SUPPLEMENTAL INFORMATION:

Title: Liver-specific ceramide reduction alleviates steatosis and insulin resistance in alcohol-fed mice

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Supplemental Figure S1: Schematic for genetically engineering inducible, liver-specific ceramide reduction in ASAH mice.

(A) The ceramidase ASAH1 reduces ceramide levels by hydrolysis to sphingosine and free fatty acid. (B) ASAH1 transcription is initiated by dox-dependent activation of reverse tetracycline-controlled transactivator (RTTA). (C) RTTA expression is repressed by a loxP-flanked stop codon. Liver-specific expression of Cre recombinase excises the stop codon, allowing RTTA expression exclusively in the liver.
Supplemental Figure S2: Ethanol consumption increases hepatic steatosis in ASAH- mice

Female ASAH- mice were pair-fed a control or ethanol diet with and without dox for 4 weeks. (A) Triglyceride levels in whole liver extracts, (B) serum ALT, and (C) fat mass measured by NMR. *p≤0.05
Supplemental Figure S3: ASAH1 overexpression and its subcellular localization.

Female ASAH+ mice were fed an Etoh-dox diet for 4 weeks. Whole liver lysate (Post-nuclear supernatant, PNS), endoplasmic reticulum (ER), lysosomes (lys), and lipid droplets (LD) were isolated from liver and assayed by immunoblotting.
| Gene symbol | Ensembl Stable ID | Gene description (Source: MGI Symbol) | Gene type | NCBI gene ID | Human gene name | log2 Fold Change | Fold Change | p value | p adj |
|-------------|-------------------|---------------------------------------|-----------|------------|----------------|----------------|-------------|---------|-------|
| Asah1       | ENSMUSG00000031991 | N-acylsphingosine amidohydrolase 1 | protein_coding | 11886 | ASAH1 | 8.390 | 335.440 | 0 | 0 |
| Sod1        | ENSMUSG00000022962 | superoxide dismutase 1, soluble | protein_coding | 20655 | SOD1 | 0.688 | 1.611 | 2.9E-10 | 2.90E-06 |
| Sel1        | ENSMUSG00000020964 | sel-1 suppressor of lin-12-like (C. elegans) | protein_coding | 20338 | SEL1L | 0.460 | 1.376 | 1.98E-06 | 0.0001 |
| Gm4837      | ENSMUSG00000030563 | predicted gene 4837 | protein_coding | 207728 | PDE2A | -2.435 | 0.186 | 1.29E-06 | 0.0040 |
| Gm4890      | ENSMUSG00000020714 | predicted gene 4890 | lncRNA | 2.966 | 7.815 | 4.91E-07 | 0.0018 |
| Gmr12       | ENSMUSG000000341408 | G-protein coupled receptor 12 | protein_coding | 14735 | GPR12 | -2.435 | 0.186 | 1.29E-06 | 0.0040 |
| Cnla5       | ENSMUSG00000012748 | collagen, type IV, alpha 5 | protein_coding | 12830 | COL4A5 | -0.992 | 0.503 | 3.35E-06 | 0.0088 |
| 903021J03Rk | ENSMUSG00000046138 | RIKEN cDNA 903021J03 gene | protein_coding | 20613 | KIAA2089 | 0.657 | 1.523 | 4.66E-06 | 0.0108 |
| Der3        | ENSMUSG000000060902 | Der1-like domain family, member 3 | protein_coding | 70377 | DER3 | 4.036 | 16.401 | 6.79E-06 | 0.0140 |
| Gm10925     | ENSMUSG000000100862 | predicted gene 10926 | pseudogene | 2.050 | 4.140 | 7.83E-06 | 0.0145 |
| Sic25a53    | ENSMUSG000000043448 | solute carrier family 25, member 53 | protein_coding | 67062 | SLC25A53 | -2.205 | 0.217 | 6.40E-05 | 0.0091 |
| Zfp991      | ENSMUSG000000079316 | zinc finger protein 991 | protein_coding | 666532 | ZNF91 | -2.052 | 0.241 | 6.27E-05 | 0.0991 |

**Supplemental Table S1:** RNAseq analysis reveals ceramide reduction increases SOD1 expression.

Female ASAH- and ASAH+ mice were fed an EtOH-dox diet for 4 weeks. RNA extracted from whole liver was assayed by RNAseq. P-adj is False Discovery Rate corrected p-value, calculated by DESeq2 using the Benjamini Hochberg method. Genes listed have p-adj <0.1.