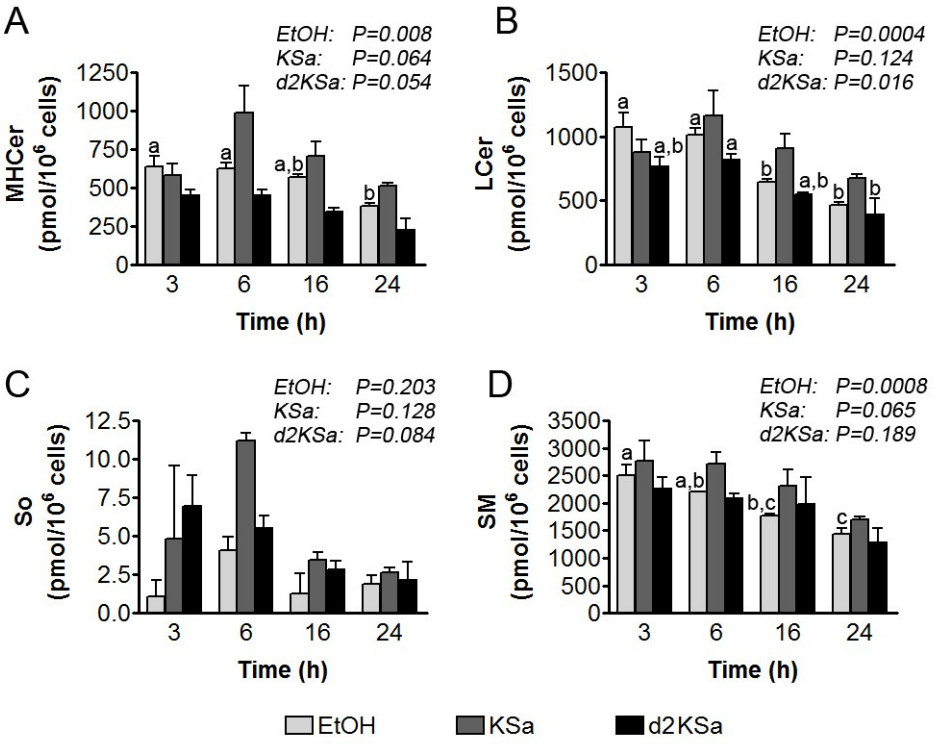
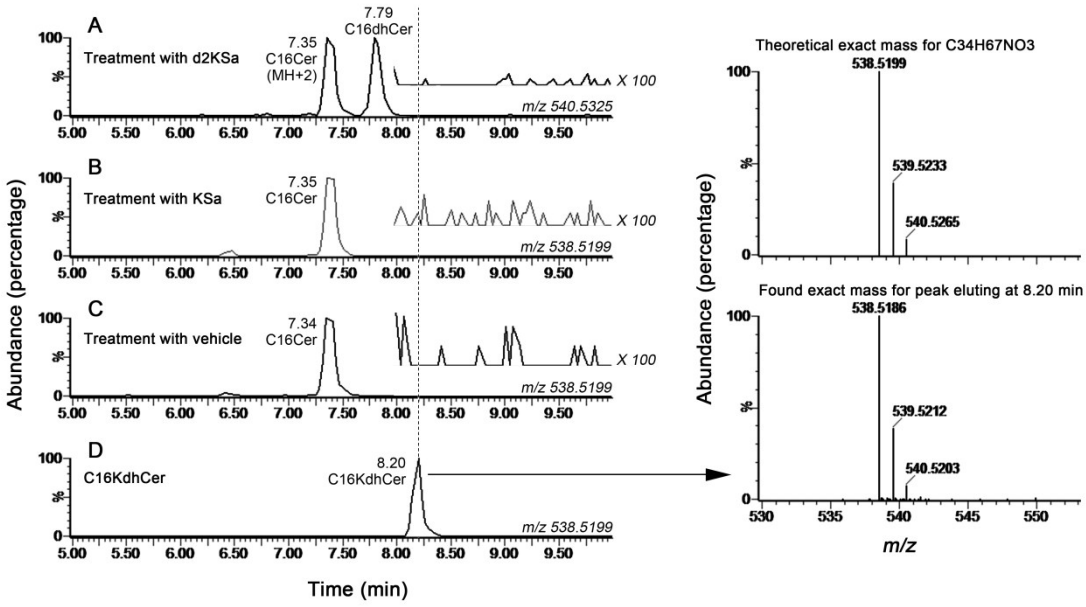
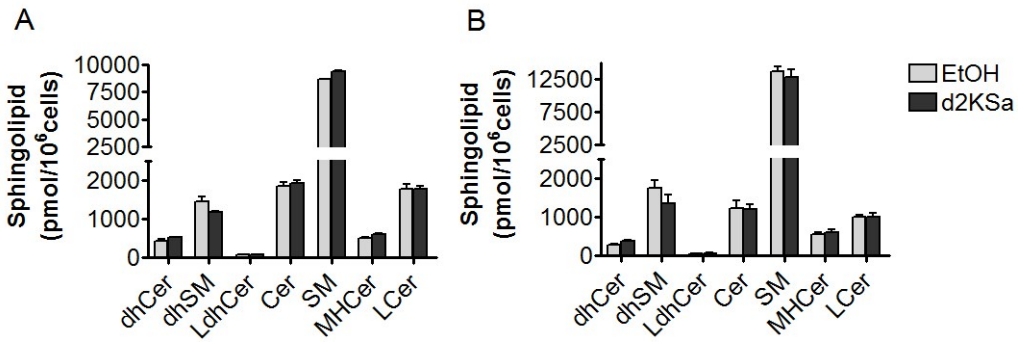
SUPPLEMENTARY FIGURES



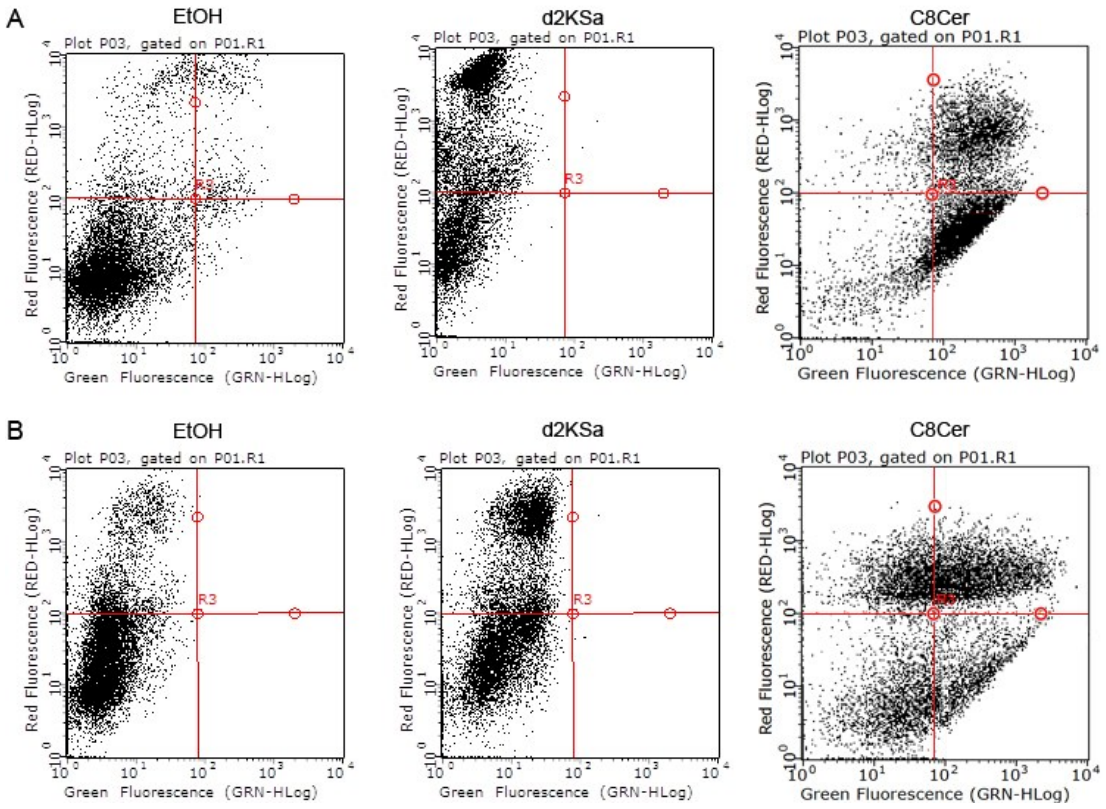
Supplementary Figure 1. Effect of KSa and d2KSa on sphingolipid levels in HGC27 cells. Cells were treated with 5 μM of KSa (dark grey bars), d2KSa (black bars) or vehicle (light grey bars) for the specified times and then cells were collected and processed for LC/MS lipid analysis. Data correspond to the mean ± SD of three independent experiments with triplicates. Data corresponding to each different treatment over time were analyzed by one-way ANOVA test followed by Bonferroni's multiple comparison post-test when ANOVA $P<0.05$. In the latter case, different letters denote statistically significant difference between means at $P<0.05$ as found by this test.



Supplementary Figure 2. Metabolization of KSa and d2KSa by direct *N*-acylation in HGC27 cells. Cells were treated with 5 μM of d2KSa (A) KSa (B), or vehicle (C) for 6 h and then cells were collected and processed for LC/MS lipid analysis. Left, chromatograms generated by selection of the ions indicated on the right above each trace, which correspond to the species indicated next below the retention times. Synthetic C16KdhCer is shown in D. Right, theoretical and experimental exact mass of peak at 8.20 min of chromatogram D, corresponding to C16KdhCer. All species were analyzed under ESI-positive mode. Other ketodihydrosphingolipids were also absent in the chromatrograms when monitoring the ions listed in the Supplementary Table. C16Cer, *N*-palmitoylsphingosine, C16dhCer, *N*-palmitoylsphinganine, C16KdhCer, *N*-palmitoylketosphinganine. Dideuterated *N*-palmitoylketosphinganine (C16d2KdhCer) (C34H65D2NO3, exact mass 540.5325; see Supplementary Table) should elute at 8.20 min in chromatogram A.



Supplementary Figure 3. Effect of d2KSa on natural sphingolipid levels in T98G (A) and U87MG (B) cells. Cells were treated with with 12 μM (T98G) or 20 μM (U87MG) d_2KSa (black bars) or vehicle (light grey bars, controls) for 6 h and then cells were collected and processed for LC/MS lipid analysis. Data correspond to the mean ± SD of three independent experiments with triplicates. None of the species changes significantly as compared to controls.



Supplementary Figure 4. Quantification of apoptosis by flow cytometry in T98G (A) and U87MG (B) cells exposed to d2KSa or C8Cer (positive control of apoptosis). Cells were treated with 12 μM (T98) or 20 μM (U87MG) d_2KSa or 20 μM or C8Cer for 24 h (C,D) and analyzed by flow cytometry after IP/Annexin V staining. Images show representative dot plot panels. Treatment with d2KSa results in increased number of necrotic cells (PI–positive and annexin V–negative; top left quadrants), while treatment with C8Cer produces early apoptotic cells (PI–negative and annexin V–positive; bottom right quadrants) and late apoptotic cells (PI–positive and annexin V–positive; top right quadrants).