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SUPPLEMENTARY MATERIAL

A novel methylated analogue of *L*-Mimosine exerts its therapeutic potency through ROS production and ceramide-induced apoptosis in malignant melanoma

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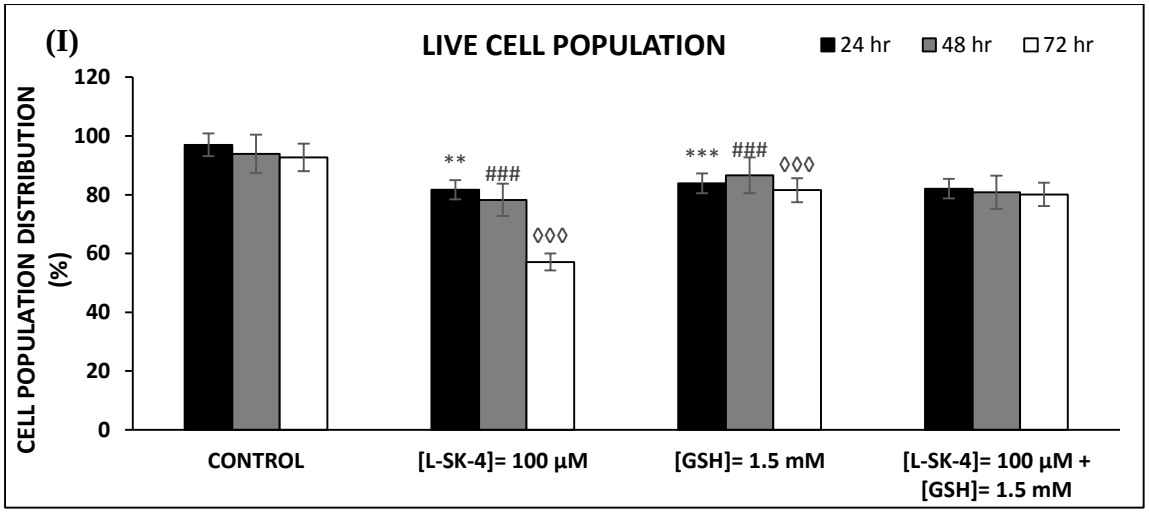
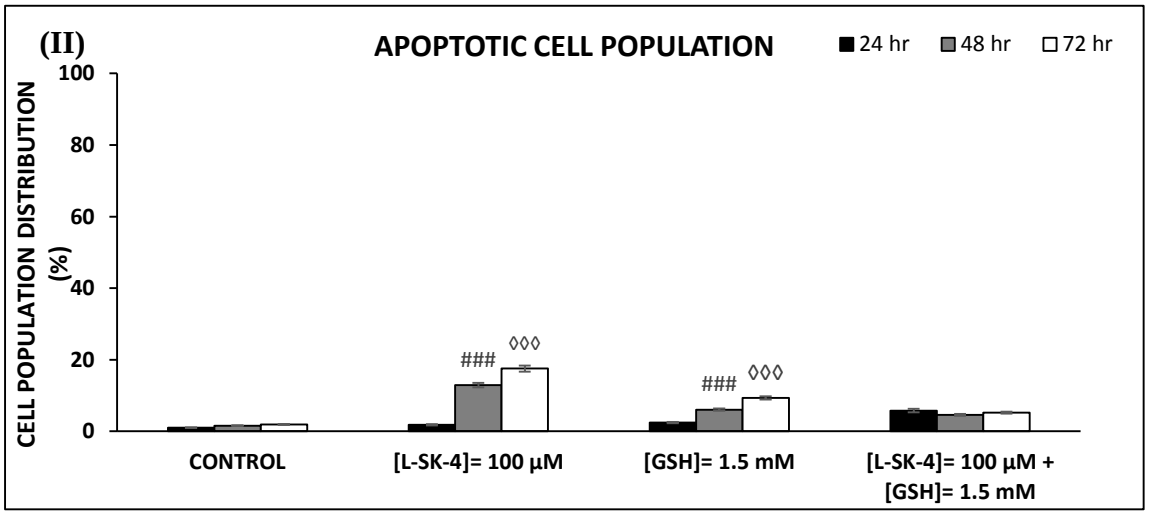




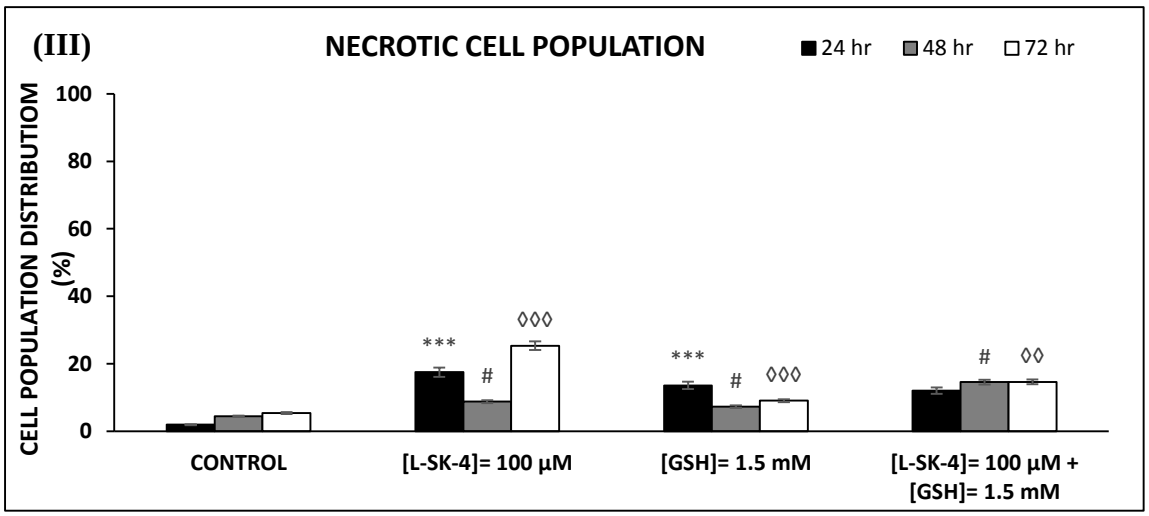
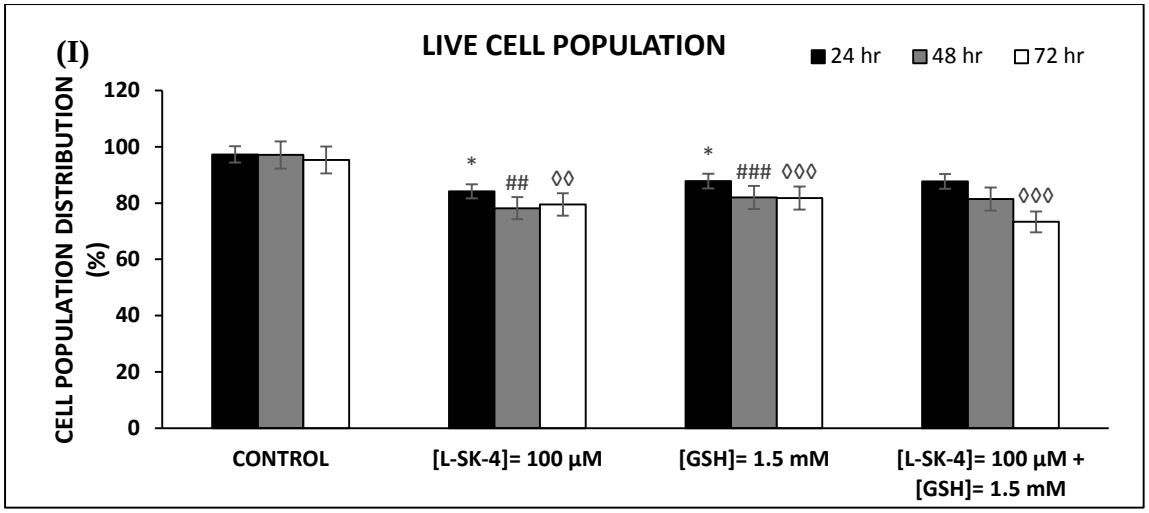
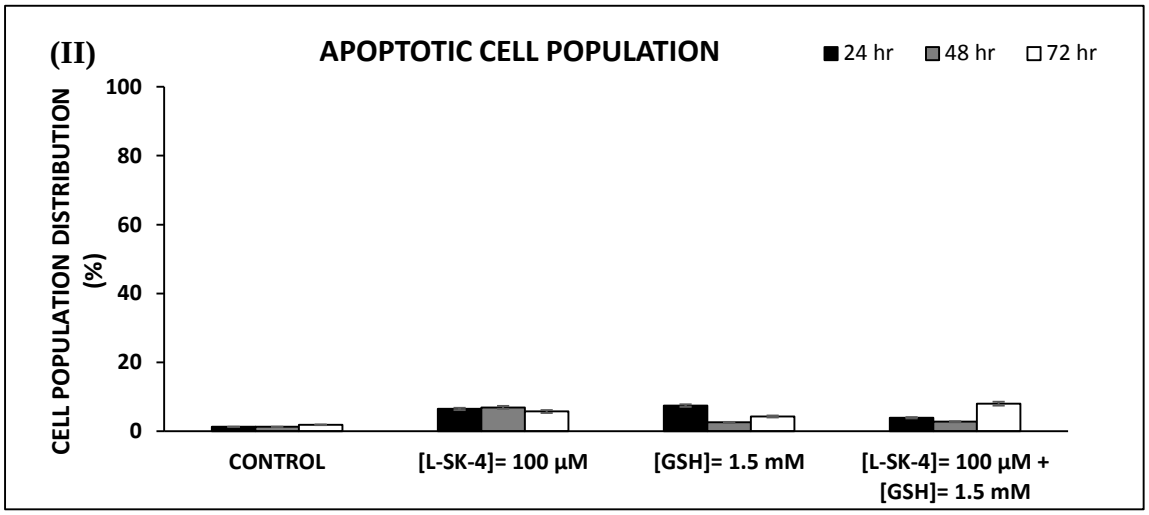


Figure S1: GSH prevents apoptotic induction in VMM-1 cells. Briefly cells were treated with 100 µM of *L*-SK-4 in the presence or absence of 1.5 mM of GSH for 24, 48 and 72 hrs. A flow cytometry-based approach was utilized for identifying live **(I)**, apoptotic **(II)** and necrotic **(III)** cell populations which were quantitated as percentages. Data shown are means of ± SD of 3 replicates from three independent experiments.





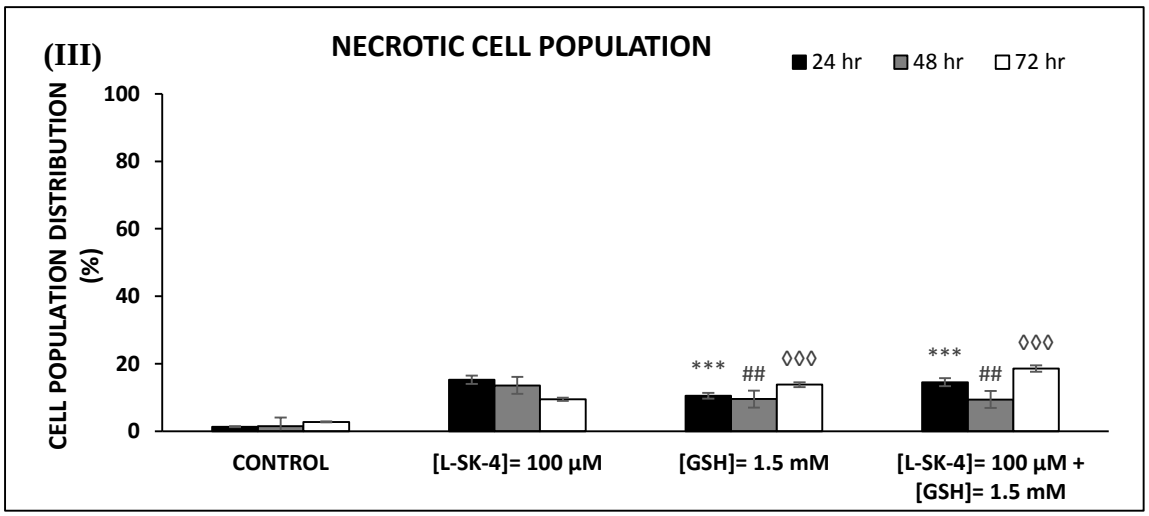
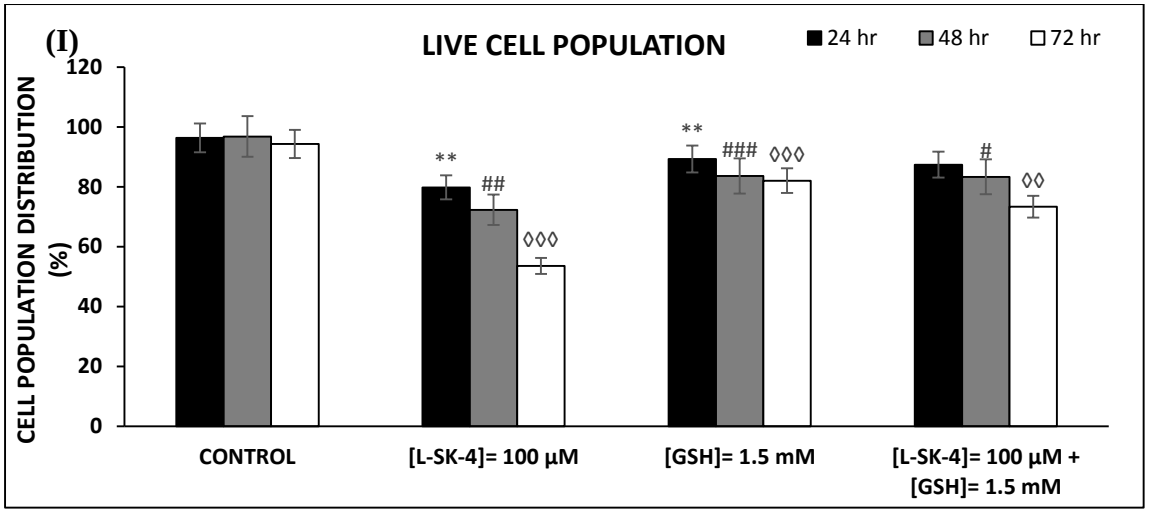
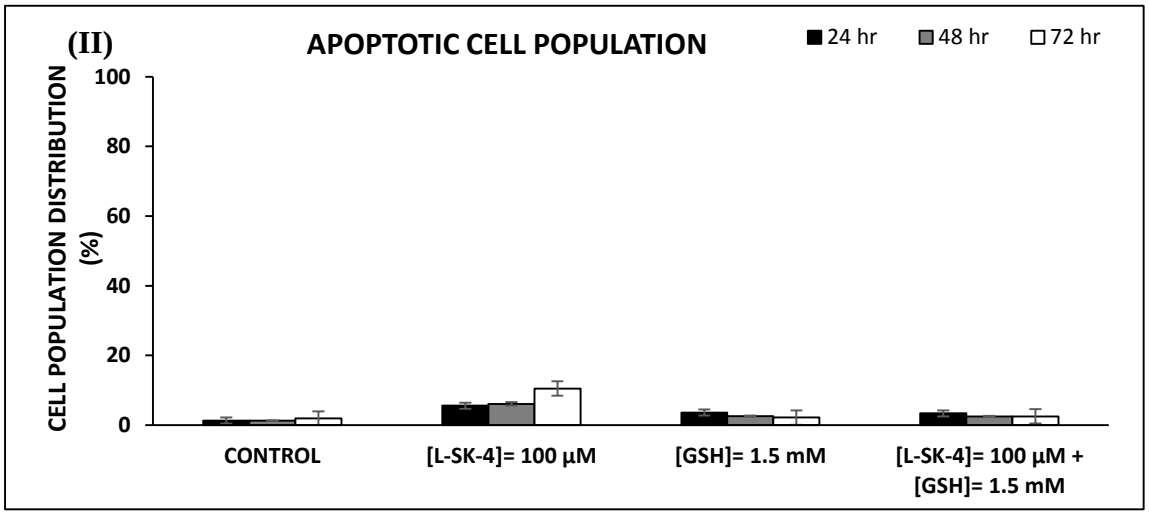


Figure S2. GSH prevents apoptotic induction in Hs 294T cells. Briefly cells were treated with 100 µM of *L*-SK-4 in the presence or absence of 1.5 mM of GSH for 24, 48 and 72 hrs. A flow cytometry-based approach was utilized for identifying live **(I)**, apoptotic **(II)** and necrotic **(III)** cell populations which were quantitated as percentages. Data shown are means of ± SD of 3 replicates from three independent experiments.





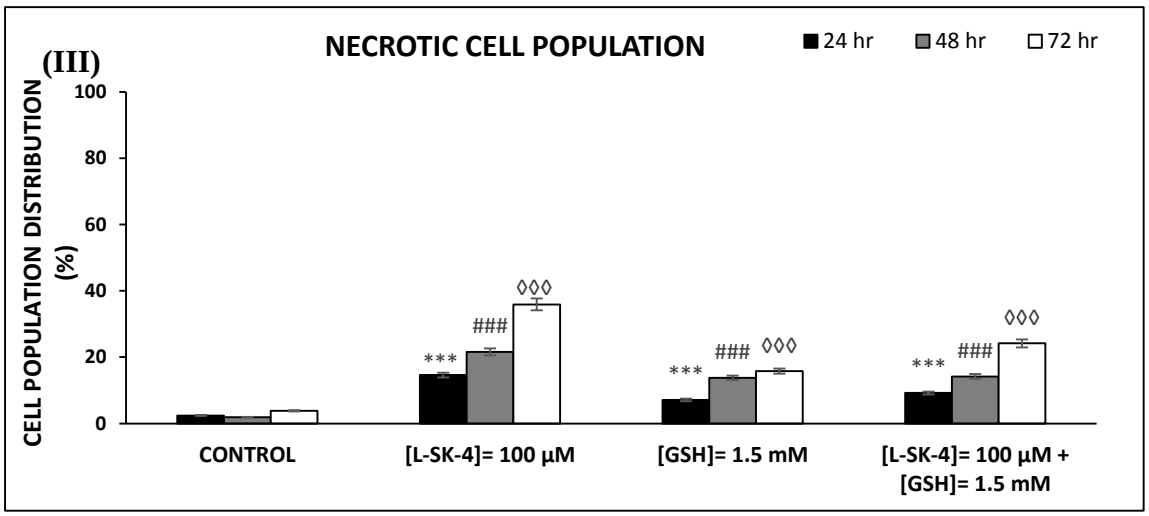


Figure S3. GSH prevents apoptotic and necrotic induction in B16F-10 cells. Briefly cells were treated with 100 µM of *L*-SK-4 in the presence or absence of 1.5 mM of GSH for 24, 48 and 72 hrs. A flow cytometry-based approach was utilized for identifying live **(I)**, apoptotic **(II)** and necrotic **(III)** cell populations which were quantitated as percentages. Data shown are means of ± SD of 3 replicates from three independent experiments.

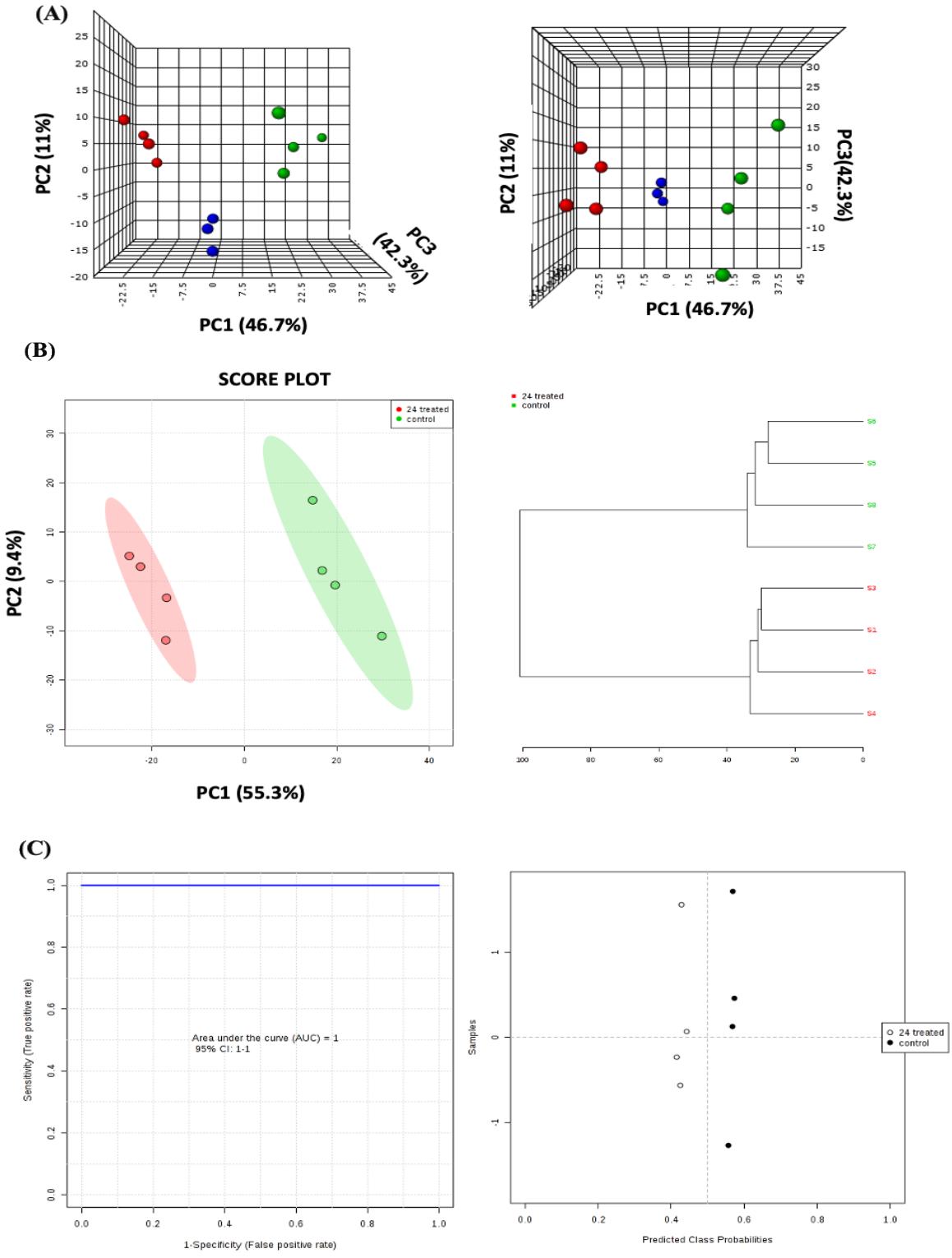


Figure S4: PCA visualization, raw data set approx. 1800 lipid MS features detected (MS1 profiling) n=4. Planar separation can be observed between treated (red spheres), control (green spheres) and quality control (blue spheres) (x~ 6% relative standard deviation (r.s.d) –analysis stability assessment. Extraction blank were also imbedded for background ion subtractions prior to PCA visualization. Control and treatment 95% confidence level are highlighted between each groups, approx. 1800 MS features**.**

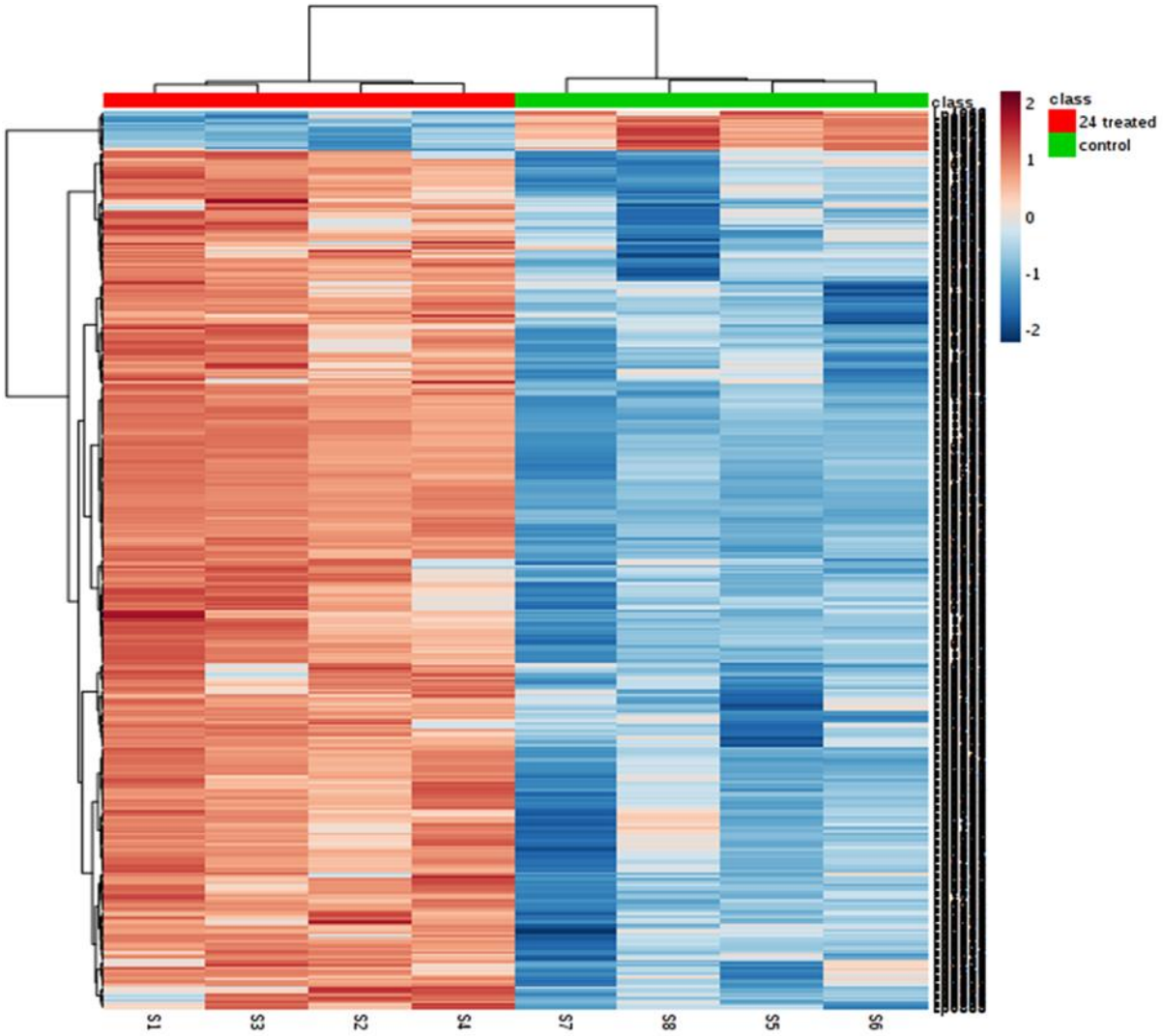


Figure S5: Heat map representation of the top 400 discriminate features, identified via ROC analysis, showing the dysregulation of the lipidome profile of A375 cells exposed to 100 µM *L*-SK-4, for 24 hrs, compared to the respective untreated control.

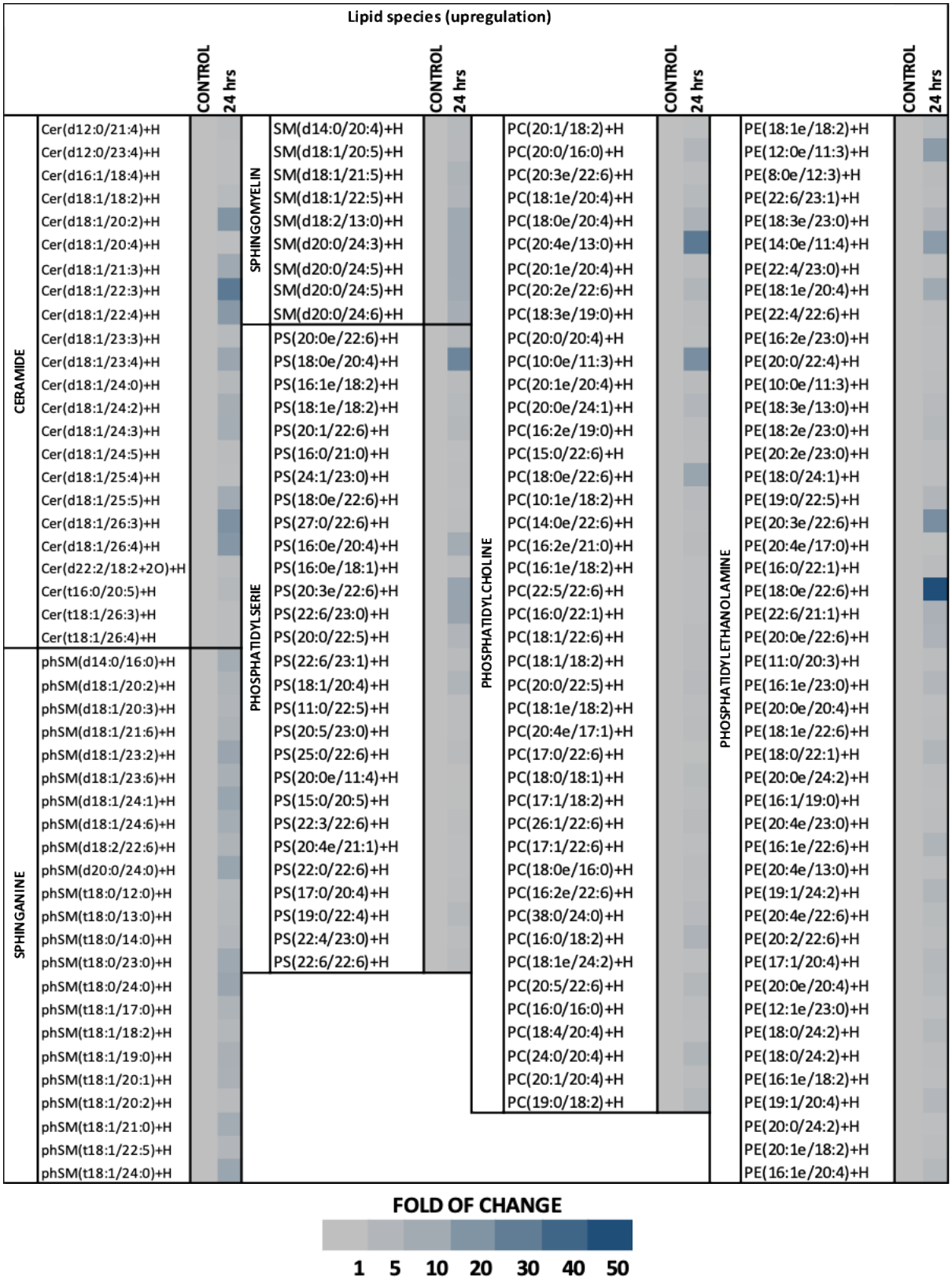


Figure S6: Heat map representation of the sphingolipids' profile of A375 cells between untreated and 24 hrs post treatment with 100 µM *L*-SK-4 experimental conditions.