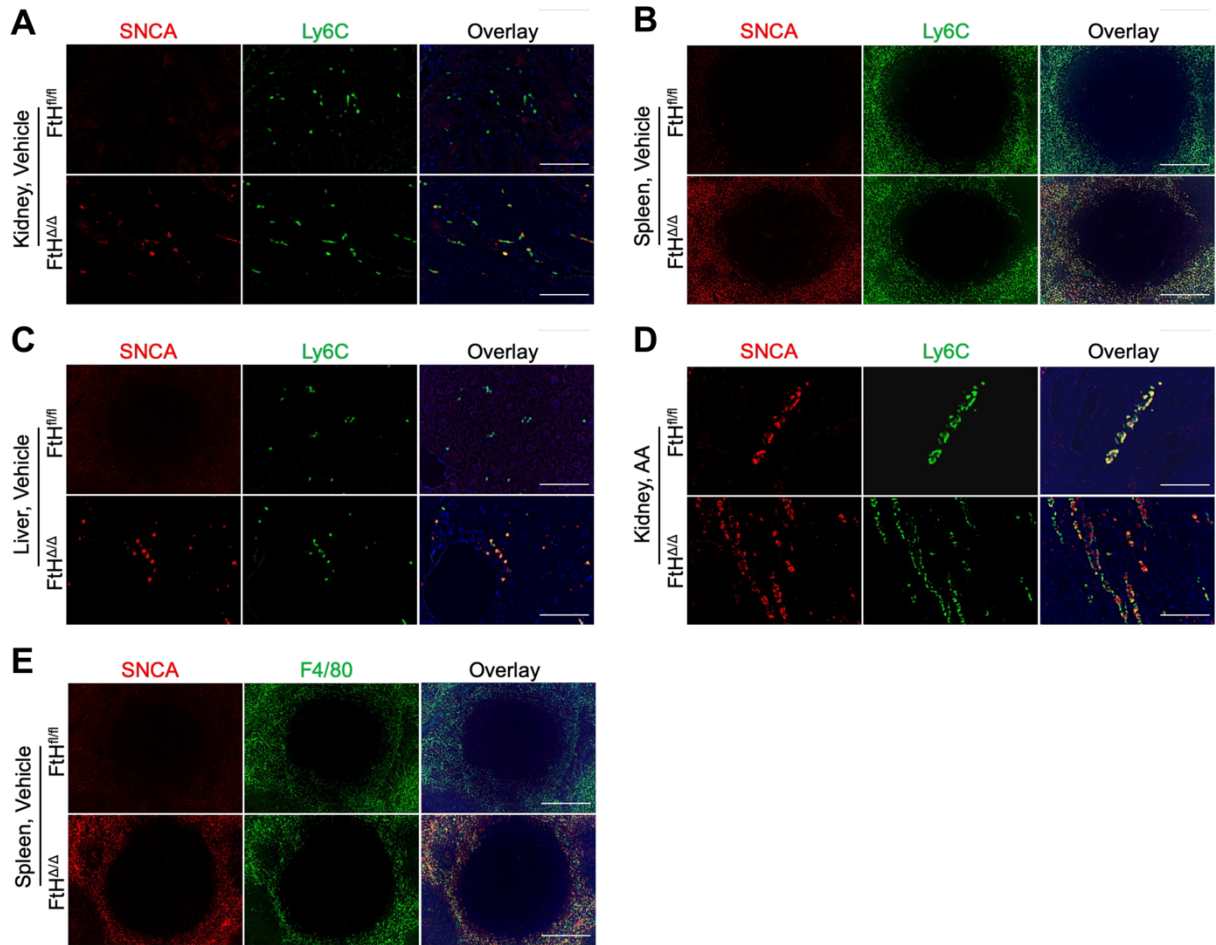
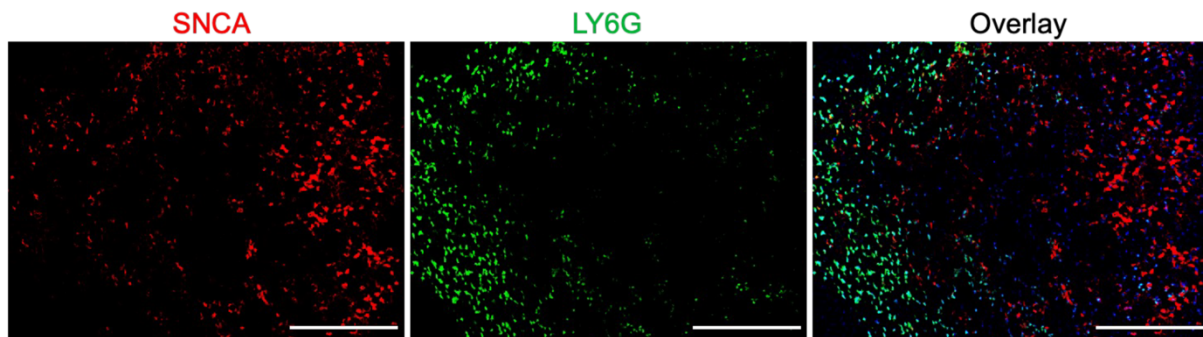


**Figure S1.** (A) Bulk RNA-sequencing data of FtH<sup>fl/fl</sup> and FtH<sup>Δ/Δ</sup> kidneys under baseline conditions highlighting genes that encode key iron regulatory proteins. Hepcidin (Hamp) levels were undetectable. (n = 4). Normalized count data of signature genes are plotted. ns = not significant, \*\*P < 0.01. (B, C) FtH<sup>fl/fl</sup> and FtH<sup>Δ/Δ</sup> livers (B) and spleens (C) were harvested under baseline conditions and mRNA expression levels of *Lcn2* (Ngal), *Hamp* (hepcidin), *Slc11a2* (DMT1), *Slc40a1* (Fpn), and *Tfrc* (transferrin receptor 1) were analyzed using qRT-PCR. Data are normalized to *Gapdh* and represented as fold change, FtH<sup>fl/fl</sup> Vs. FtH<sup>Δ/Δ</sup>. ns = not significant, \*P < 0.05, \*\*P < 0.01, \*\*\*P < 0.001. (D) Serum iron concentrations in FtH<sup>fl/fl</sup> and FtH<sup>Δ/Δ</sup> under quiescent conditions were measured and reported in μg/dL. ns = not significant. (E) Immunofluorescence staining of SNCA in kidneys of FtH<sup>fl/fl</sup> and FtH<sup>Δ/Δ</sup> mice under baseline conditions. Scale bar = 50 μm.

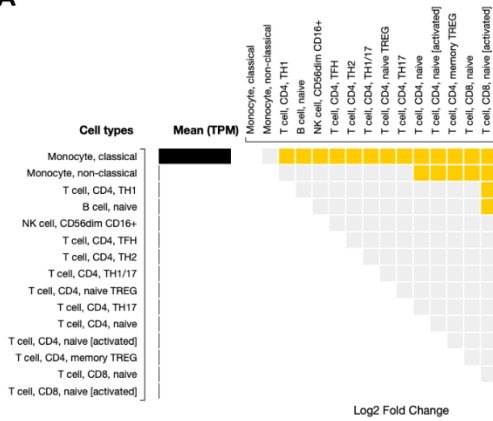


**Figure S2.** (A-C) Immunofluorescence staining of SNCA and Ly6C in the kidney, spleen, and liver of  $Fth^{fl/fl}$  and  $Fth^{\Delta/\Delta}$  mice under baseline conditions. (D) Upregulation of SNCA in  $Ly6C^+$  cells following AA-induced injury in both genotypes. Scale bars: kidney= 25  $\mu$ m, spleen= 200  $\mu$ m, liver= 25  $\mu$ m. (E) Immunofluorescence assay illustrating co-expression of SNCA and F4/80 in spleen of  $Fth^{\Delta/\Delta}$  mice. Scale bar = 200  $\mu$ m.

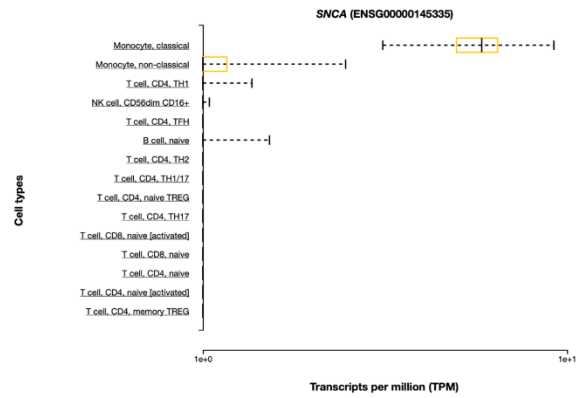


**Figure S3.** Immunofluorescence assays were performed using anti-SNCA and anti-LY6G antibodies to determine the pattern of expression of these proteins in spleen of FtH<sup>ΔΔ</sup> mice. As illustrated neutrophils are not the primary source of SNCA expression in FtH<sup>ΔΔ</sup> spleens. Scale bar = 100  $\mu$ m.

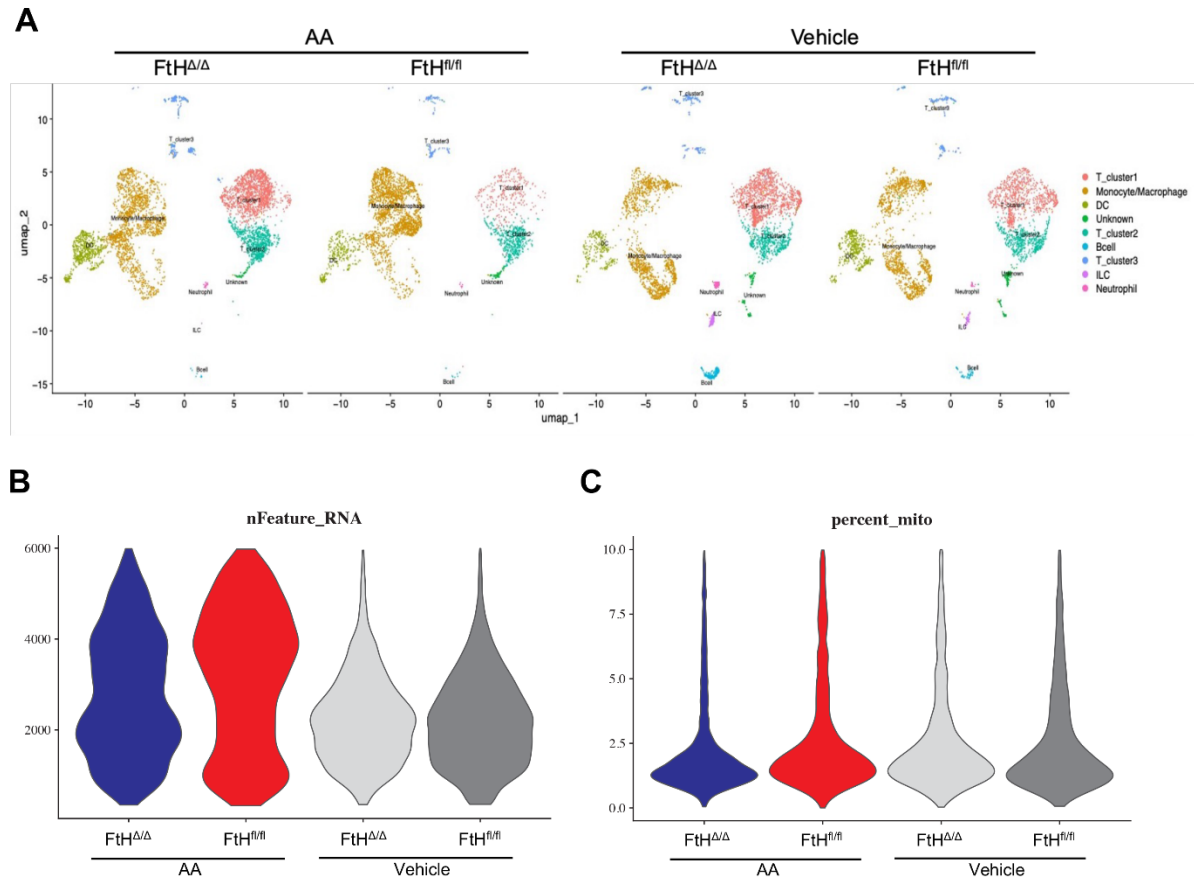
**A**



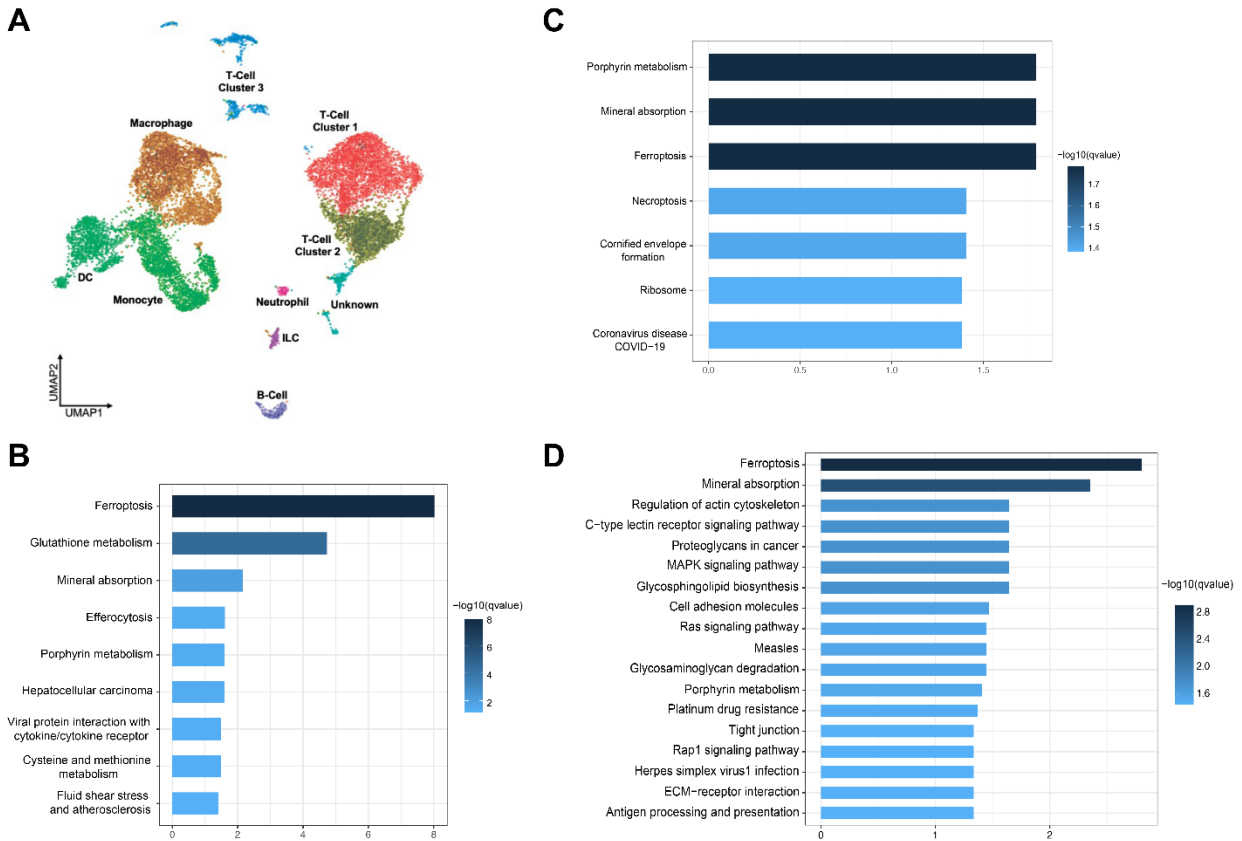
**B**



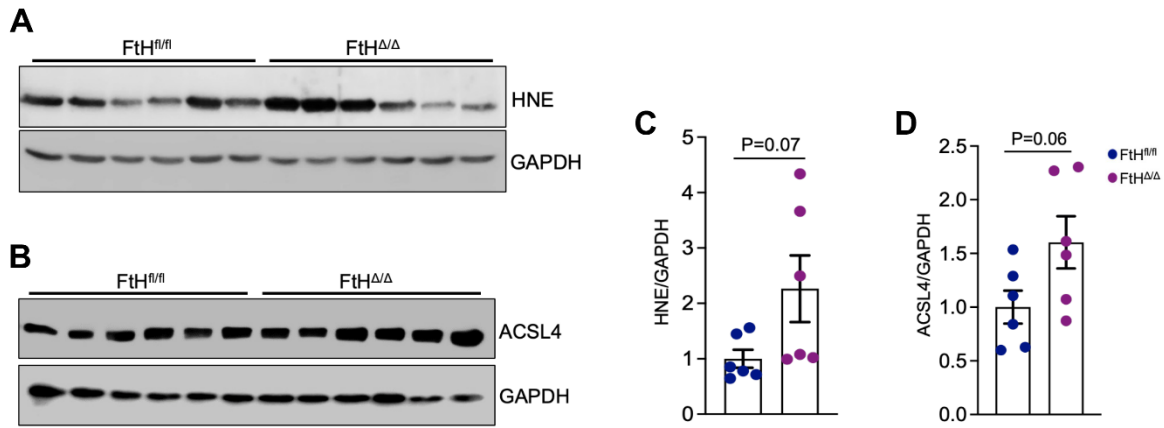
**Figure S4.** Expression of SNCA in leukocytes as determined by (A) log2 fold change, and (B) transcripts per million reveals monocytes as the major cellular source of expression. Data was generated via accessing publicly available transcriptome analysis platform. (<https://dice-database.org>)



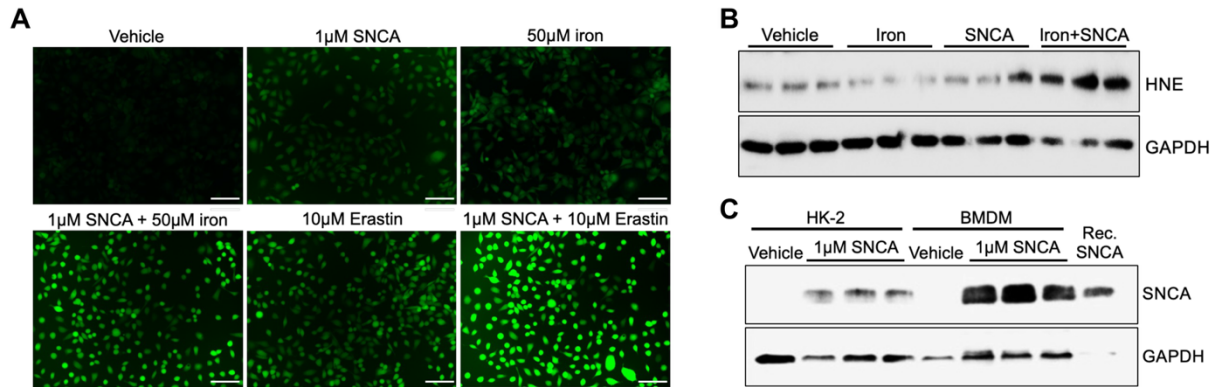
**Figure S5.** (A) UMAP projection of isolated CD45<sup>+</sup> (leukocytes) from kidneys of both genotypes, treated with vehicle or AA, passing rigid quality control filtering and after dataset integration, yielding 9 distinct cell clusters (n= 1 per genotype and treatment condition). (B, C) Violin plots showing (B) the number of informative genes per single cell, and (C) the percentage of mitochondrial genes per single cell, all split by batches.



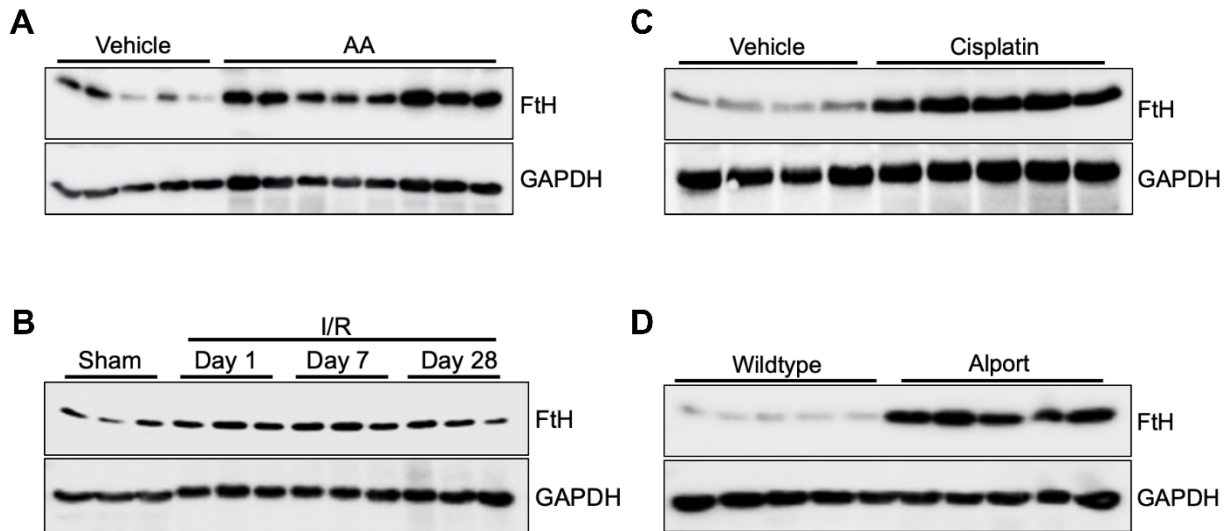
**Figure S6.** (A) Uniform manifold approximation and projection (UMAP) plot showing the clustering of immune cells (CD45<sup>+</sup>) in the kidney based on single-cell RNA sequencing. The analysis identifies ten distinct clusters, with contaminating kidney cells and clusters representing less than 1% removed. Specifically, monocytes and MΦ were clustered as distinct populations to enable independent assessment of pathway enrichment under each experimental condition. (B) Pathway enrichment analysis of differentially expressed genes in FtH deficient monocytes versus wildtype monocytes under vehicle-treated conditions. (C) Pathway enrichment analysis of differentially expressed genes in FtH deficient monocytes versus wildtype monocytes following AA administration. (D) Pathway enrichment analysis of differentially expressed genes in MΦ derived from FtH $\Delta/\Delta$  kidneys versus MΦ derived from FtH $^{fl/fl}$  kidneys following AA administration.



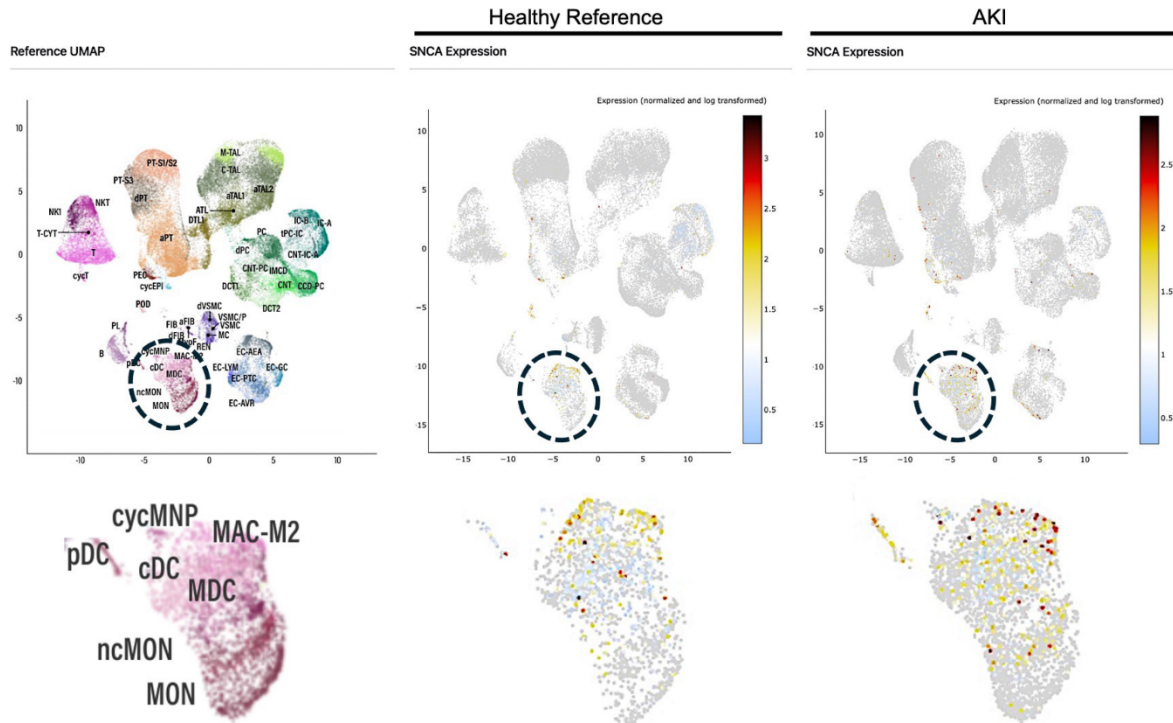
**Figure S7.** (A, B) FtH<sup>fl/fl</sup> and FtH<sup>Δ/Δ</sup> kidneys were harvested under baseline conditions and protein expression of (A) HNE, and (B) ACSL4 were examined via Western blotting. (C, D) Densitometric analysis indicates an increasing trend for both (C) HNE, and (D) ACSL4 in baseline FtH<sup>Δ/Δ</sup> kidneys; however, these results did not achieve statistical significance.



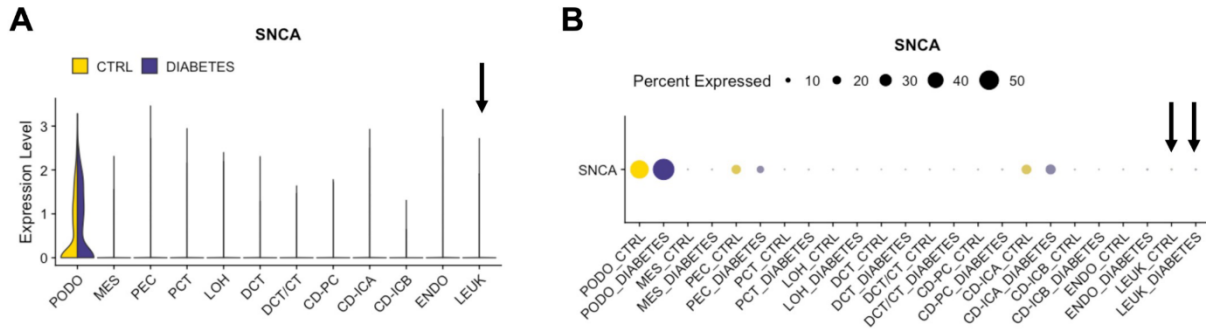
**Figure S8.** Representative images demonstrating intracellular ROS using DCFDA in HK-2 cells. DCFDA staining was performed at 16 hours post treatment with SNCA alone, iron alone, SNCA and iron, Erastin alone, Erastin and SNCA, at denoted concentrations. Scale bar = 200  $\mu$ m. (B) HK-2 cells were treated with vehicle, 50  $\mu$ M iron alone, 1  $\mu$ M SNCA alone, and combination of 50  $\mu$ M iron and 1  $\mu$ M SNCA for 16 hours. Cell lysates were used to detect HNE levels. GAPDH was used as loading control. (C) To detect SNCA levels western blotting was performed using protein lysates of HK-2 cells and BMDMs subjected to 1  $\mu$ M SNCA for 16 hours. GAPDH was used as loading control.



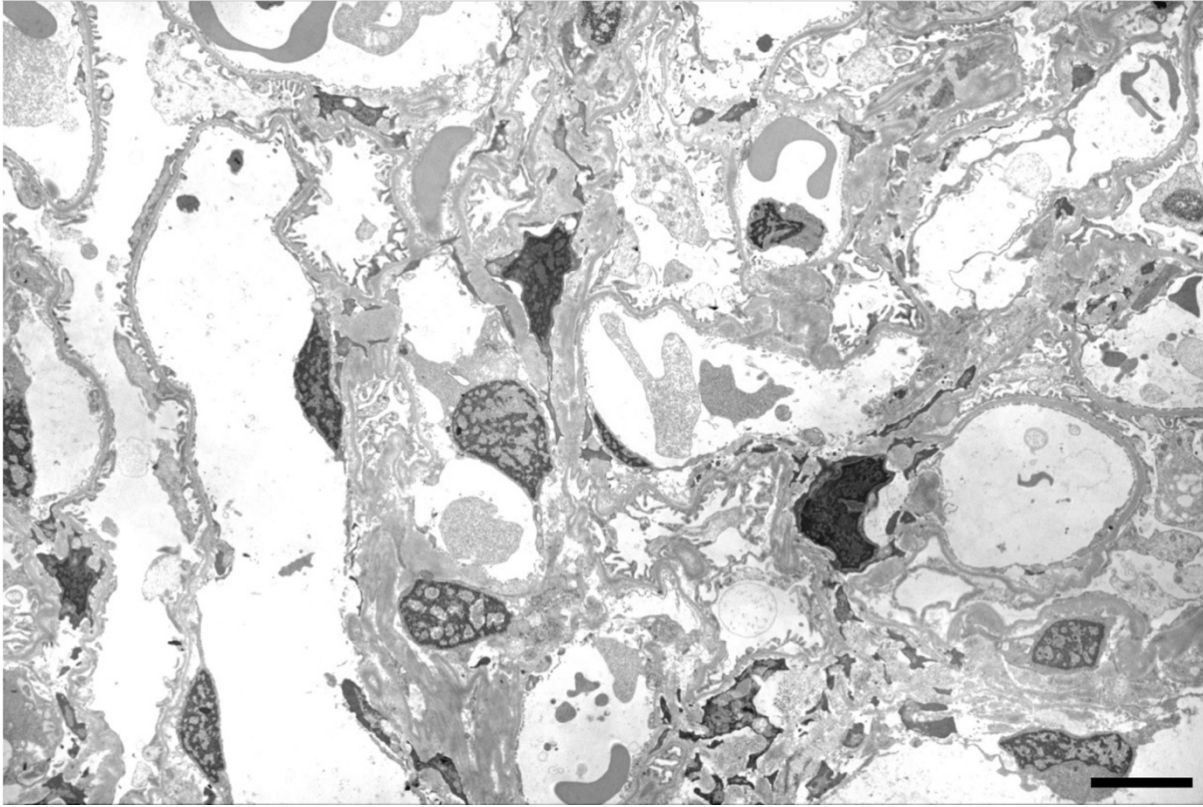
**Figure S9.** (A-D) Western blot analysis of Fth in kidney lysates from four different mouse models of kidney disease: (A) Wildtype mice were treated with vehicle or aristolochic acid (AA), and kidney lysates were collected six weeks post-AA administration. (B) Wildtype mice subjected to 20 minutes of ischemia-reperfusion (I/R) injury. Kidney samples were collected at indicated time-points. (C) Protein expression of Fth in wildtype kidneys treated with cisplatin as described in methods. (D) Fth expression in kidneys from wildtype (Col4a3<sup>+/+</sup>) and Alport (Col4a3<sup>-/-</sup>) mice at twelve weeks of age. GAPDH was used as loading control.



**Figure S10.** Reference UMAP, the left panel, shows different annotated cell types. The black dash sequesters cells of monocytic lineage subpopulation of leukocytes and was isolated for better visualization. The middle panel shows expression levels of SNCA in healthy kidneys and the right panel demonstrates enrichment of SNCA during AKI. Data was generated via accessing kidney precision medicine project website. (<https://www.kpmp.org>)



**Figure S11.** (A) violin plot of Sc-RNAseq demonstrates enrichment of SNCA in control and diabetic kidneys that is almost exclusively seen in podocytes. Arrow indicates expression of SNCA in isolated leukocytes. (B) Dotplot analysis confirms lack of enrichment of SNCA in leukocytes as indicated by arrows. Data was generated via accessing kidney interactive transcriptomics platform developed by Dr. Humphreys. (<https://humphreyslab.com/SingleCell/>)



**Figure S12.** Representative images of kidney biopsies with an isolated diagnosis of TBM demonstrate thinning of the basement membrane. Scale bar = 5  $\mu\text{m}$ .

**Supplemental Table 1.** Cluster of cells that significantly express *Snca* mRNA in kidneys of healthy individuals

| cluster Abbrev | cluster Name  | cell Count | Mean Exp | pct Cells Expressing | fold Change | p Val     | p Val Adj |
|----------------|---|------------|----------|----------------------|-------------|-----------|-----------|
| MDC            | Monocyte-derived Cell                                 | 1017       | 0.68     | 37.2                 | 3.21        | 4.32E-135 | 1.39E-130 |
| POD            | Podocyte  | 201        | 1.95     | 52.2                 | 4.63        | 5.32E-133 | 1.71E-128 |
| IC-A           | Intercalated Cell Type A                              | 1980       | 0.322    | 18.6                 | 2.07        | 1.16E-52  | 3.72E-48  |
| MAC-M2         | M2-Macrophage   | 465        | 0.852    | 19.6                 | 3.43        | 8.51E-42  | 2.74E-37  |
| PEC            | Parietal Epithelial Cell                              | 242        | 0.565    | 21.5                 | 2.74        | 2.88E-26  | 9.24E-22  |
| cDC            | Classical Dendritic Cell                              | 298        | 0.647    | 18.1                 | 2.95        | 8.44E-23  | 2.71E-18  |
| pDC            | Plasmacytoid Dendritic Cell                           | 36         | 0.666    | 38.9                 | 2.99        | 2.39E-22  | 7.69E-18  |
| CNT-IC-A       | Connecting Tubule Intercalated Cell Type A            | 712        | 0.171    | 14                   | 1           | 3.96E-18  | 1.27E-13  |
| cycMNP         | Mononuclear Phagocyte ( <i>cycling</i> <sup>2</sup> ) | 14         | 0.59     | 42.9                 | 2.92        | 1.39E-13  | 4.46E-09  |
| MON            | Monocyte  | 459        | 0.223    | 10.5                 | 1.39        | 1.06E-07  | 0.00339   |

**Supplemental Table 2.** Cluster of cells that significantly express *Snca* mRNA in kidneys of patients with AKI

| cluster Abbrev | cluster Name  | cell Count | mean Exp | pct Cells Expressing | fold Change | p Val    | p Val Adj |
|----------------|---|------------|----------|----------------------|-------------|----------|-----------|
| PEC            | Parietal Epithelial Cell                              | 178        | 0.858    | 28.7                 | 3.88        | 5.69E-73 | 1.83E-68  |
| MAC-M2         | M2-Macrophage   | 794        | 0.588    | 21.2                 | 3.47        | 5.88E-70 | 1.89E-65  |
| POD            | Podocyte  | 57         | 1.99     | 47.4                 | 5.08        | 7.86E-67 | 2.53E-62  |
| cyc MNP        | Mononuclear Phagocyte ( <i>cycling</i> <sup>2</sup> ) | 108        | 0.713    | 25.9                 | 3.59        | 5.08E-42 | 1.63E-37  |
| cDC            | Classical Dendritic Cell                              | 1012       | 0.339    | 12                   | 2.61        | 8.18E-34 | 2.63E-29  |
| pDC            | Plasmacytoid Dendritic Cell                           | 158        | 0.699    | 20.3                 | 3.57        | 8.01E-30 | 2.57E-25  |
| MDC            | Monocyte-derived Cell                                 | 582        | 0.449    | 13.4                 | 2.99        | 8.58E-29 | 2.76E-24  |

**Supplemental Table 3.** Characteristics of analyzed human kidney biopsy tissues

|                              | <b>TBM<br/>(n=6)</b> | <b>AIN<br/>(n=6)</b> | <b>ACR<br/>(n=6)</b> |
|------------------------------|----------------------|----------------------|----------------------|
| Age [years]                  | 46±5.76              | 47±10.79             | 43±6.0               |
| Gender: female [%]           | 100%                 | 50%                  | 66.6%                |
| Max serum creatinine [mg/dL] | 0.9±0.15             | 8.7±4.24             | 4.0±0.76             |

**Supplemental Table 4.** List of primary antibodies and dilutions used in Immunoblotting, Immunohistochemistry, and Immunofluorescence experiments

| <b>Primary Antibody</b>   | <b>Source</b>             | <b>Catalog Number</b> | <b>Dilution for Immunoblotting</b>         |
|---|---------------------------|-----------------------|--|
| Purified Mouse Anti- $\alpha$ -Synuclein  | BD Biosciences            | Cat #610787           | 1:1000                                     |
| Anti-ACSL4 (FACL4) antibody [EPR8640]   | Abcam                     | Cat #ab155282         | 1:2500                                     |
| Anti-ferritin heavy chain Antibody (C6)   | Santa Cruz Biotechnology  | Cat #sc-517438        | 1:10,000 spleens, 1:1000 all other samples |
| Anti-Glyceraldehyde-3-Phosphate Dehydrogenase Antibody, clone 6C5                           | Millipore Sigma           | Cat #MAB374           | 1:10,000                                   |
| Anti-4 Hydroxynonenal antibody  | Abcam                     | Cat #ab46545          | 1:2500                                     |
| Ferritin Light Chain Polyclonal Antibody  | Invitrogen                | Cat #PA5-19059        | 1:1000                                     |
| <b>Primary Antibody</b>   | <b>Source</b>             | <b>Catalog Number</b> | <b>Dilution for Immunohistochemistry</b>   |
| $\alpha$ -Synuclein (D37A6) XP <sup>®</sup> Rabbit mAb                                      | Cell Signaling Technology | Cat #4179S            | 1:200                                      |
| Anti-4 Hydroxynonenal antibody  | Abcam                     | Cat #ab46545          | 1:1000                                     |
| <b>Primary Antibody</b>   | <b>Source</b>             | <b>Catalog Number</b> | <b>Dilution for Immunofluorescence</b>     |
| $\alpha$ -Synuclein (D37A6) XP <sup>®</sup> Rabbit mAb                                      | Cell Signaling Technology | Cat #4179S            | 1:200                                      |
| CD11b Monoclonal Antibody (M1/70), Super Bright <sup>™</sup> 600, eBioscience <sup>™</sup>  | ThermoFisher Scientific   | Cat #63011282         | 1:200                                      |
| Alexa Fluor <sup>®</sup> 700 anti-mouse Ly-6G Antibody                                      | BioLegend                 | Cat #127622           | 1:200                                      |
| Alexa Fluor <sup>®</sup> 488 Conjugate. anti-mouse F4/80 (D4C8V) Rabbit Monoclonal Antibody | Cell Signaling Technology | Cat #27076            | 1:200                                      |
| BD Pharmingen <sup>™</sup> FITC Rat Anti-Mouse Ly-6C  | BD Biosciences            | Cat #553104           | 1:200                                      |

**Supplemental Table 5.** List of oligonucleotide sequences

| <b>q-PCR primer sequences</b>      |  |                      |                                    |
|------------------------------------|--|----------------------|------------------------------------|
| <b>Species</b>                     | <b>Gene Name</b>                       |                      | <b>Primer Sequence 5' -&gt; 3'</b> |
| Mouse                              | <i>Gapdh</i>                           | FWD                  | ATCATCCCTGCATCCACT                 |
| Mouse                              | <i>Gapdh</i>                           | REV                  | ATCCACGACGGACACATT                 |
| Mouse                              | <i>Nfe2l2</i> (Nrf2)                   | FWD                  | CAGCATAGAGCAGGACATGGAG             |
| Mouse                              | <i>Nfe2l2</i> (Nrf2)                   | REV                  | GAACAGCGGTAGTATCAGCCAG             |
| Mouse                              | <i>Slc40a1</i> (ferroportin)           | FWD                  | GTGGAGTACTTCTTGCTCTGG              |
| Mouse                              | <i>Slc40a1</i> (ferroportin)           | REV                  | CTGCTTCAGTTCTGACTCCTC              |
| Mouse                              | <i>Slc7a11</i> (xCT)                   | FWD                  | GATTCATGTCCACAAGCACAC              |
| Mouse                              | <i>Slc7a11</i> (xCT)                   | REV                  | GAGCATCACCATCGTCAGAG               |
| Mouse                              | <i>Lcn2</i> (Ngal)                     | FWD                  | AACATTTGTTCCAAGCTCCAGGGC           |
| Mouse                              | <i>Lcn2</i> (Ngal)                     | REV                  | CAAAGCGGGTGAAACGTTCCCTTCA          |
| Mouse                              | <i>Hamp</i> (hepcidin)                 | FWD                  | CAGCACCACCTATCTCCATCAAC            |
| Mouse                              | <i>Hamp</i> (hepcidin)                 | REV                  | CAGATGGGGAAGTTGGTGTCTC             |
| Mouse                              | <i>Slc11a2</i> (DMT-1)                 | FWD                  | TGTCACCGTCAGTATCCCA                |
| Mouse                              | <i>Slc11a2</i> (DMT-1)                 | REV                  | ATGGCTGAGCCAATGACTTC               |
| Mouse                              | <i>Tfrc</i> (Transferrin Receptor-1)   | FWD                  | GGATTCATGAGTGGCTACCT               |
| Mouse                              | <i>Tfrc</i> (Transferrin Receptor-1)   | REV                  | CTGACTTGTCTGTCTCCTCC               |
| <b>Genotyping primer sequences</b> |  |                      |                                    |
| Mouse                              | Cyclization recombinase (Cre)          | FWD                  | GCCAGGCGTTTTCTGAGCATAC             |
| Mouse                              | Cyclization recombinase (Cre)          | REV                  | CACCATTGCCCTGTTTCACTATC            |
| Mouse                              | Ferritin heavy chain 1 ( <i>FtH1</i> ) | <i>FtH1</i> flox FWD | CCATCAACCGCCAGATCAAC               |
| Mouse                              | Ferritin heavy chain 1 ( <i>FtH1</i> ) | <i>FtH1</i> flox REV | CGCCATACTCCAGGAGGAAC               |