**Supplemental Materials and Methods**

*CUT&RUN-seq*

Protein A-MNase was kindly provided by Dr. Steven Henikoff (Fred Hutchinson Cancer Research Center, Seattle, WA, USA). One million erythroblasts were sorted from wild-type and *Gata1s* embryos for analysis of GATA1 and GATA1s chromatin occupancy and half million erythroblasts were collected for H3K27me3 analysis. Briefly, nuclei were isolated in NE buffer (20 mM HEPES-KOH, pH 7.9, 10 mM KCl, 0.5 mM spermidine, 0.1% Triton X-100, 20% Glycerol and protease inhibitor cocktails from Roche), captured with BioMag Plus Concanavalin A (Polysciences, 86057-3) in binding buffer (20 mM HEPES-KOH, pH 7.9, 10 mM KCl, 1 mM $CaCl_2$, 1 mM $MnCl_2$ and protease inhibitor cocktails from Roche) and incubated with primary antibodies in wash buffer (20 mM HEPES-KOH, pH 7.5, 150 mM NaCl, 0.5 mM spermidine, 0.1% BSA and protease inhibitor cocktails from Roche) for 2 hours at 4°C with rotation. Antibodies included anti-GATA1/GATA1s (Abcam, ab11852, 1:100), anti-H3K27me3 (Millipore, 07-449, 1:100) and rabbit control IgG (Abcam, ab46540). After washing the nuclei twice with wash buffer, pA-MNase was added at 1:400 (Batch #6) and incubated for 1 hour at 4°C. Digestion was carried out on ice water. $CaCl_2$ was then added to a final concentration of 2 mM to activate pA-MNase. The digestion time was 15 minutes for GATA1/GATA1s and 10 minutes for H3K27me3. The reaction was stopped by addition of equal volume of 2x STOP buffer (200 mM NaCl, 20 mM EDTA, 4 mM EGTA, 50 μg/mL RNase A and 40 μg/mL glycogen). DNA was released into the supernatant and extracted using Phenol/Chloroform.

A modified protocol of NEBNext Ultra II DNA Library Prep Kit for Illumina (NEB, E7645) was used to prepare the CUT&RUN-seq library. Briefly, the dA-tailing temperature was decreased to 50°C, and the reaction time was increased to 1 hour to compensate for lower enzymatic activity. Next, 2.1x AMPure XP beads (Beckman, A63881) were added for cleanup of adaptor-ligated DNA without size selection. After PCR amplification, the reaction was cleaned up with AMPure XP beads.

We applied an *in-house* shell script to process the sequencing data. Briefly, TrimGalore (http://www.bioinformatics.babraham.ac.uk/projects/trim_galore/) was used to remove adaptor sequence. We applied Bowtie 2[1] to align reads to the reference genome (UCSC mm9). We used MACS1.4[2] to call the binding peaks. P values of differentially enriched binding peaks between wild type and *Gata1s* were calculated using DESeq2[3].

*ATAC-seq*

ATAC-seq reactions were performed as previous described[4], but the time for transposase reaction was increased to 30 minutes. Paired-end sequences were merged and aligned to the mouse reference genome (UCSC mm9). We used TrimGalore to remove adaptor sequence and applied Bowtie 2 to align reads of the ATAC-seq samples to the reference genome (UCSC mm9). We used MACS1.4 to call the binding peaks. P-values of differentially enriched ATAC-seq peaks between wild type and *Gata1s* were calculated using DESeq2. Peaks with at least 10 reads within the called peak region were ordered by log2-transformed fold change statistics according to DESeq2 for downstream analysis. GSEA analysis was applied in weighted mode against the gene sets collection in MSigDB (v5.1)[5]. Gene sets with size over 5,000 genes or smaller than 10 genes were excluded for further analysis. Each gene set was permuted 1,000 times to calculate P value and FDR values. The ChIPSeq profiles and heatmaps (Figure 3B) are drawn with fluff bandplot and heatmap function with default parameters[6].

*Flow cytometry, sorting and intracellular staining of RUNX1*

Cells were suspended in sterile FACS buffer (PBS, 0.5% BSA, 2 mM EDTA) and stained with indicated surface markers for 30 minutes at 4°C, then sorted R1/2 ($CD71^{hi}Ter119^{neg/med}$) and R3 ($CD71^{hi}Ter119^{hi}$) populations of erythroblasts using a FACSAria IIu (BD). All flow cytometry analysis was performed on a BD LSR II flow cytometer, and the results were analyzed using FlowJo. Antibodies included c-kit-APC-eFluor 450 (eBioscience, 48-1171-80, 1:100), Ter119-APC (BioLegend, 116212, 1:100), CD41-PE/Cy7 (Biolegend, 133916, 1:100), CD42-DyLight 649 (Emfret Analytics, M040-3, 1:10), CD16/32-PerCP-Cy5.5 (BD Biosciences, 560540, 1:100), CD71-PE (BD Biosciences, 553267, 1:100). For intracellular staining of RUNX1, cells were stained with surface markers as above, fixed with 4% formaldehyde in PBS and stained with RUNX-PE (eBioscience, 12-9816-80) in Saponin buffer (1% BSA and 0.05% saponin in PBS) for 1 hour at 4°C.

*Western blot*

Lysates from human $CD34^{+}$ erythroid differentiation were provided by Amittha Wickrema (University of Chicago, Chicago, IL, USA). Fetal liver cell lysates and G1-ER cell lysates were prepared according to standard methods, fractionated on NuPAGE Bis-Tris protein gels (Invitrogen, NP0335BOX), and transferred to Immobilion-P PVDF Membranes (Millipore, IPVH00010) membranes. Antibodies included anti-GATA1/GATA1s (Abcam, ab11852, 1:1,000), HSC70 (Santa Cruz Biotechnology Inc., sc-7298, 1:1,000), RUNX1 (Santa Cruz Biotechnology

Inc., sc-365644, 1:1,000), H3 (Abcam, ab1791, 1:1,000), H4 (Abcam, ab10158, 1:1,000), H3K9me3 (Abcam, ab8898, 1:1,000) and H3K27me3 (Millipore, 07-449, 1:1,000 and ABE44, 1:1000). Membranes were incubated with primary antibodies and HRP-conjugated secondary antibodies, then developed by ECL (Amersham). Band intensities were quantified using ImageJ software.

**References**


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3. Love MI, Huber W, Anders S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol*. 2014;15(12).
4. Volk A, Liang K, Suraneni P, et al. A CHAF1B-Dependent Molecular Switch in Hematopoiesis and Leukemia Pathogenesis. *Cancer Cell*. 2018;34(5):707-723 e707.
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6. Georgiou G, van Heeringen SJ. fluff: exploratory analysis and visualization of high-throughput sequencing data. *PeerJ*. 2016;4:e2209.

**Supplemental Figure Legends**

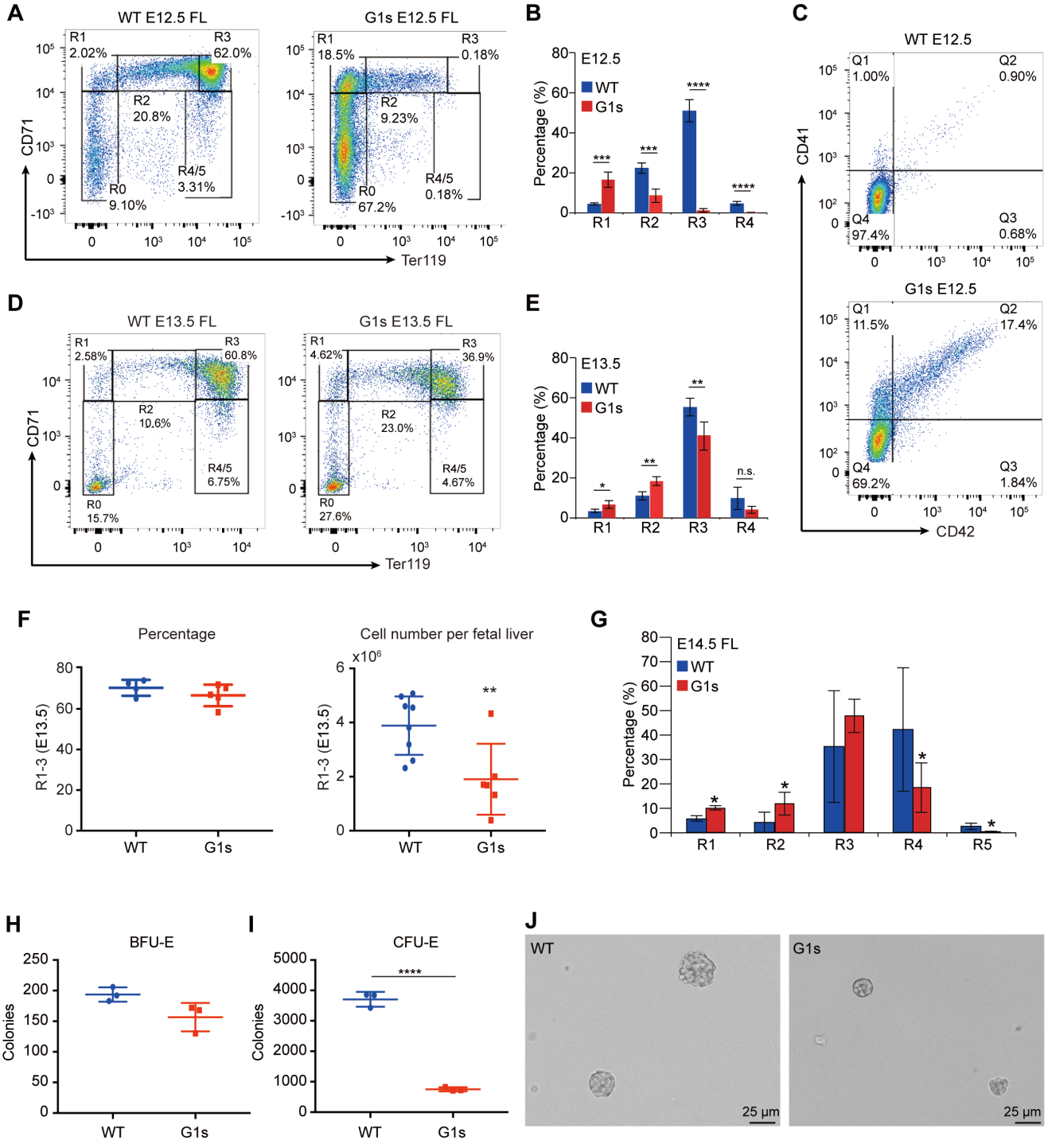
**Figure S1. Erythroid phenotype of *Gata1s* embryos. (A)** Representative flow cytometry plots assessment of erythropoiesis in E12.5 fetal livers using antibodies against CD71 and Ter119. Fetal liver cells were divided into subsets R0 to R5 containing increasingly-mature erythroblasts. **(B)** Bar graph depicting mean (± SD) percentages of R1 to R5 populations from experiments in (A), N ≥ 3. **(C)** Representative dot plots showing the frequencies of immature megakaryocytes ($CD41^+CD42^-$) and mature megakaryocytes ($CD41^+CD42^+$) in fetal livers from WT or G1s E12.5 embryos. **(D)** Flow cytometry assessment of the maturing erythroid populations in E13.5 fetal liver from WT and G1s embryos. **(E)** Bar graph depicting mean (± SD) percentages of R1 to R5 populations from experiments in (D), N ≥ 3. **(F)** Mean (± SD) percentages (left) and total cell numbers per fetal liver of R1/2/3 (R1-3) erythroblasts from E13.5 embryos. Individual values for biological replicates are shown. **(G)** Bar graph depicting mean (± SD) percentages of R1 to R5 populations from E14.5 WT and G1s as determined by flow cytometry, N ≥ 3. **(H)** Numbers of burst-forming unit-erythroid (BFU-E) from 30,000 Ter119-depleted WT or G1s fetal liver cells (E13.5). Individual values for three replicates are shown. Middle bar depicts averages, upper and lower bars depict SD. **(I)** Numbers of colony-forming unit (CFU-E) from 20,000 Ter119-depleted WT or G1s fetal liver cells (E13.5). Individual values for three replicates are shown. Middle bar depicts averages, upper and lower bars depict SD. **(J)** Photos of the representative CFU-E colonies from WT or G1s fetal liver cells cultured in methylcellulose-based medium. Images were collected on a Bio-Rad ZOE fluorescent cell imager. n.s. = not significant, $*P \leq 0.05$, $**P \leq 0.01$, $***P \leq 0.001$, $****P \leq 0.0001$ (unpaired Student's *t*-test).

**Figure S2. Enriched pathways of genes nearest to sites that were gained or lost by GATA1s as determined by GSEA.** X-axis indicates the statistical significance measured by -log10-transformed FDR (False Discovery Rate).

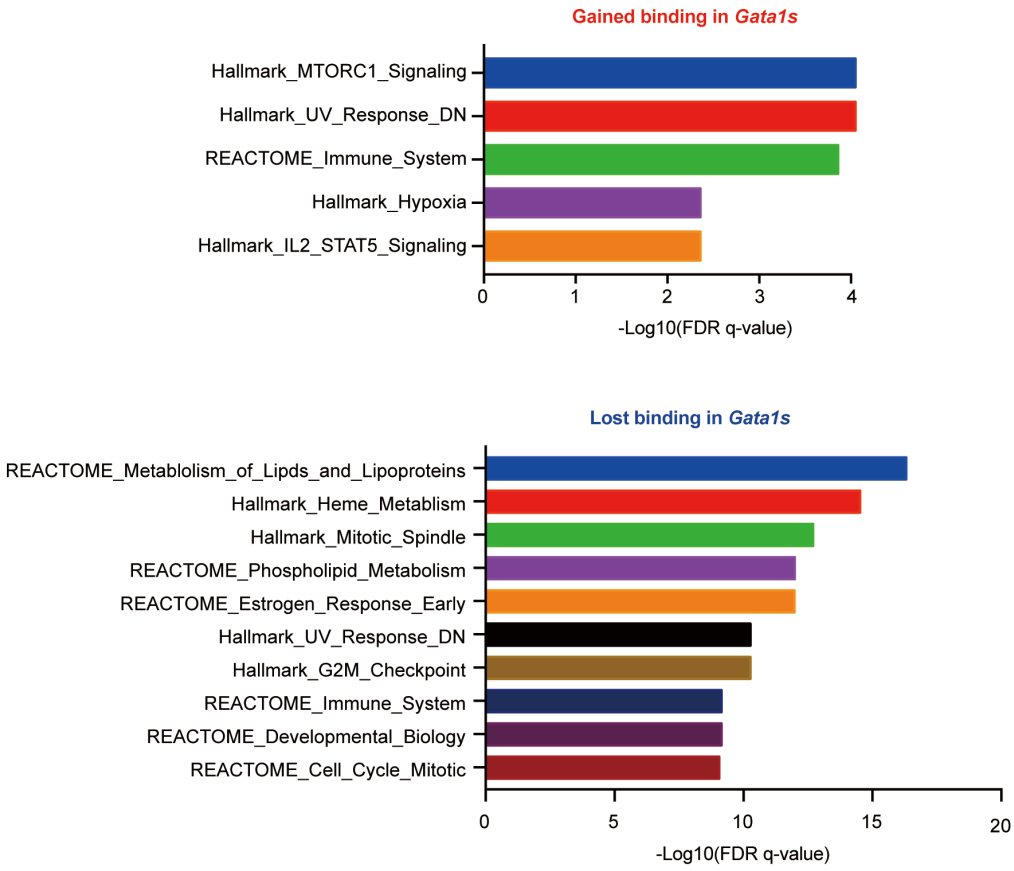
**Figure S3. mTORC1, hypoxia and cell cycle related pathways are enriched in *Gata1s* fetal liver erythroblasts.** Gene set enrichment analysis (GSEA) of differentially expressed genes from WT R1/2 versus G1s R1/2 **(A)** or WT R3 versus G1s R3 **(B)** from RNA-seq. NES, Normalized Enrichment Score; FDR, False Discovery Rate.

**Figure S4. Gene ontology analysis of genes that are differentially expressed and methylated at H3K27.** X-axis indicates the statistical significance measured by -log10-transformed FDR (False Discovery Rate).

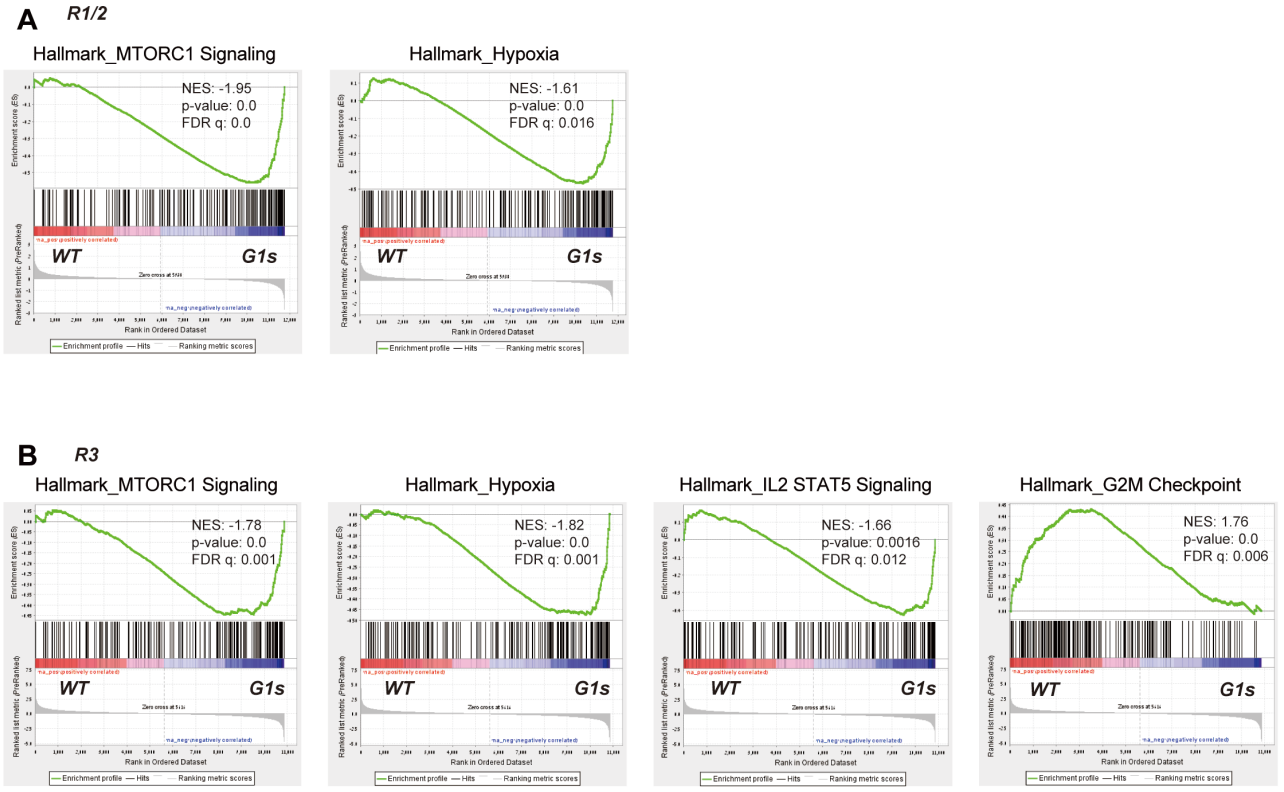
**Figure S5. Generation of *Gata1s/Gata2*$^{het}$ double transgenic mice.** *Gata2*$^{het}$ mice were mated with *Gata1s* hemizygous or homozygous mice to generate *Gata1/Gata2, Gata1/Gata2*$^{het}$, *Gata1s/Gata2* and *Gata1s/Gata2*$^{het}$ mice. ♀ female, ♂ male.




**Supplemental Figure S1**




**Supplemental Figure S2**

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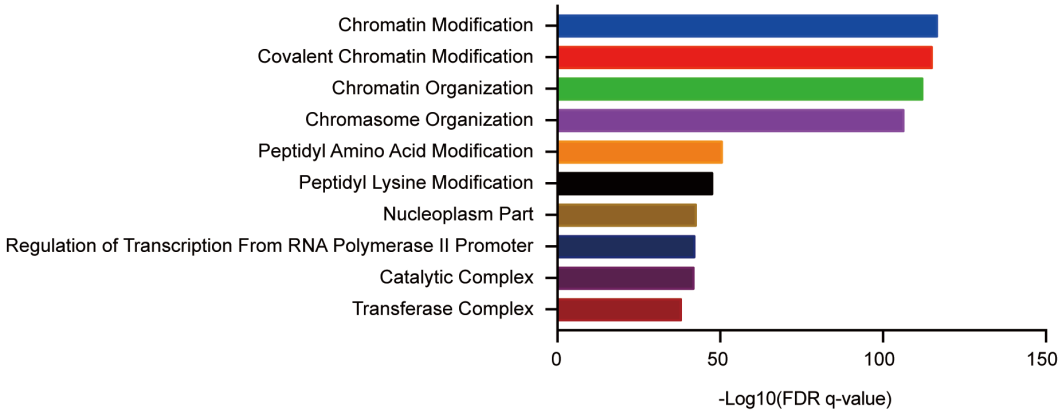
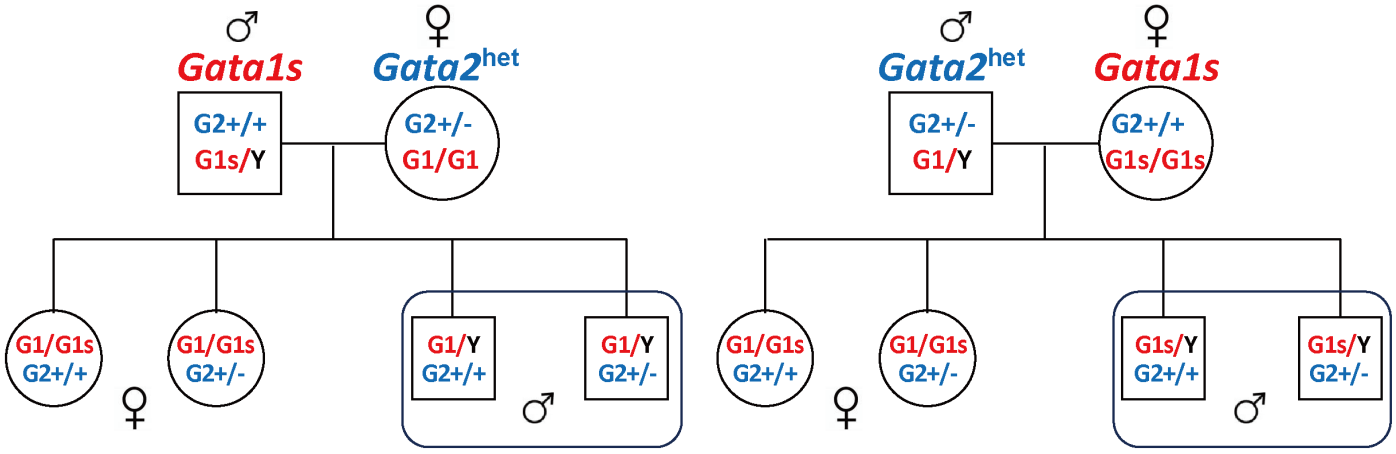



**Supplemental Figure S4**




**Supplemental Figure S5**