

Figure S1

Pathway interactions between the tricarboxylic acid (TCA) cycle, heme synthesis, heme trafficking, and epigenome modification. The TCA cycle occurs in the mitochondria, and the rate-limiting first enzyme of heme synthesis, aminolevulinic acid synthase (ALAS), condenses the TCA intermediate succinyl CoA together with glycine to form 5-aminolevulinic acid (ALA). Feline leukemia virus subgroup C receptor (FLVCR) exports free heme. Mutations in succinate dehydrogenase (SDH) and fumarate hydratase (FH) cause excess succinate and fumarate, which inhibit TET family DNA demethylases and JumonjiC domain-containing histone lysine demethylases (KDM) and cause DNA and histone hypermethylation. PBG, porphobilinogen; HMB, hydroxymethylbilane; URO III, uroporphyrinogen III; COPRO III, coproporphyrinogen III; PPG IX, protoporphyrinogen IX; PPIX, protoporphyrin IX; Fe²⁺, ferrous iron; CO, carbon monoxide.

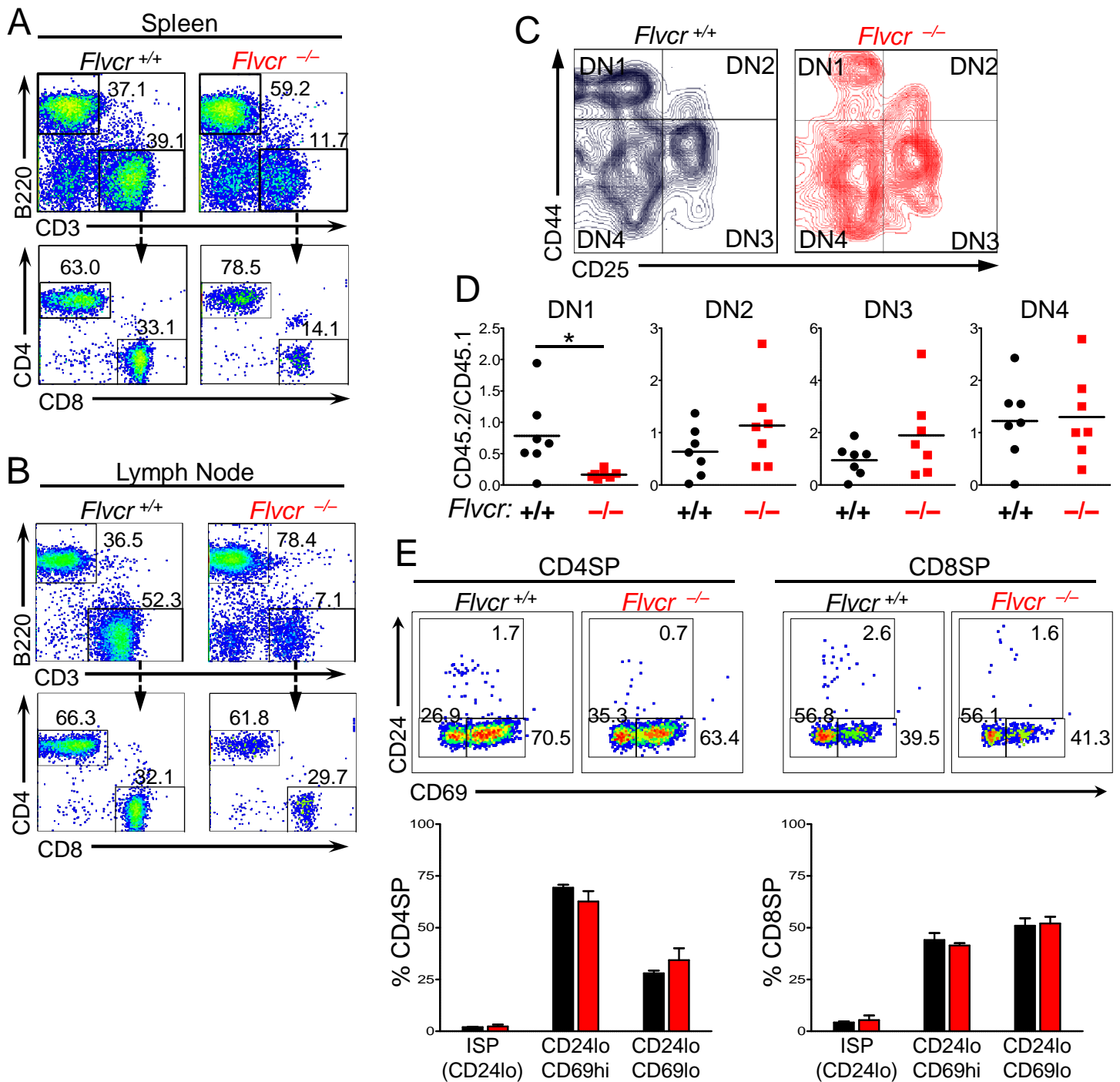


Figure S2

Flow cytometric analysis of lymphocytes in the spleen (**A**) and lymph nodes (**B**) of *Flvcr*^{+/+} and *Flvcr*^{-/-} mice. B220 versus CD3 staining is shown in the upper panels with the relative frequency of cells in each gate shown. The lower panel shows CD4 and CD8 staining on cells in the CD3⁺ gates from the upper panel. (**C**) Flow cytometric analysis of CD44 and CD25 expression of CD4-CD8⁻ (DN) thymocytes from lethally irradiated recipients of competitive BM transplants (CD45.2:CD45.1 with CD45.2 either *Flvcr*^{+/+} or *Flvcr*^{-/-}); flow plots show CD45.2-gated *Flvcr*^{+/+} or *Flvcr*^{-/-} DN. Development proceeds from the DN1 (CD44⁺CD25⁻) to DN2 (CD44⁺CD25⁺) to DN3 (CD44⁻CD25⁺) to the DN4 (CD44⁻CD25⁻) stages. (**D**) CD45.2:CD45.1 ratio for the gated populations with each symbol representing an individual mouse. *p=0.02. (**E**) Representation flow cytometric analysis of CD24 and CD69 expression on *Flvcr*^{+/+};CD4-cre and control CD4SP (left) and CD8SP (right). Below is shown the proportion of SP in each population where ISP are early pre-DP precursors and the CD24loCD69hi and CD24loCD69lo are progressive stages of SP maturation. Bars show the mean and s.e.m. of 4 mice per group and are representative of 2 experiments.

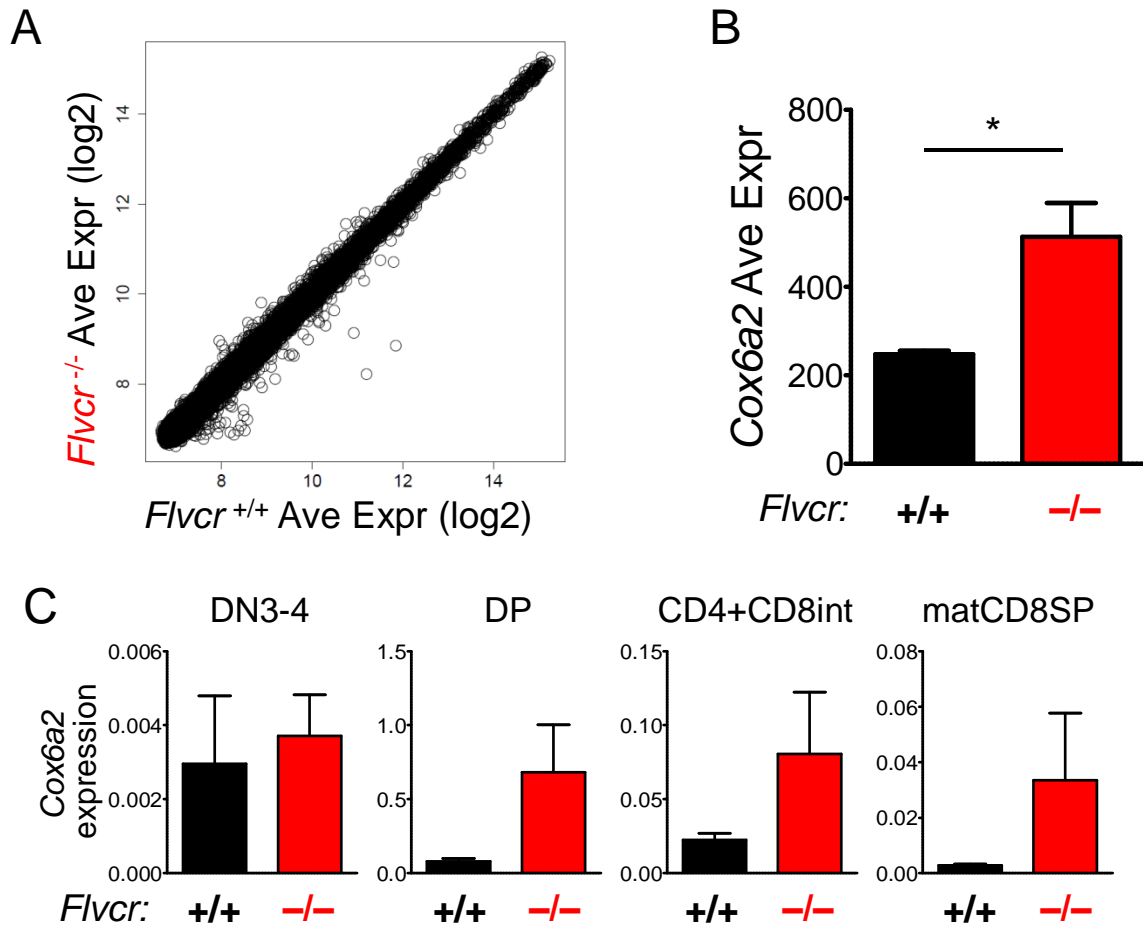


Figure S3

mRNA expression profiling from *Flvcr*^{-/-} or *Flvcr*^{+/+} DP shows a striking lack of differentially-expressed genes. (A) Probe level average expression (Ave Expr) (log2) by *Flvcr*^{-/-} DP is plotted against *Flvcr*^{+/+} DP. (B) Probe level average expression of *Cox6a2* by sorted *Flvcr*^{-/-} or *Flvcr*^{+/+} DP obtained from microarray analysis. Bars show mean and s.e.m of 3 replicates per group. *p=0.02. (C) qRT-PCR was used to validate up-regulation of the *Cox6a2* gene expression in sorted OT-I; *Flvcr*^{fllox/fllox}; *Lck-cre* compared to OT-I; *Flvcr*^{+/+}; *Lck-cre* thymocytes. The sorted samples include late DN3-4 (gated on CD44-CD4-CD8- thymocytes), DP (gated on CD4+CD8+), the CD4+CD8int (developmental stage between DP and matCD8), and matCD8SP (CD24-TCRβ^{high}CD8+). *Cox6a2* expression in sorted thymic subsets is calculated relative to whole C57BL/6 thymus and bars show the mean and s.e.m of 5 mice per group.

| Target ID | logFC (KO/WT) | <i>Flvcr</i> -KO AveExpr | <i>Flvcr</i> -WT AveExpr | p-value | adj.p.val |
|---------------|---------------|--------------------------|--------------------------|----------|-----------|
| COX6A2 | 1.02 | 8.97 | 7.95 | 2.58E-12 | 2.80E-09 |
| CDK5RAP1 | 0.93 | 9.81 | 8.88 | 1.20E-10 | 9.08E-08 |
| PDLIM4 | 0.85 | 8.86 | 8.01 | 4.00E-09 | 2.52E-06 |
| HIST1H2BJ | 0.82 | 11.57 | 10.75 | 1.46E-08 | 7.61E-06 |
| CDK5RAP1 | 0.78 | 9.36 | 8.58 | 7.32E-08 | 3.36E-05 |
| PLAC8 | 0.68 | 8.76 | 8.08 | 2.68E-06 | 9.43E-04 |
| HIST1H2BF | 0.65 | 11.81 | 11.16 | 7.78E-06 | 2.45E-03 |
| HIST1H4H | 0.64 | 9.31 | 8.68 | 1.21E-05 | 3.73E-03 |
| HIST1H3D | 0.63 | 11.60 | 10.97 | 1.42E-05 | 4.31E-03 |
| HIST1H2BE | 0.60 | 10.92 | 10.32 | 3.35E-05 | 9.75E-03 |
| HIST1H2AB | 0.60 | 9.20 | 8.61 | 4.00E-05 | 1.12E-02 |
| HIST1H2BH | 0.59 | 11.30 | 10.71 | 4.73E-05 | 1.28E-02 |
| HIST1H2BN | 0.58 | 10.64 | 10.05 | 5.67E-05 | 1.48E-02 |
| H1FO | 0.58 | 8.60 | 8.03 | 7.03E-05 | 1.78E-02 |
| HIST1H4D | 0.57 | 8.95 | 8.38 | 8.38E-05 | 2.02E-02 |
| HIST1H3H | 0.57 | 8.65 | 8.08 | 9.65E-05 | 2.29E-02 |
| HIST1H4K | 0.56 | 9.38 | 8.82 | 1.04E-04 | 2.42E-02 |
| HIST1H2BK | 0.55 | 10.77 | 10.22 | 1.40E-04 | 3.16E-02 |
| HIST1H3E | 0.55 | 11.62 | 11.07 | 1.45E-04 | 3.18E-02 |
| C85492 | 0.55 | 9.71 | 9.16 | 1.55E-04 | 3.35E-02 |
| HIST1H3H | 0.54 | 10.30 | 9.76 | 2.10E-04 | 4.23E-02 |
| HIST1H2BE | 0.54 | 11.54 | 11.00 | 2.19E-04 | 4.30E-02 |
| HIST1H3A | 0.54 | 11.02 | 10.48 | 2.21E-04 | 4.30E-02 |
| ADSSL1 | 0.53 | 9.03 | 8.49 | 2.28E-04 | 4.38E-02 |
| ADSSL1 | 0.53 | 10.12 | 9.59 | 2.65E-04 | 4.84E-02 |
| 4930486L24RIK | 0.53 | 9.63 | 9.10 | 2.65E-04 | 4.84E-02 |
| HIST1H3C | 0.53 | 8.80 | 8.27 | 2.66E-04 | 4.84E-02 |
| KCNMB4 | -0.53 | 7.99 | 8.52 | 2.68E-04 | 4.84E-02 |
| SEPP1 | -0.53 | 9.89 | 10.42 | 2.52E-04 | 4.76E-02 |
| JUN | -0.54 | 8.04 | 8.58 | 2.21E-04 | 4.30E-02 |
| SDC4 | -0.54 | 8.40 | 8.94 | 1.93E-04 | 3.95E-02 |
| CEBPB | -0.54 | 8.56 | 9.10 | 1.86E-04 | 3.86E-02 |
| IGHG | -0.54 | 7.18 | 7.73 | 1.85E-04 | 3.86E-02 |
| SPARC | -0.54 | 7.11 | 7.65 | 1.83E-04 | 3.86E-02 |
| IGF1R | -0.55 | 7.99 | 8.54 | 1.42E-04 | 3.17E-02 |
| E430033B07RIK | -0.56 | 11.18 | 11.74 | 1.30E-04 | 2.98E-02 |
| LY6D | -0.57 | 12.69 | 13.27 | 7.53E-05 | 1.84E-02 |
| TUBA3A | -0.58 | 7.57 | 8.15 | 7.22E-05 | 1.79E-02 |
| LOC626347 | -0.58 | 6.85 | 7.43 | 6.84E-05 | 1.76E-02 |
| LOC333685 | -0.59 | 7.65 | 8.24 | 5.07E-05 | 1.35E-02 |
| 9530027K23RIK | -0.59 | 7.11 | 7.70 | 4.73E-05 | 1.28E-02 |
| LOC214575 | -0.60 | 10.46 | 11.05 | 3.87E-05 | 1.11E-02 |
| IGL-V1 | -0.62 | 7.27 | 7.89 | 1.95E-05 | 5.79E-03 |

Table S1

List of differentially-expressed genes between *Flvcr*^{-/-} or *Flvcr*^{+/+} DP determined by microarray. logFC denotes the average log₂ ratio of *Flvcr*^{-/-} (KO) or *Flvcr*^{+/+} (WT) DP. *Flvcr*-KO AveExpr denotes the average log₂ signal intensity across all *Flvcr*^{-/-} DP and *Flvcr*-WT AveExpr the average log₂ signal intensity across all *Flvcr*^{+/+} DP. p-value denotes the raw p-value while the adj. p-value denotes the adjusted p-value (Benjamini and Hochberg false-discovery rate). Genes with |log₂ (ratio)| ≥ 0.585 (± 1.5-fold on linear scale) and adjusted p-value < 0.05 were included and are ordered by logFC.

Table S1 (continued)

| Target ID | logFC (KO/WT) | Flvcr-KO AveExpr | Flvcr-WT AveExpr | p-value | adj.p-value |
|---|---------------|------------------|------------------|----------|-------------|
| IL17RE | -0.66 | 8.68 | 9.34 | 5.49E-06 | 1.77E-03 |
| LOC272683 | -0.66 | 6.87 | 7.53 | 5.48E-06 | 1.77E-03 |
| LOC672342 | -0.66 | 6.99 | 7.65 | 5.34E-06 | 1.77E-03 |
| CCL4 | -0.66 | 7.53 | 8.19 | 4.73E-06 | 1.63E-03 |
| SCN4B | -0.70 | 9.11 | 9.80 | 1.67E-06 | 6.02E-04 |
| PODXL2 | -0.71 | 8.39 | 9.10 | 9.79E-07 | 3.62E-04 |
| LOC676136 | -0.73 | 7.01 | 7.74 | 5.64E-07 | 2.14E-04 |
| INTS7 | -0.74 | 9.41 | 10.14 | 3.73E-07 | 1.45E-04 |
| SLC7A11 | -0.74 | 9.52 | 10.26 | 3.57E-07 | 1.42E-04 |
| IGKV3-2_X16954_IG_KAPPA_VARIABLE_3-2_18 | -0.74 | 7.11 | 7.85 | 3.28E-07 | 1.34E-04 |
| GRAMD3 | -0.75 | 7.49 | 8.24 | 2.71E-07 | 1.14E-04 |
| DUSP1 | -0.75 | 10.76 | 11.51 | 2.15E-07 | 9.29E-05 |
| LOC213684 | -0.77 | 6.89 | 7.66 | 1.05E-07 | 4.69E-05 |
| INSL5 | -0.79 | 9.37 | 10.16 | 4.58E-08 | 2.17E-05 |
| 1110036O03RIK | -0.80 | 8.04 | 8.84 | 3.19E-08 | 1.56E-05 |
| LOC384422 | -0.82 | 6.79 | 7.61 | 1.60E-08 | 8.06E-06 |
| LOC636752 | -0.84 | 7.14 | 7.98 | 7.02E-09 | 3.80E-06 |
| MFSD7B | -0.84 | 10.30 | 11.15 | 5.83E-09 | 3.27E-06 |
| BC038167 | -0.85 | 8.64 | 9.50 | 4.49E-09 | 2.62E-06 |
| BC020108 | -0.85 | 9.64 | 10.49 | 4.29E-09 | 2.60E-06 |
| LOC669053 | -0.89 | 7.05 | 7.94 | 8.23E-10 | 5.42E-07 |
| NPHS2 | -0.89 | 8.66 | 9.56 | 7.21E-10 | 4.96E-07 |
| FOS | -0.93 | 8.98 | 9.91 | 1.81E-10 | 1.31E-07 |
| LOC637227 | -0.94 | 7.15 | 8.09 | 9.25E-11 | 7.37E-08 |
| IGL-V1 | -0.97 | 7.92 | 8.90 | 1.89E-11 | 1.59E-08 |
| XKRX | -0.98 | 8.62 | 9.60 | 1.66E-11 | 1.48E-08 |
| LOC240672 | -1.00 | 9.30 | 10.30 | 6.97E-12 | 6.60E-09 |
| LOC100046793 | -1.01 | 6.90 | 7.91 | 3.35E-12 | 3.38E-09 |
| LOC100047162 | -1.06 | 7.43 | 8.49 | 3.13E-13 | 3.64E-10 |
| TMIE | -1.07 | 8.12 | 9.18 | 1.85E-13 | 2.33E-10 |
| BAMBI-PS1 | -1.08 | 10.72 | 11.80 | 1.02E-13 | 1.41E-10 |
| LOC676222 | -1.20 | 7.29 | 8.49 | 1.24E-16 | 1.87E-13 |
| LOC384413 | -1.26 | 6.93 | 8.19 | 2.98E-18 | 5.02E-15 |
| LOC636944 | -1.35 | 7.08 | 8.43 | 1.37E-20 | 2.59E-17 |
| IGH-VJ558 | -1.35 | 7.22 | 8.57 | 1.12E-20 | 2.41E-17 |
| LOC100047316 | -1.39 | 6.88 | 8.27 | 7.97E-22 | 2.01E-18 |
| LOC100046793 | -1.40 | 6.98 | 8.37 | 5.51E-22 | 1.67E-18 |
| LOC636944 | -1.54 | 6.97 | 8.52 | 2.16E-26 | 8.18E-23 |
| SMOC1 | -1.79 | 9.14 | 10.92 | 8.06E-35 | 4.07E-31 |
| LOC100047628 | -2.98 | 8.22 | 11.20 | 1.70E-93 | 1.29E-89 |
| IGK-C | -2.99 | 8.85 | 11.84 | 1.71E-94 | 2.60E-90 |