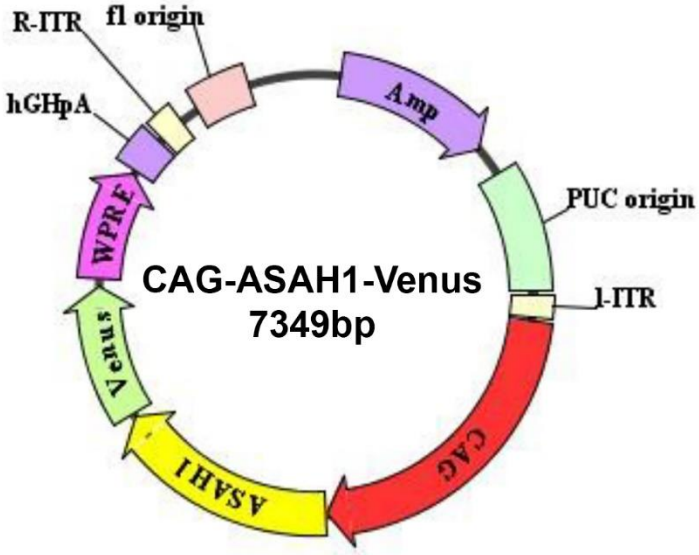
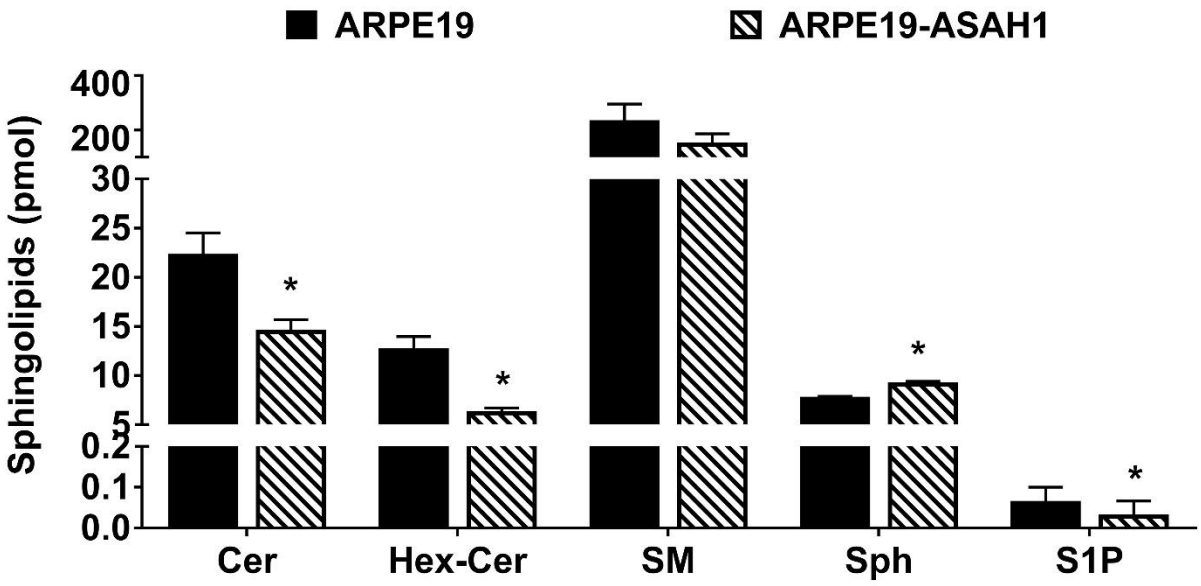
SUPPLMENT FIGURE S1



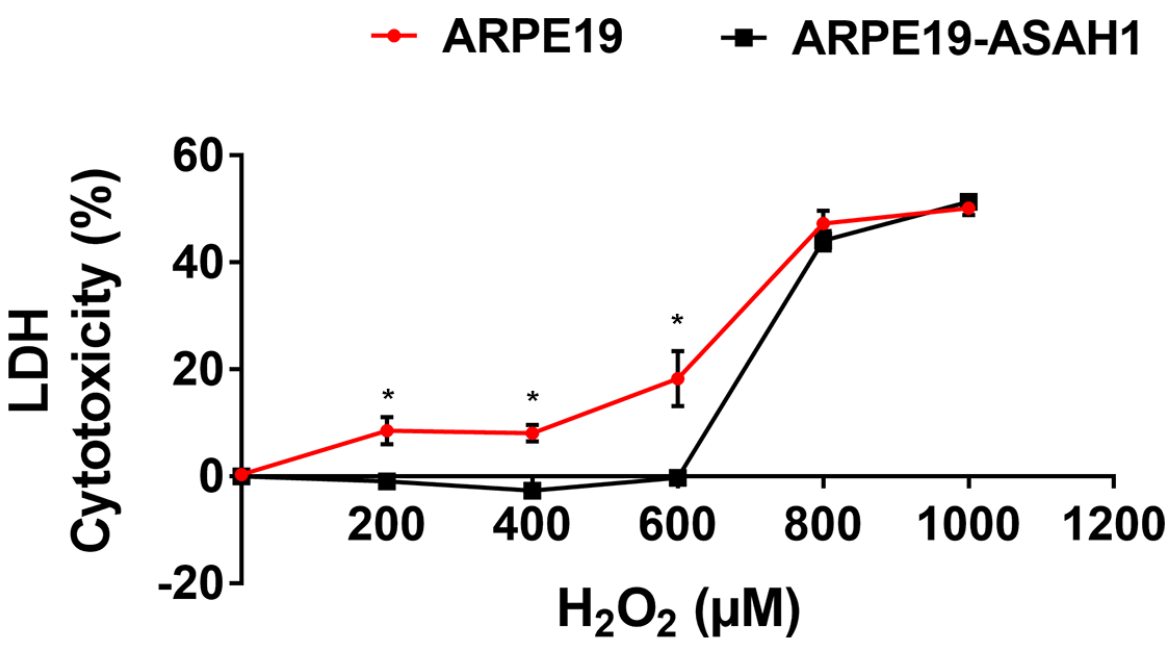
Supplemental Figure S1. **ASAH1 Cloning vector.** Human *Acylsphingosine amidohydrolase 1(ASAH1)* cDNA (Genbank Accession No. BC016481.1) was cloned from ARPE19 cells and was fused to the upstream DNA segment encoding the fluorescent protein, Venus. Recombinant plasmids were linearized by restriction digestion and electroporated into ARPE19 cell to generate the stable cell line ARPE19-ASAH1.

SUPPLEMENT FIGURE S2



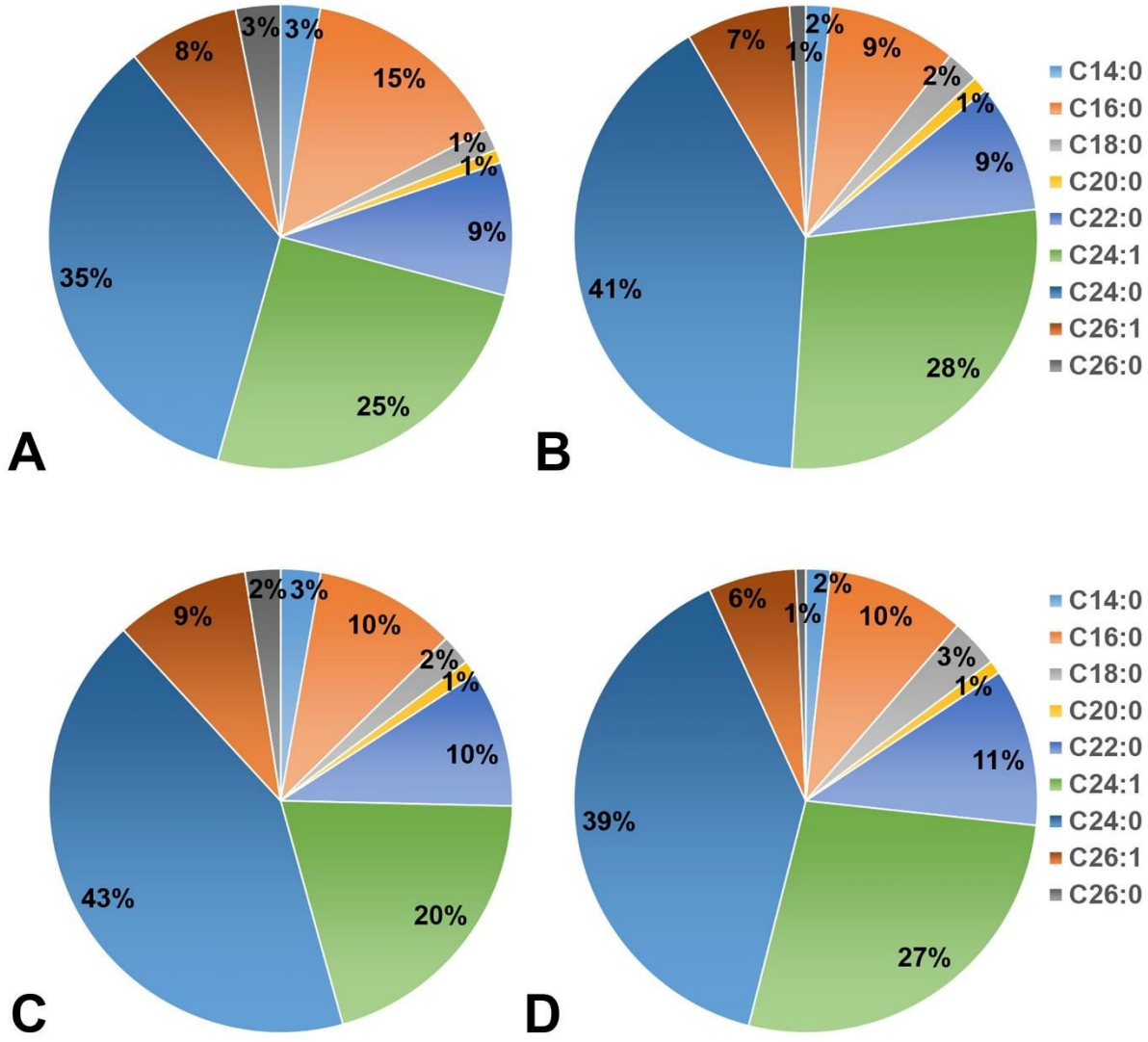
Supplemental Figure S2. **Analysis of major sphingolipids in the media from ARPE19 and ARPE19-ASAH1 cells.** ARPE19 and ARPE19-ASAH1 cells were grown in serum-free media for 24 hours and 1.5 mL cell-free media was analyzed for sphingolipids. Bar graphs represent mean overall sphingolipid concentrations ± SEM. There was significantly less total Ceramide (Cer), Hexosyl-ceramide (Hex-Cer), and Sphingosine 1-phosphate (S1P), but significantly more Sphingosine (Sph) detected in the media from ARPE19-ASAH1 cells. There was no change detected in Sphingomyelin (SM) levels. n=4; *$p<0.05$.

SUPPLEMENT FIGURE S3



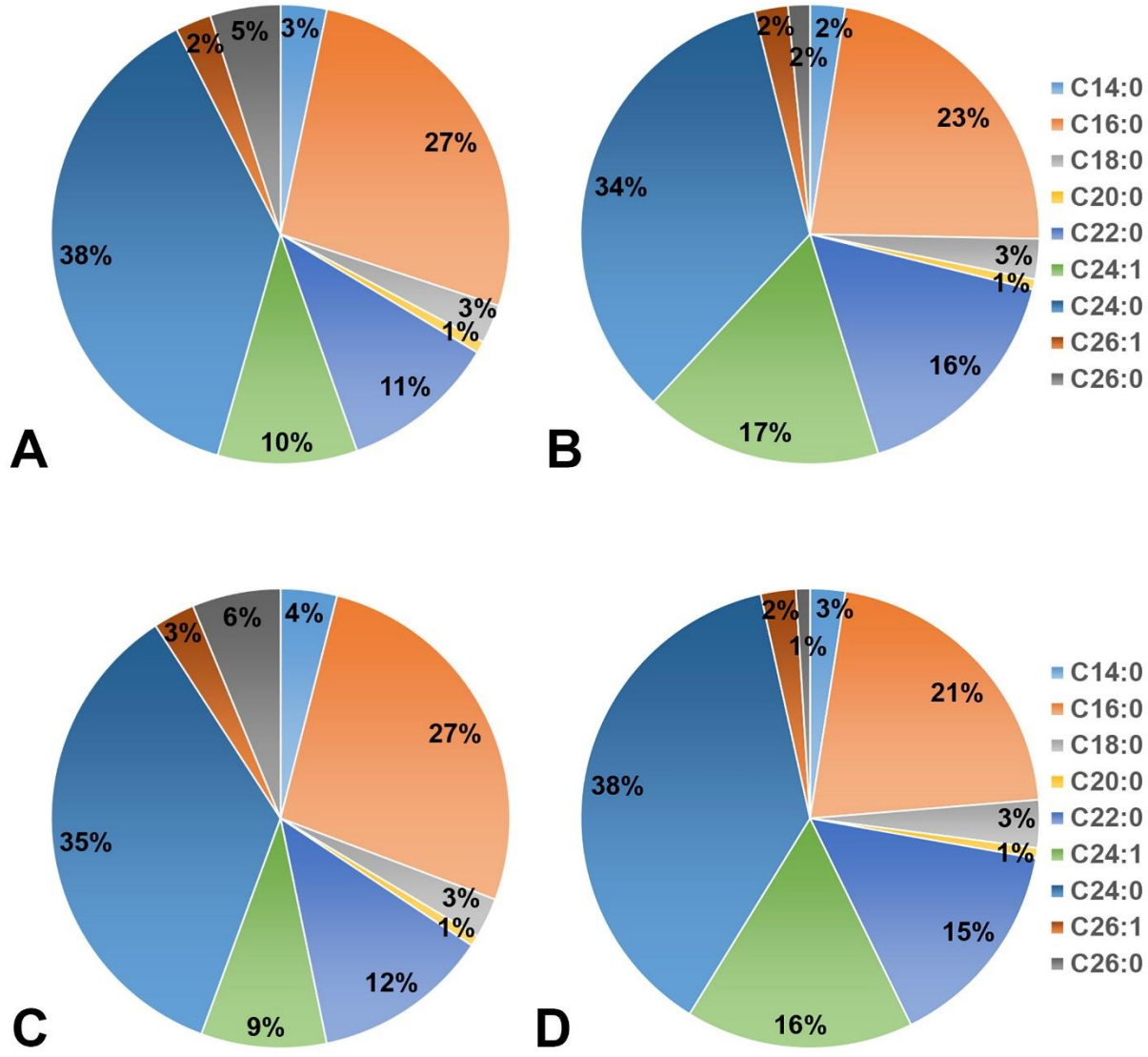
Supplemental Figure S3. **LDH assay for H_2O_2 cytotoxicity.** ARPE19 and ARPE19-ASAH1 cells were treated with varying concentrations of H_2O_2 and assayed for cytotoxicity. Significant protection is noted at concentrations up to 800 µM H_2O_2 in ARPE19-ASAH1 cells. ARPE19 n=6; ARPE19-ASAH1 n=6. *p<0.05.

SUPPLEMENT FIGURE S4



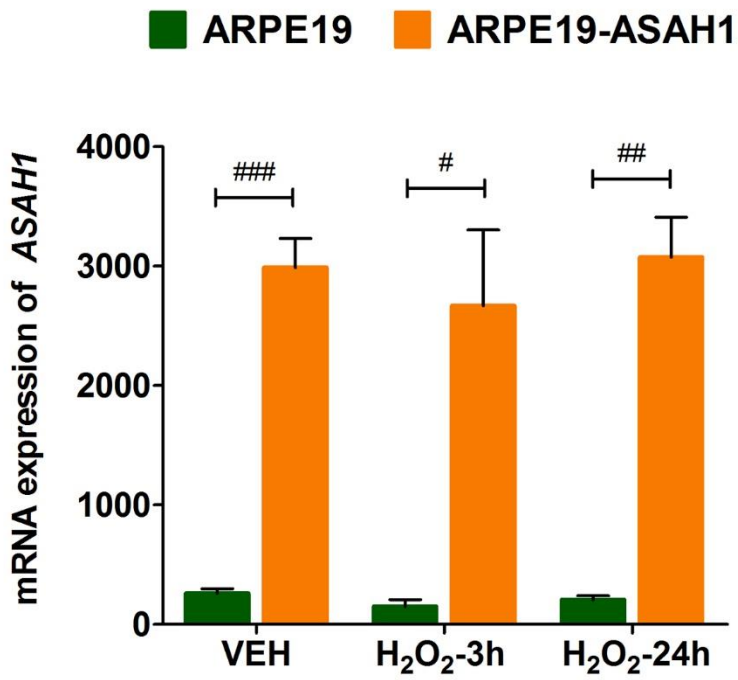
Supplemental Figure S4. **Compositional analysis of Ceramide species.** Samples were processed to collect sphingolipids and analyzed using LC-MS/MS. Ceramide species were quantified with respect to the different chain lengths. Pie charts represent mole percent composition of each lipid species with respect to its own chain length variants within same group. **A)** ARPE19 + Vehicle **B)** ARPE19 + H_2O_2. **C)** ARPE19-ASAH1 + Vehicle **D)** ARPE19-ASAH1 + H_2O_2.

SUPPLEMENT FIGURE S5



Supplemental Figure S5. **Compositional analysis of Hexosyl-Ceramide species.** Samples were processed to collect sphingolipids and analyzed using LC-MS/MS. Hexosyl-Ceramide species were quantified with respect to the different chain lengths. Pie charts represent mole percent composition of each lipid species with respect to its own chain length variants within same group. **A)** ARPE19 + Vehicle **B)** ARPE19 + H_2O_2. **C)** ARPE19-ASAH1 + Vehicle **D)** ARPE19-ASAH1 + H_2O_2.

SUPPLEMENT FIGURE S6



Supplemental Figure S6. **No change in *ASAH1* mRNA expression within cell types upon H_2O_2 treatment.** There was a significantly greater amount of ASAH1 in the transfected cell line compared to ARPE19. Overall, no changes with H_2O_2 administration were seen in the transgene expression. #p<0.05; ##p<0.01; ###p<0.001.