

A Comprehensive Profiling of Cellular Sphingolipids in Mammalian Endothelial and Microglial Cells Cultured in Normal and High-Glucose Conditions

Figure S1:

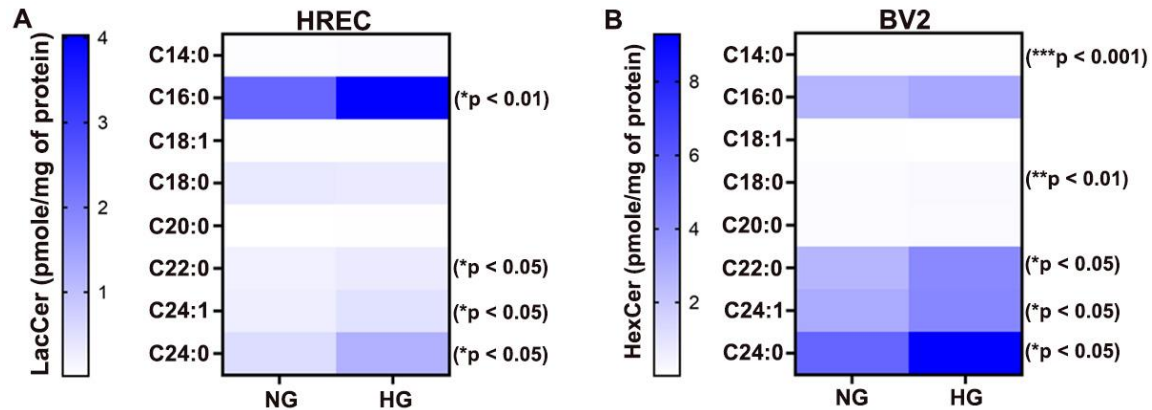


Figure S1: Heat map of Glycosphingolipids in high glucose-cultured HREC and BV2 cells. Different carbon chains of Lactosyl-Ceramide (LacCer) (pmole/mg protein) in Normal glucose (NG) and High glucose (HG)-cultured HREC (A). Different carbon chains of Hexosyl-Ceramide (HexCer) (pmole/mg protein) in NG and HG-cultured BV2 (B). *p < 0.05, **p < 0.01, ***p < 0.001, Mean \pm SEM, n=6.

Figure S2:

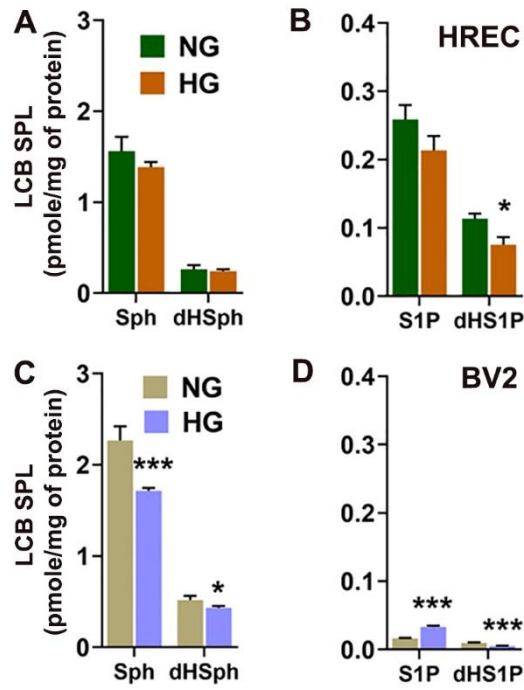


Figure S2: Analysis of different Long Chain Base (LCB) Sphingolipid (SPL) classes in high glucose cultured HREC and BV2 cells. Levels of LCB-SPL (Sphingosine, Sph; dihydrosphingosine, dHSph; sphingosine-1-phosphate, S1P and dihydro S1P, dHS1P) (pmole/mg protein) in Normal Glucose (NG) and High glucose (HG)-cultured HREC (A, B) and BV2 (C, D). * $p < 0.05$, *** $p < 0.001$, Mean \pm SEM, $n=6$.

Figure S3:

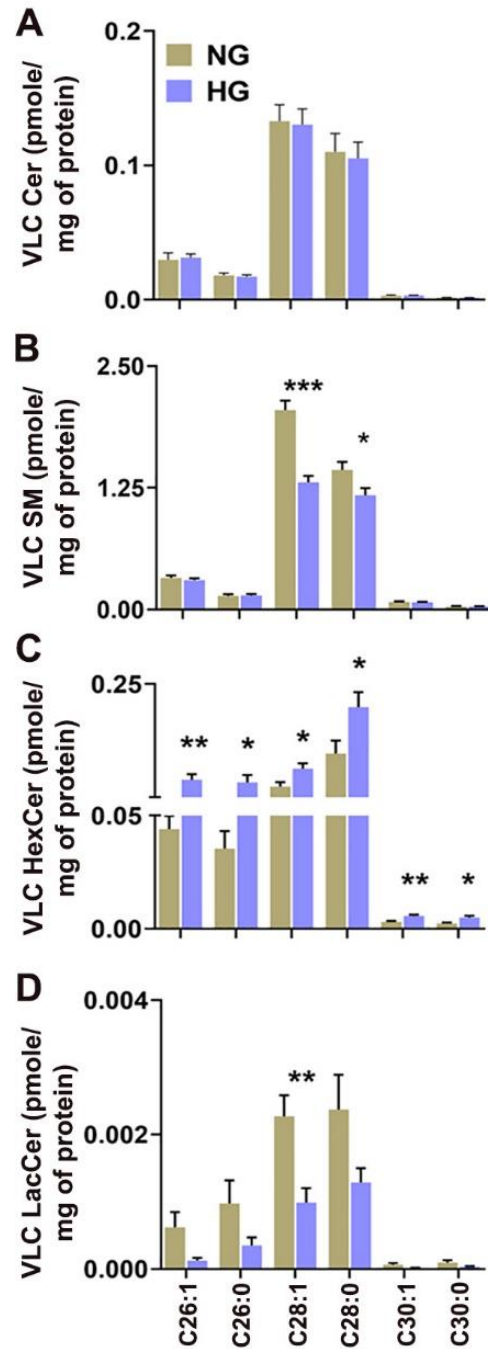


Figure S3: Analysis of different Very Long Chain (VLC) Sphingolipid classes of high glucose-cultured BV2 cells. Total concentration (pmole/mg protein) of different carbon chain length of VLC Ceramide (VLC Cer) (**A**), VLC Sphingomyelin (VLC SM) (**B**), VLC Hexosyl-Ceramide (VLC HexCer) (**C**), VLC Lactosyl-Ceramide (VLC LacCer) (**D**) in normal glucose (NG) and high glucose (HG) cultured cells. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, Mean \pm SEM, $n=6$.

Supplementary Table S1: Primers used and their sequences for HREC (Human origin)

Name of Genes	Sequences
<i>B4GALT5</i>	Forward: CCAGAGGGTGACACAGGAAA Reverse: GGCGTCGTATGTGATGTTTG
<i>B4GALT6</i>	Forward: AAGTGGGCTGACAGTGGAAC Reverse: CTGACGCTCCTTGAATACC
<i>CERS2</i>	Forward: TCTATATCACGCTGCCCTG Reverse: CTTGCCACTGGTCAGGTAGA
<i>CERS4</i>	Forward: GCATTGAGGACTTTCCTTACCT Reverse: ACCACTCGTTGAAACTGGAC
<i>ELOVL4</i>	Forward: TCCAGAAATATCTTTGGTGG Reverse: GTTAAGGCCCAGTTCAATT
<i>SMPD1</i>	Forward: GAAGAGCTGGAGCTGGAATTA Reverse: CTGGGTCAGATTCAGGATGTAG
<i>SMPD2</i>	Forward: CTT ACC CAG CAC ATC TAC ACT C Reverse: AGC ACG TAG TCA ATG CGG ACA
<i>SPHK1</i>	Forward: AGCACCGATAAGGAGCTGAA Reverse: AGATTCAGCCTCAGCCAAA
<i>SPHK2</i>	Forward: TTCCGGAAGAAAGGGATCT Reverse: TAGGCTGGTAGGAGCAAGGA
<i>SPTLC1</i>	Forward: AGGGATTCTGATCCTCTGGATA Reverse: GTTGCCACTCTTCAATCAGTTC
<i>SPTLC2</i>	Forward: TATGGAGCTGGAGTGTGCAG Reverse: GCCAACAAGAGCAGGAATGT
<i>ICAM1</i>	Forward: ATTCCCAGCAGACTCCAATG Reverse: GGCTGCTACCACAGTGATGA
<i>PECAM1</i>	Forward: TGAGGGTGAAGGTGATAGCC Reverse: GGGTTTGCCCTCTTTTCTC
<i>VEGFA</i>	Forward: ATCTTCAAGCCGTCCTGTGT Reverse: AAATGCTTTCTCCGCTCTGA
<i>IL6</i>	Forward: ACTCACCTCTTCAGAACGAA Reverse: GAAGCATCCATCTTTTTCAG
TNF α	Forward: CTTCTGGCTCAAAAAGAGAA Reverse: GTCAGGGATCAAAGCTGTAG
IL18	Forward: AATAAAGATGGCTGCTGAAC Reverse: CCTTGGTCAATGAAGAGAAC
RPL19	Forward: TCACAGCCTGTACCTGAAGG Reverse: TCGTGCTTCCTTGGTCTTAG
GAPDH	Forward: TGAAGGTCGGAGTCAAGGG Reverse: AGAGTTAAAAGCAGCCCTGGTG

Supplementary Table S2: Primers used and their sequences for BV2 (Rodent origin)

Name of the Genes	Sequence (5'-3')
<i>CerS2</i>	Forward: GAACGACTATGGCTGCCTGT Reverse: AGGGGTGTTGCCACATAAAG
<i>CerS4</i>	Forward: TGGTTACCACCCAATGTCAC Reverse: TGGACACTGCTTCATCCTGA
<i>Gcs</i>	Forward: GCCATGCAAACTCTGGTTC Reverse: TGGACACCCCTGAGTTGAAT
<i>Sptlc1</i>	Forward: TTTCCGGTTTAAAAGTGGTG Reverse: CTGATGCTTGGAGGAGGAAG
<i>Sptlc2</i>	Forward: CATTGAGTCCAGAGCCAGAT Reverse: ACACACTGTCCTGGGAGGAA
<i>Sphk1</i>	Forward: GATGCATGAGGTGGTGAATG Reverse: AACAGCAGTGTGCAGTTGAT
<i>Sphk2</i>	Forward: GAAGGCATTGTCACTGTGTC Reverse: GCAGAGAAGAAGCGAGCAGT
<i>Smpd1</i>	Forward: CAGTTCTTTGGCCCACTCA Reverse: TCCGGGGTAGTTTCCATCTA
<i>Smpd2</i>	Forward: ACGTGCTTTACAAGGCAGTC Reverse: AGGACACACAGCAACACCAG
<i>Icam1</i>	Forward: TCAAACGGGAGATGAATGGT Reverse: AGTTTTAGGGCCTCCTCCTG
<i>Pecam1</i>	Forward: ATGACCCAGCAACATTCACA Reverse: TCGACAGGATGGAAATCACA
<i>Vegfa</i>	Forward: ATCTTCAAGCCGTCCTGTGT Reverse: AAATGCTTTCTCCGCTCTGA
<i>IL-6</i>	Forward: TGTGCAATGGCAATTCTGAT Reverse: GGAAGTTGGGGTAGGAAGGA
<i>IL-18</i>	Forward: CAGCCTGTGTTGAGGATATG Reverse: TCACAGCCAGTCCTCTTACT
<i>Tnf-α</i>	Forward: CTCAAACTCGAGTGACAAGC Reverse: GTGGGTGAGGAGCACGTAGT
<i>Rpl19</i>	Forward: TCACAGCCTGTACCTGAAGG Reverse: TCGTGCTTCCTTGGTCTTAG
<i>Gapdh</i>	Forward: CTGAACGGGAAGCTCACTG Reverse: ACCACCCTGTTGCTGTAGC