Supplemental Data

Figure S1. CerS down-regulation by targeted siRNA causes multiple changes in non-targeted CerS mRNA levels.

Figure S2. Heatmap of statistically signficant changes in sphingolipids following siCerS1-6 knockdown.

Figure S3. Effect of CerS2 and CerS6 knockdow on in vitro CerS activity.

Figure S4. CerS1 down-regulation increases non-C18-glycosphingolipids without significantly decreasing C18-ceramide.

Figure S5. siCerS3 increases multiple HexCer species.

Figure S6. siCerS4 decreases C24:1-Cer levels and increases C26:0-sphingolipids.

Figure S7. siCerS5 increases multiple SM species and C26:1-sphingolipids.

Figure S8. Some CerS knockdowns increase total levels of a particular sphingolipid class but do not significantly elevate sphingoid bases.

Figure S1.

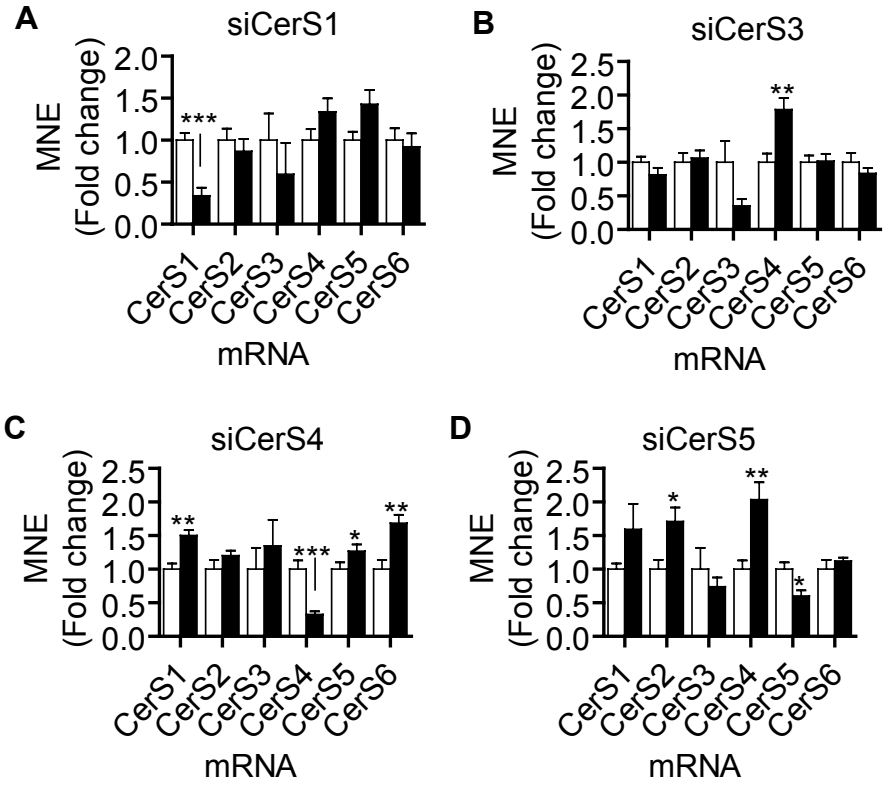

Figure S1. CerS down-regulation by targeted siRNA causes multiple changes in non-targeted CerS mRNA levels. MCF-7 cells were transfected with 5 nM siRNA targeted against CerS1 (A), CerS3 (B), CerS4 (C), or CerS5 (D) (black bars) or siControl (white bars) for 48 hours. Cells were harvested and RNA was extracted for Q-PCR analysis of expression of CerS1-6. Q-PCR data are normalized to β-actin mRNA expression and data are mean ± SEM for three independent experiments: *$p < 0.05$, **$p < 0.01$, ***$p < 0.01$ versus siControl.

Figure S2.

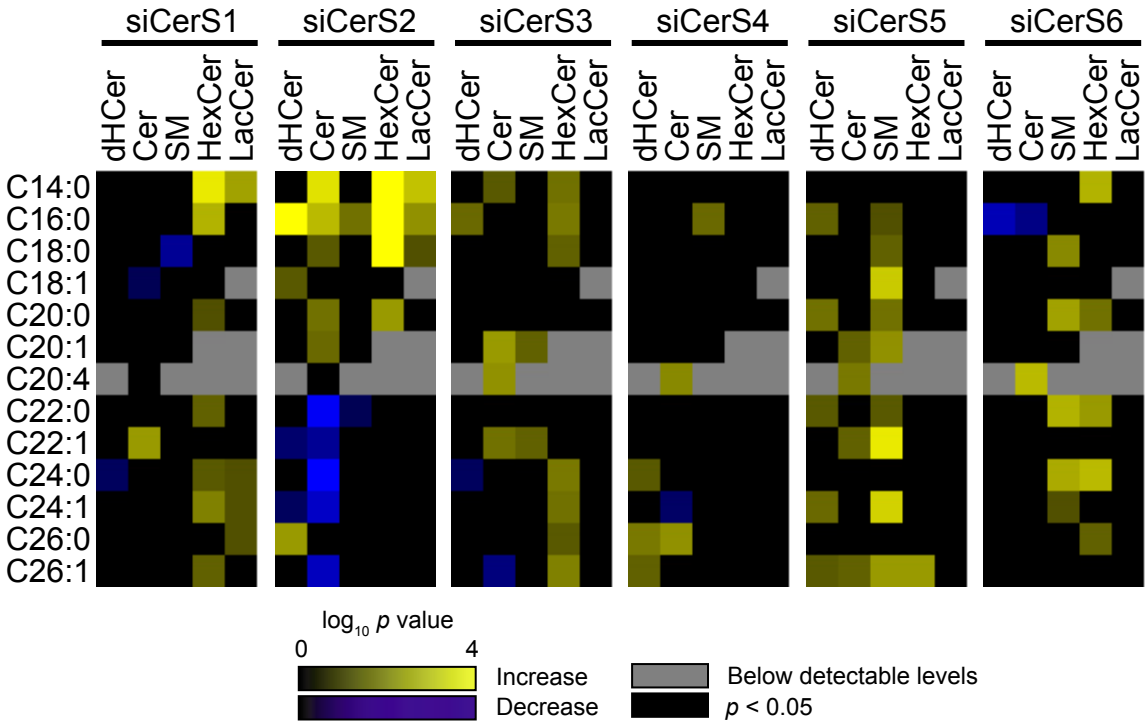

Figure S2. Heatmap of statistically signficant changes in sphingolipids following siCerS1-6 knockdown. MCF-7 cells were transfected with 5 nM siRNA targeted against CerS1-6 and sphingolipid masses were determined by HPLC/MS. Sphingolipid levels were normalized to the amount of total lipid phosphate. (A) Sphingolipid mass changes are displayed as a heatmap of the -$\log_{10}$ of the *p* value calcuted from Student's *t* test of siCerS vs. siControl. Gray boxes indicate that the lipid was below detectable levels (BDL). Yellow boxes indicate a significant increase and blue boxes indicate a significant decrease. Black indicates no significant change, and gray indicated that the lipid was below detectable levels. Data represent three to five independent experiments.

Figure S3.

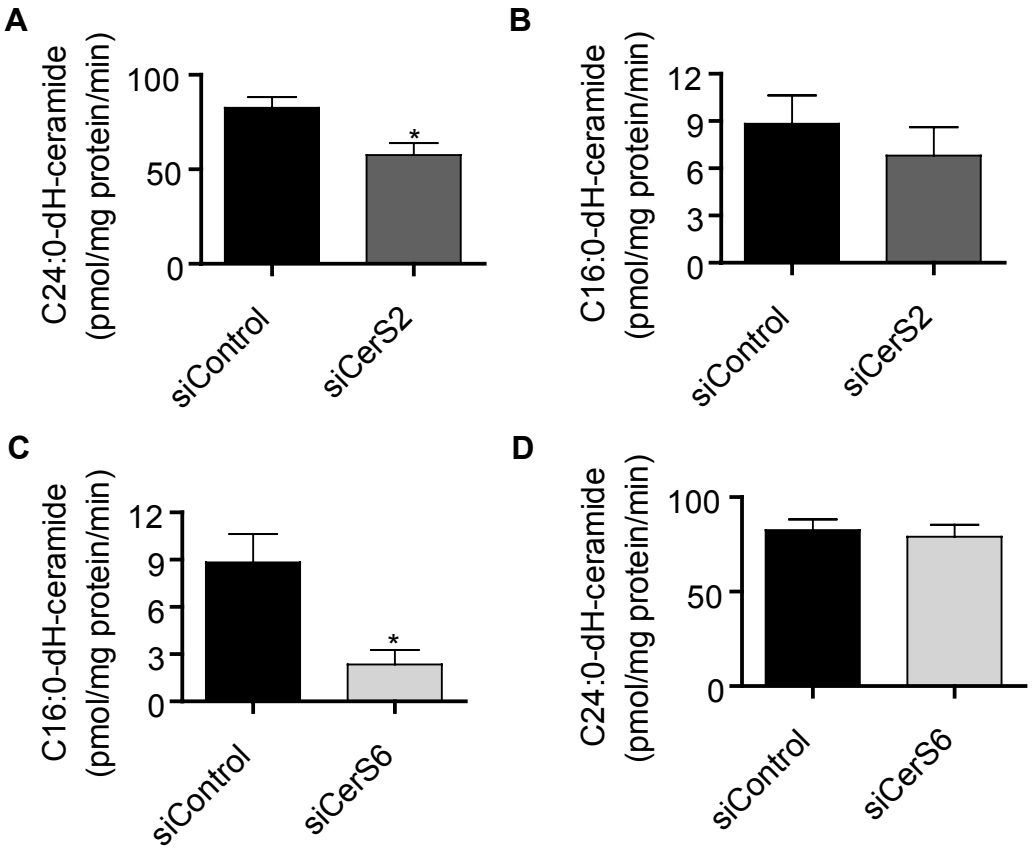

Figure S3. Effect of CerS2 and CerS6 knockdow on *in vitro* CerS activity. MCF-7 cells were transfected with 5 nM siRNA targeted against CerS2 (A-B) or CerS6 (C-D) for 48 hours after which *in vitro* CerS activity was determined as described in *Materials and Methods*. Reactions were conducted using 17C-dHSph and lignoceroyl(C24:0)-CoA (A,D) or palmitoyl(C16:0)-CoA (B,C). Data represent mean fold change ± SEM for four experiments assayed in duplicate. Statistical signifcance was determined using an un-paired Student's *t* test vs. siControl.*$p < 0.05$ versus siControl.

Figure S4.

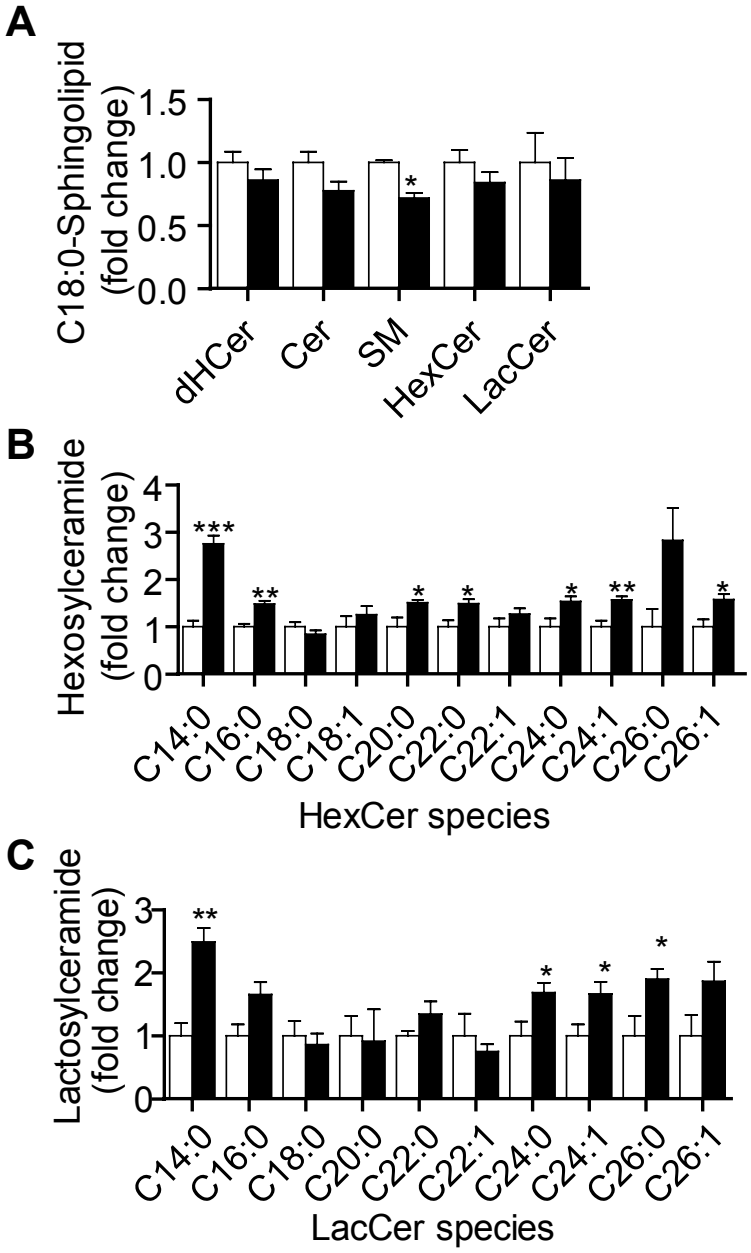

Figure S4. CerS1 down-regulation increases non-C18-glycosphingolipids without significantly decreasing C18-ceramide. The effects of siCerS1 (black bars) on C18:0-containing sphingolipids (A), all HexCer species (B), and all LacCer species (C) compared to siControl (white bars) were determined as described in Figure S2. (A) siCerS1 reduces C18:0-SM without significantly reducing other C18:0-sphingolipids. (B-C) siCerS1 increases several non-C18:0 long-chain HexCer (B) and very long-chain LacCer (C) species. Sphingolipid levels are normalized to the amount of total lipid phosphate and represent the mean fold change ± SEM for three to five independent experiments. *$p < 0.05$, **$p < 0.01$, ***$p < 0.01$ versus siControl.

Figure S5.

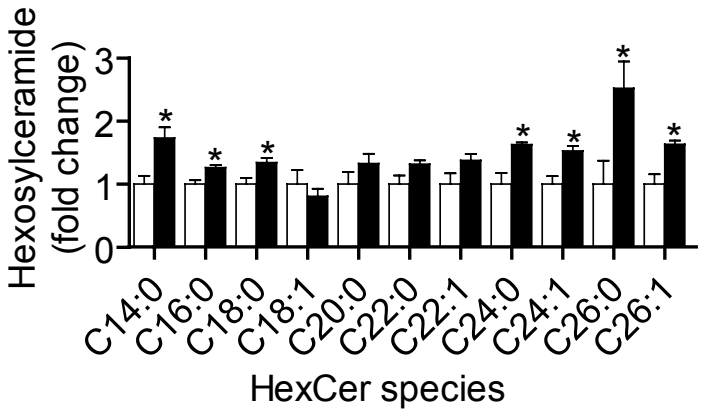

Figure S5. siCerS3 increases multiple HexCer species. The effects of siCerS3 (black bars) on HexCer species compared to siControl (white bars) was determined as described in Figure S2. CerS3 knockdown increases long-chain and very long-chain HexCer species. Sphingolipid levels are normalized to the amount of total lipid phosphate. Data are mean ± SEM for three to five independent experiments. *$p < 0.05$ versus siControl.

Figure S6.

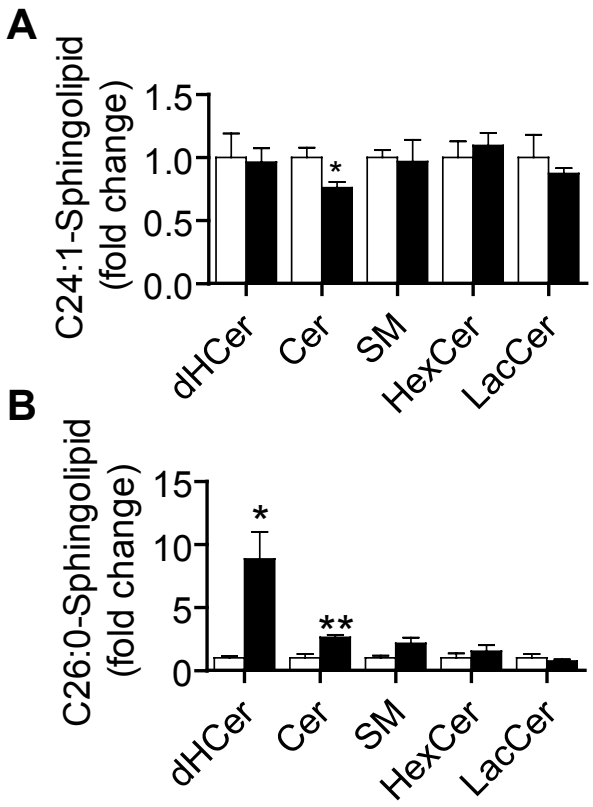

Figure S6. siCerS4 decreases C24:1-Cer levels and increases C26:0-sphingolipids. The effects of siCerS4 (black bars) on C24:1- (A) and C26:0-sphingolipids (B) compared to siControl (white bars) were determined as described in Figure S2. (A) siCerS4 reduces C24:1-Cer levels without decreasing other C24:1-sphingolipid levels. (B) siCerS4 increases C26:0-dHCer and C26:0-Cer levels. The mean C26:0-SM level was also increased but this change did not meet our criterion for significane by a slight margin ($p = 0.0513$). Data are normalized to the amount of lipid phosphate present in each sample. Sphingolipid levels are normalized to the amount of total lipid phosphate. Data are mean ± SEM for three to five independent experiments. *$p < 0.05$, **$p < 0.01$ versus siControl.

Figure S7.

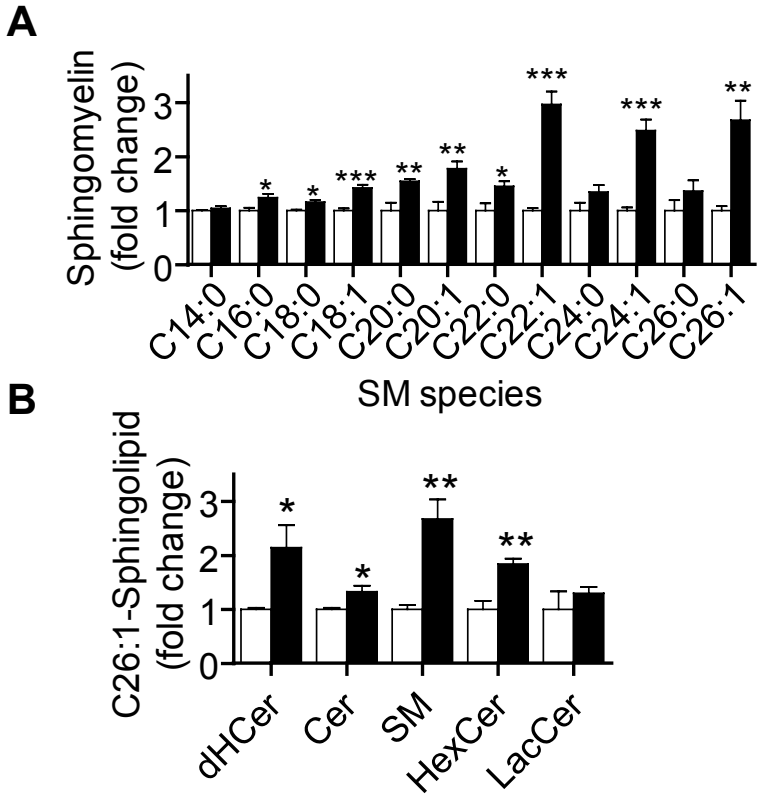

Figure S7. siCerS5 increases multiple SM species and C26:1-sphingolipids. The effects of siCerS5 (black bars) on SM (A) and C26:1-sphingolipids (B) compared to siControl (white bars) were determined as described in Figure S2. (A) siCerS5 induced the accumulation of several SM species. (B) siCerS5 increased C26:1-sphingolipid including dHCer, Cer, SM, and HexCer. Sphingolipid levels are normalized to the amount of total lipid phosphate. Data are mean ± SEM for three to five independent experiments. *$p < 0.05$, **$p < 0.01$, ***$p < 0.01$ versus siControl.

Figure S8.

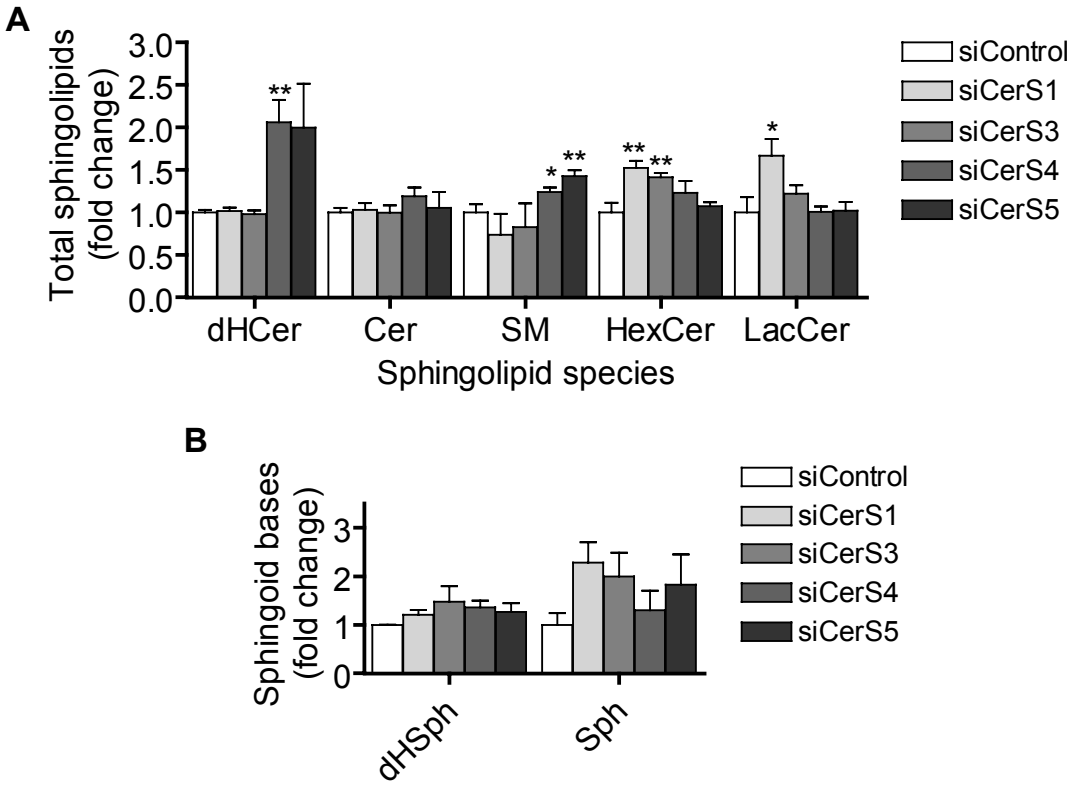

Figure S8. Some CerS knockdowns increase total levels of a particular sphingolipid class but do not significantly elevate sphingoid bases. MCF-7 were transfected with siCerS1, siCerS3, siCerS4, or siCerS5 or siControl as indicated in Figure S2 and the total levels of different sphingolipid classes (A) were determined by HPLC/MS. (A) CerS1 knockdown elevated total HexCer and LacCer levels. siCerS3 caused a minor stimulation of overall HexCer levels. siCerS4 induced a significant elevation in dHCer. siCerS5 caused an overall increase in SM. (B) dHSph and Sph levels were determined following CerS knockdown. No statistically signficant changes were observed. Sphingolipid levels are normalized to the amount of total lipid phosphate. Data are mean ± SEM for three to five independent experiments. *$p < 0.05$, **$p < 0.01$, ***$p < 0.01$ versus siControl.