0
Condition[T.LioRps19het]	Stellate	0
Condition[T.LioRps19het]	Unknown1	-0.435799
Condition[T.LioRps19het]	Unknown2	0

Rpl5 Effects:

Covariate	Cell Type	Final Parameter
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Condition[T.LioRpl5het]	Basophil	0
Condition[T.LioRpl5het]	EP	0
Condition[T.LioRpl5het]	GMP	0
Condition[T.LioRpl5het]	Gran	0
Condition[T.LioRpl5het]	HSPC	0
Condition[T.LioRpl5het]	Hepatocyte	0
Condition[T.LioRpl5het]	Kupffer	0
Condition[T.LioRpl5het]	LEC	0
Condition[T.LioRpl5het]	MEP	0
Condition[T.LioRpl5het]	Mk	0
Condition[T.LioRpl5het]	OrthoE	0
Condition[T.LioRpl5het]	PolyE	0
Condition[T.LioRpl5het]	Primitive Erythrocyte	0
Condition[T.LioRpl5het]	ProE	0
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Condition[T.LioRpl5het]	Unknown2	0

Supplementary Table 1. Results of scCODA analyses.