Supplement #1 for

A simple method for visualization of sphingolipid and glycosphingolipid pathway transcriptomic data to predict metabolomic differences: Application to cancer

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Supplement 1 Table 1 Glycosyltransferase genes

Homo sapiens	Gene Name	Abbreviation	References
Gene			
UGCG	Ceramide glucosyltransferase	GlcCer Synthase, UGCG, GlcT-I, GCS	[1]
UGT8, CGT	GalCer synthase	CGT	[2]
β4GALTI, B4GALT1	β-*N*-acetylglucosaminyl-glycopeptide β-1,4-galactosyltransferase I	β4GalT-I, B4GALT1	[3]
B4GALT2, β4Gal-T2	β-*N*-acetylglucosaminyl-glycopeptide β-1,4-galactosyltransferase II	β4GalT-II, B4GALT2	[4]
B4GALT3	β-*N*-acetylglucosaminyl-glycopeptide β-1,4-galactosyltransferase IIII	β4GalT-III,	[4]
B4GALT4	β-N-acetylglucosaminyl-glycopeptide β-1,4-galactosyltransferase IV	β4GalT-IV	
B4GALT5	β-N-acetylglucosaminyl-glycopeptide β-1,4-galactosyltransferase V	β4GalT-V	[5]
B4GALT6	β-N-acetylglucosaminyl-glycopeptide β-1,4-galactosyltransferase VI	β4GalT-VI	[6]
B3GALT1	β3-galactosyltrasferase I	β3GalT-I	
B3GALT2	β3-galactosyltrasferase II	β3GalT-II	[7]
B3GALT4, GalT4, β3GalT4	β3-galactosyltrasferase IV	β3GalT-IV, GM1/GD1b/GA1 synthase, β3GalT, Gal-T2	[7]
B3GalT5	β3-galactosyltrasferase V	β3GalT-V	[8]
A3GALT2	*N*-acetyllactosaminide 3-α-galactosyltransferase	α3GalT, iGb_3 synthase	[9]
β3GlcNAcT1 (iGnT1)	β3-N-Acetylglucosaminyltrasferase (iGnT)	β3GalNAcT, iGnT	[10]
β3GlcNAcT2			
β3GlcNAcT3			
β3GlcNAcT4			
β3GlcNAcT5			
IGnT	β6-N-Acetylglucosaminyltrasferase (IGnT)	β6GalNAcT, IGnT	
Core2GlcNAcT-I	Core 2 β6-N-Acetylglucosaminyltransferase I	β6GlcNAcT, Core2GlcNAcT-I, GNT	[11]
B4GALNT1	β4-N-Acetylgalactosaminyltrasferase	β4GalNAcT, GM2/GD2 synthase	[12]
	Histoblood group A transferase		[13]
	Histoblood group B transferase		
Forssman synthase	Forssman Glycolipid Synthase	α3GalNAcT	[14, 15]
	para-Forssman Glycolipid Synthase		Y. Yoda et al. / J. Biochem (Tokyo) 88 (1980) 1887-1890
Fut1, Fut2	α1/2-Fucosyltransferase	FUT1, H, HH, HSC	[16]
FUT2		FUT2, SE, Se2, sej	[17]
FUT3, Fuc-TIII	α3/4-Fucosyltransferase	FUT3, Lewis enzyme, LE, Les, CD174, MGC131739	[18]
FUT4, ELFT,	α3-Fucosyltransferase-IV	FUT4, CD15, ELFT,	[19]

FCT3A, FUC-TIV		FCT3A, FUC-TIV	
FUT5, FUC-TV	α3-Fucosyltransferase-V	FUT5, FUC-TV	[20]
FUT6	α3-Fucosyltransferase-VI	FUT6, FT1A, FLJ40754	[21]
FUT7, Fuc-TVII	α3-Fucosyltransferase-VII	FUT7	[22]
FUT9, Fuc-TIX	α3-Fucosyltransferase-IX	FUT9, FUC-TIX	[23, 24]
	Sialyltransferase 3	SAT-III	
SIAT4B	ST3Gal-II	SAT-IV	[25, 26]
SIAT9	ST3Gal-V	GM3 synthase, SAT-I	[27]
ST6GALNAC3	ST6GalNAc-III	STY	[28]
ST6GALNAC5	ST6GalNAc-V	GD1α synthase	[29]
ST6GALNAC6	ST6GalNAc-VI		[30]
ST8SIA1, SIAT8A, GD3 synthase	ST8Sia-I	GD3 synthase, SAT-II	[31]
ST8SIA3	ST8Sia-III	GT3 synthase	[32]
ST8SIA5	ST8Sia-V	SAT-V/SAT-III, GQ1b/GT1a/GD1c synthase	[33]
GlcAT-P, B3GAT1	HNK-1 Glucuronlytransferase		[34]
	HNK-1 Sulfotransferase	HNK-1 SulfoT	[35]
GAL3ST1, CST	βGal 3-Osulfotransferase-1	Gal3ST-1	[36]
A4galt, Gb3 synthase	LacCer 4-α-galactosyltransferase	Gb3/CD77 synthase, α1,4-GalT	[37]
beta3GalNAc-T1	Gb3 3-β-N-acetylgalactosaminyltransferase	Globoside synthase	[38]
SLC33A1, ACATN	O-Acetyltransferase		[39]

Table1: List of modifications in the altered pathway maps for different branches of sphingolipid biosynthesis. The reason and suitable reference is provided for each change.

1. Ichikawa S, Sakiyama H, Suzuki G, Hidari KI, Hirabayashi Y: **Expression cloning of a cDNA for human ceramide glucosyltransferase that catalyzes the first glycosylation step of glycosphingolipid synthesis**. *Proc Natl Acad Sci U S A* 1996, **93**(10):4638-4643.
2. Bosio A, Binczek E, Le Beau MM, Fernald AA, Stoffel W: **The human gene CGT encoding the UDP-galactose ceramide galactosyl transferase (cerebroside synthase): cloning, characterization, and assignment to human chromosome 4, band q26**. *Genomics* 1996, **34**(1):69-75.
3. Lee PL, Kohler JJ, Pfeffer SR: **Association of beta-1,3-N-acetylglucosaminyltransferase 1 and beta-1,4-galactosyltransferase 1, trans-Golgi enzymes involved in coupled poly-N-acetyllactosamine synthesis**. *Glycobiology* 2009, **19**(6):655-664.
4. Almeida R, Amado M, David L, Levery SB, Holmes EH, Merkx G, van Kessel AG, Rygaard E, Hassan H, Bennett E *et al*: **A family of human beta4-galactosyltransferases. Cloning and expression of two novel UDP-galactose:beta-n-acetylglucosamine beta1, 4-galactosyltransferases, beta4Gal-T2 and beta4Gal-T3**. *J Biol Chem* 1997, **272**(51):31979-31991.
5. Kumagai T, Tanaka M, Yokoyama M, Sato T, Shinkai T, Furukawa K: **Early lethality of beta-1,4-galactosyltransferase V-mutant mice by growth retardation**. *Biochem Biophys Res Commun* 2009, **379**(2):456-459.

6. Wandall HH, Pizette S, Pedersen JW, Eichert H, Levery SB, Mandel U, Cohen SM, Clausen H: **Egghead and brainiac are essential for glycosphingolipid biosynthesis in vivo**. *J Biol Chem* 2005, **280**(6):4858-4863.
7. Amado M, Almeida R, Carneiro F, Levery SB, Holmes EH, Nomoto M, Hollingsworth MA, Hassan H, Schwientek T, Nielsen PA *et al*: **A family of human beta3-galactosyltransferases. Characterization of four members of a UDP-galactose:beta-N-acetyl-glucosamine/beta-nacetyl-galactosamine beta-1,3-galactosyltransferase family**. *J Biol Chem* 1998, **273**(21):12770-12778.
8. Isshiki S, Togayachi A, Kudo T, Nishihara S, Watanabe M, Kubota T, Kitajima M, Shiraishi N, Sasaki K, Andoh T *et al*: **Cloning, expression, and characterization of a novel UDP-galactose:beta-N-acetylglucosamine beta1,3-galactosyltransferase (beta3Gal-T5) responsible for synthesis of type 1 chain in colorectal and pancreatic epithelia and tumor cells derived therefrom**. *J Biol Chem* 1999, **274**(18):12499-12507.
9. Keusch JJ, Manzella SM, Nyame KA, Cummings RD, Baenziger JU: **Expression cloning of a new member of the ABO blood group glycosyltransferases, iGb3 synthase, that directs the synthesis of isoglobo-glycosphingolipids**. *J Biol Chem* 2000, **275**(33):25308-25314.
10. Sasaki K, Kurata-Miura K, Ujita M, Angata K, Nakagawa S, Sekine S, Nishi T, Fukuda M: **Expression cloning of cDNA encoding a human beta-1,3-N-acetylglucosaminyltransferase that is essential for poly-N-acetyllactosamine synthesis**. *Proc Natl Acad Sci U S A* 1997, **94**(26):14294-14299.
11. Sekine M, Nara K, Suzuki A: **Tissue-specific regulation of mouse core 2 beta-1,6-N-acetylglucosaminyltransferase**. *J Biol Chem* 1997, **272**(43):27246-27252.
12. Nagata Y, Yamashiro S, Yodoi J, Lloyd KO, Shiku H, Furukawa K: **Expression cloning of beta 1,4 N-acetylgalactosaminyltransferase cDNAs that determine the expression of GM2 and GD2 gangliosides**. *J Biol Chem* 1992, **267**(17):12082-12089.
13. Yamamoto F, Marken J, Tsuji T, White T, Clausen H, Hakomori S: **Cloning and characterization of DNA complementary to human UDP-GalNAc: Fuc alpha 1----2Gal alpha 1----3GalNAc transferase (histo-blood group A transferase) mRNA**. *J Biol Chem* 1990, **265**(2):1146-1151.
14. Kijimoto S, Ishibashi T, Makita A: **Biosynthesis of Forssman hapten from globoside by alpha-N-acetylgalactosaminyltransferase of guinea pig tissues**. *Biochem Biophys Res Commun* 1974, **56**(1):177-184.
15. Yoda Y, Ishibashi T, Makita A: **Isolation, characterization, and biosynthesis of Forssman antigen in human lung and lung carcinoma**. *J Biochem* 1980, **88**(6):1887-1890.
16. Sarnesto A, Kohlin T, Hindsgaul O, Thurin J, Blaszczyk-Thurin M: **Purification of the secretor-type beta-galactoside alpha 1----2-fucosyltransferase from human serum**. *J Biol Chem* 1992, **267**(4):2737-2744.
17. Kelly RJ, Rouquier S, Giorgi D, Lennon GG, Lowe JB: **Sequence and expression of a candidate for the human Secretor blood group alpha(1,2)fucosyltransferase gene (FUT2). Homozygosity for an enzyme-inactivating nonsense mutation commonly correlates with the non-secretor phenotype**. *J Biol Chem* 1995, **270**(9):4640-4649.
18. Kukowska-Latallo JF, Larsen RD, Nair RP, Lowe JB: **A cloned human cDNA determines expression of a mouse stage-specific embryonic antigen and the Lewis blood group alpha(1,3/1,4)fucosyltransferase**. *Genes Dev* 1990, **4**(8):1288-1303.
19. Kumar R, Potvin B, Muller WA, Stanley P: **Cloning of a human alpha(1,3)-fucosyltransferase gene that encodes ELFT but does not confer ELAM-1 recognition on Chinese hamster ovary cell transfectants**. *J Biol Chem* 1991, **266**(32):21777-21783.
20. Weston BW, Nair RP, Larsen RD, Lowe JB: **Isolation of a novel human alpha (1,3)fucosyltransferase gene and molecular comparison to the human Lewis blood group alpha**

(1,3/1,4)fucosyltransferase gene. Syntenic, homologous, nonallelic genes encoding enzymes with distinct acceptor substrate specificities. *J Biol Chem* 1992, **267**(6):4152-4160.

21. Koszdin KL, Bowen BR: **The cloning and expression of a human alpha-1,3 fucosyltransferase capable of forming the E-selectin ligand**. *Biochem Biophys Res Commun* 1992, **187**(1):152-157.
22. Natsuka S, Gersten KM, Zenita K, Kannagi R, Lowe JB: **Molecular cloning of a cDNA encoding a novel human leukocyte alpha-1,3-fucosyltransferase capable of synthesizing the sialyl Lewis x determinant**. *J Biol Chem* 1994, **269**(24):16789-16794.
23. Johnson PH, Yates AD, Watkins WM: **Human salivary fucosyltransferases : evidence for two distinct alpha-3-L-fucosyltransferase activities one or which is associated with the Lewis blood group Le gene**. *Biochem Biophys Res Commun* 1981, **100**(4):1611-1618.
24. Nishihara S, Iwasaki H, Kaneko M, Tawada A, Ito M, Narimatsu H: **Alpha1,3-fucosyltransferase 9 (FUT9; Fuc-TIX) preferentially fucosylates the distal GlcNAc residue of polylactosamine chain while the other four alpha1,3FUT members preferentially fucosylate the inner GlcNAc residue**. *FEBS Lett* 1999, **462**(3):289-294.
25. Rearick JI, Sadler JE, Paulson JC, Hill RL: **Enzymatic characterization of beta D-galactoside alpha2 leads to 3 sialyltransferase from porcine submaxillary gland**. *J Biol Chem* 1979, **254**(11):4444-4451.
26. Sadler JE, Rearick JI, Paulson JC, Hill RL: **Purification to homogeneity of a beta-galactoside alpha2 leads to 3 sialyltransferase and partial purification of an alpha-N-acetylgalactosaminide alpha2 leads to 6 sialyltransferase from porcine submaxillary glands**. *J Biol Chem* 1979, **254**(11):4434-4442.
27. Kono M, Takashima S, Liu H, Inoue M, Kojima N, Lee YC, Hamamoto T, Tsuji S: **Molecular cloning and functional expression of a fifth-type alpha 2,3-sialyltransferase (mST3Gal V: GM3 synthase)**. *Biochem Biophys Res Commun* 1998, **253**(1):170-175.
28. Sjoberg ER, Kitagawa H, Glushka J, van Halbeek H, Paulson JC: **Molecular cloning of a developmentally regulated N-acetylgalactosamine alpha2,6-sialyltransferase specific for sialylated glycoconjugates**. *J Biol Chem* 1996, **271**(13):7450-7459.
29. Okajima T, Fukumoto S, Ito H, Kiso M, Hirabayashi Y, Urano T, Furukawa K: **Molecular cloning of brain-specific GD1alpha synthase (ST6GalNAc V) containing CAG/Glutamine repeats**. *J Biol Chem* 1999, **274**(43):30557-30562.
30. Okajima T, Chen HH, Ito H, Kiso M, Tai T, Furukawa K, Urano T: **Molecular cloning and expression of mouse GD1alpha/GT1aalpha/GQ1balpha synthase (ST6GalNAc VI) gene**. *J Biol Chem* 2000, **275**(10):6717-6723.
31. Eppler CM, Morre DJ, Keenan TW: **Ganglioside biosynthesis in rat liver: characterization of cytidine-5'-monophospho-n-acetylneuraminic acid:hematoside (GM3) sialyltransferase**. *Biochim Biophys Acta* 1980, **619**(2):318-331.
32. Yoshida Y, Kojima N, Kurosawa N, Hamamoto T, Tsuji S: **Molecular cloning of Sia alpha 2,3Gal beta 1,4GlcNAc alpha 2,8-sialyltransferase from mouse brain**. *J Biol Chem* 1995, **270**(24):14628-14633.
33. Kono M, Yoshida Y, Kojima N, Tsuji S: **Molecular cloning and expression of a fifth type of alpha2,8-sialyltransferase (ST8Sia V). Its substrate specificity is similar to that of SAT-V/III, which synthesize GD1c, GT1a, GQ1b and GT3**. *J Biol Chem* 1996, **271**(46):29366-29371.
34. Mitsumoto Y, Oka S, Sakuma H, Inazawa J, Kawasaki T: **Cloning and chromosomal mapping of human glucuronyltransferase involved in biosynthesis of the HNK-1 carbohydrate epitope**. *Genomics* 2000, **65**(2):166-173.
35. Chou DK, Tobet SA, Jungalwala FB: **Restoration of synthesis of sulfoglucuronylglycolipids in cerebellar granule neurons promotes dedifferentiation and neurite outgrowth**. *J Biol Chem* 1998, **273**(14):8508-8515.

36. Ogawa D, Shikata K, Honke K, Sato S, Matsuda M, Nagase R, Tone A, Okada S, Usui H, Wada J *et al*: **Cerebroside sulfotransferase deficiency ameliorates L-selectin-dependent monocyte infiltration in the kidney after ureteral obstruction**. *J Biol Chem* 2004, **279**(3):2085-2090.
37. Kojima Y, Fukumoto S, Furukawa K, Okajima T, Wiels J, Yokoyama K, Suzuki Y, Urano T, Ohta M: **Molecular cloning of globotriaosylceramide/CD77 synthase, a glycosyltransferase that initiates the synthesis of globo series glycosphingolipids**. *J Biol Chem* 2000, **275**(20):15152-15156.
38. Okajima T, Nakamura Y, Uchikawa M, Haslam DB, Numata SI, Furukawa K, Urano T: **Expression cloning of human globoside synthase cDNAs. Identification of beta 3Gal-T3 as UDP-N-acetylgalactosamine:globotriaosylceramide beta 1,3-N-acetylgalactosaminyltransferase**. *J Biol Chem* 2000, **275**(51):40498-40503.
39. Kanamori A, Nakayama J, Fukuda MN, Stallcup WB, Sasaki K, Fukuda M, Hirabayashi Y: **Expression cloning and characterization of a cDNA encoding a novel membrane protein required for the formation of O-acetylated ganglioside: a putative acetyl-CoA transporter**. *Proc Natl Acad Sci U S A* 1997, **94**(7):2897-2902.

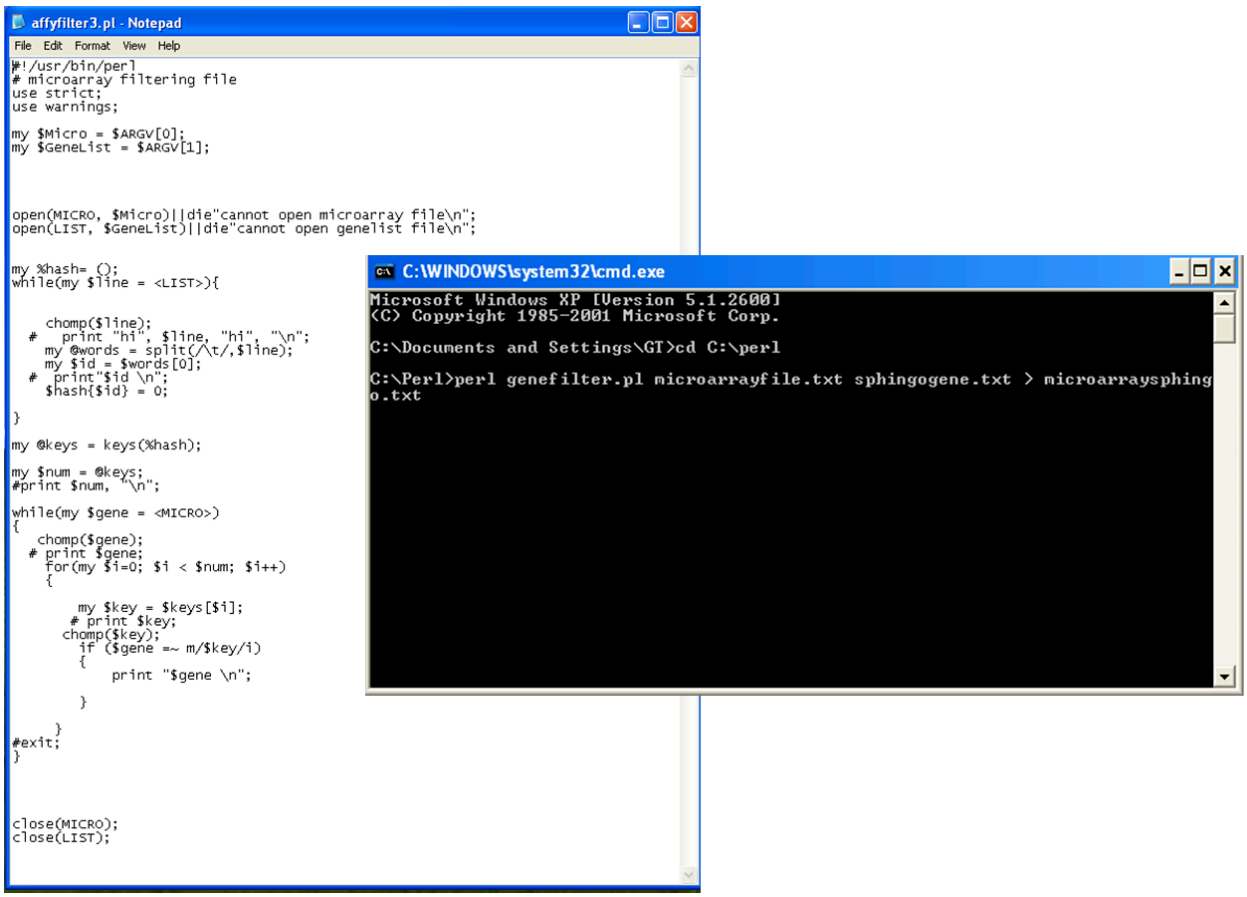
Supplement 1A: Perl program to extract normalized glycosphingolipid gene expression values

```
#!/usr/bin/Perl
# microarray filtering file
use strict;
use warnings;

my $Micro = $ARGV[0];  # Microarray file neme
my $GeneList = $ARGV[1];  # Gene or probelist file


open(MICRO, $Micro)||die"cannot open microarray file\n";
open(LIST, $GeneList)||die"cannot open genelist file\n";


my %hash= ();
while(my $line = <LIST>){


    chomp($line);
    my @words = split(/\t/,$line);
    my $id = $words[0];
    $hash{$id} = 0;

}

my @keys = keys(%hash);

my $num = @keys;

while(my $gene = <MICRO>)
{
   chomp($gene);
    for(my $i=0; $i < $num; $i++)
    {

      my $key = $keys[$i];
      chomp($key);
        if ($gene =~ m/$key/i)
        {
          print "$gene \n";

        }

     }
}

close(MICRO);
close(LIST);
```

Instructions for using Perl program for filtering sphingolipid genes: on windows PC install the Activeperl package from www.activestate.com (Perl is already installed on Mac OS in the X-terminal). Copy and paste the above script in text editor (notepad) and save it as 'genefilter.pl' in a separate folder. Next copy and paste the list of gene IDs and probe IDs into separate text file and save them as 'shingogene.txt' or 'sphingoprobe.txt' in the same folder as the Perl script. Save the normalized microarray dataset with gene expression values and gene ID or affymetrix probe IDs in a tab delineated text file in the folder along with the gene/probe list and the Perl script. To filter the sphingolipid related gene expression values from all the microarray gene probes run the Perl script on the command line (as shown in the figure below for windows PC).

'c:\perl> perl genefilter.pl microarrayfile.txt sphingogene.txt > microarraysphingo.txt'

The script generates an output text file 'microarraysphingo.txt' with the selected sphingolipid gene expression values, which is used to prepare pathvisio input dataset file.



Supplement 1B: Gene list and preselected probe IDs for Affymetrix HG-U133 plus2

A user may use either gene list or preselected probe ids to extract gene expression information

Gene Symbol	Probe Set ID
A4GALT	219488_at
ABO	214504_at
AGA	204332_s_at
AOAH	205639_at
ASAH1	1555419_a_at
B3GALNT2	1562391_at
B3GALT1	222969_at
B3GALT2	210121_at
B3GALT4	210205_at
B3GALT5	206947_at
B3GALT6	1553959_a_at
B3GNT1	203188_at
B3GNT2	219326_s_at
B3GNT3	204856_at
B3GNT4	221240_s_at
B3GNT5	1554835_a_at
B3GNT6	1552833_at
B3GNT7	1552965_a_at
B3GNT8	237338_at
B4GALNT1	206435_at
B4GALNT2	1552903_at
B4GALNT3	1553727_at
B4GALNT4	238080_at
B4GALT1	216627_s_at
B4GALT2	209413_at
B4GALT3	210243_s_at
B4GALT4	210540_s_at
B4GALT6	206232_s_at
BGLAP	206956_at
CERK	218421_at

CERKL	243366_s_at
ELOVL1	218028_at
ELOVL2	220029_at
ELOVL3	234513_at
ELOVL4	219532_at
ELOVL5	208788_at
ELOVL6	210868_s_at
FUT1	206109_at
FUT10	235472_at
FUT11	238551_at
FUT2	208505_s_at
FUT9	207696_at
FVT1	202419_at
GAL3ST1	205670_at
GAL3ST2	1553046_s_at
GAL3ST3	1553257_at
GAL3ST4	219815_at
GALNT2	217787_s_at
GBGT1	231780_at
GCNT1	205505_at
GLA	214430_at
GM2A	209727_at
GM2A_1	215890_at
HEXA	1559932_at
HEXB	201944_at
LASS1	229448_at
LASS2	222212_s_at
LASS3	1554253_a_at
LASS4	218922_s_at
LASS5	224951_at
LASS6	242019_at
NAGA	202943_s_at
PHCA	222688_at

PPAP2A	209147_s_at
PPAP2B	232324_x_at
PPAP2C	209529_at
SGPL1	212322_at
SGPP2	244780_at
SMPD1	209420_s_at
SMPD1_2	217171_at
SMPD3	231732_at
SMS	202043_s_at
SPHK1	219257_s_at
SPHK2	209857_s_at
SPTLC1_2	1554053_at
SPTLC1	202278_s_at
SPTLC2	203127_s_at
SPTLC2_1	203128_at
SPTLC2_2	216203_at
SPTLC3_2	220456_at
SPTLC3	227752_at
ST3GAL1	208322_s_at
ST3GAL2	205346_at
ST3GAL3	1555171_at
ST3GAL4	203759_at
ST3GAL5	203217_s_at
ST3GAL6	213355_at
ST6GAL1	214971_s_at
ST6GAL2	1555123_at
ST6GALNAC1	227725_at
ST6GALNAC2	204542_at
ST6GALNAC3	235334_at
ST6GALNAC4	228163_at
ST6GALNAC5	220979_s_at
ST6GALNAC6	222571_at
ST8SIA1	210073_at

ST8SIA5 206258_at

TRAM2 1554383_a_at

UGCG 204881_s_at

UGT8 208358_s_at

COL4A3BP 223466_x_at

ASAH3 1553929_at

FUT3 216010_x_at

SLC33A1 203164_at

B3GALNT1 223374_s_at

A3GALT2

ASAH2 231791_at

DEGS1 209250_at

DEGS2 236496_at

SGMS1 212989_at

SGMS2 242963_at

Supplement 1C

Application of data to Pathvisio:

Formatting the input file

Gene expression data for sphingolipid specific genes extracted with the perl Script (Supplement 1A) should be formatted into a pathvisio input file with three essential columns and saved as a comma separated file (.csv). An example is given below

GeneID	SystemCode	Fold
1552833_at	X	-1.143967291
1552903_at	X	1.010328449
1552965_a_at	X	1.422851697
1553046_s_at	X	1.220136212
1553257_at	X	1.113669687
1553727_at	X	4.407868628
1553959_a_at	X	1.83540546
1554053_at	X	-2.338778491
1554253_a_at	X	1.068452133
1554383_a_at	X	1.139011527
1554835_a_at	X	2.045004684
1555123_at	X	2.645276048
1555171_at	X	-1.123596112
1555419_a_at	X	-2.284137975

GeneID is the gene identifier or probe ID

SystemCode -- type of gene identifier

Fold – the calculated fold change for the specific gene probe.

Further information about the tile format can be obtained from www.pathvisio.org

Instruction of download of maps from wikipathways

Visit www.wikipathways.org

Browse for pathway and click for Sphingolipid metabolism

In the download link select PathVisio (.gmpl)

Save the pathway maps in a folder in the pathvisio directory

Creating Pathway diagrams with expression data in Pathvisio.

Open the pathway map in Pathvisio browser

Create a expression data using formatted expression file

In the menu select Data → Import expression data

Select the 'input file' (previously prepared .csv file) and 'output file'

Follow the steps by clicking next

Choose data delimiter → 'comma' , Next

Select 'systemcode' then Next

Visualization of expression values

Select the dataset Data → 'select expression data'

Select Data → 'visualization options'

Define the color criteria

After the completion the map should be colored according to the selected criteria.

For further details about the steps one can refer the Pathvisio user tutorial at www.pathvisio.org

Supplement #2

for

A simple method for visualization of "omic" datasets for sphingolipid metabolism to predict potentially interesting differences

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Screen capture figures for online tutorial

This tutorial is available on the web site:

http://sphingolab.biology.gatech.edu/sphingoPathvisioTutorial1.html

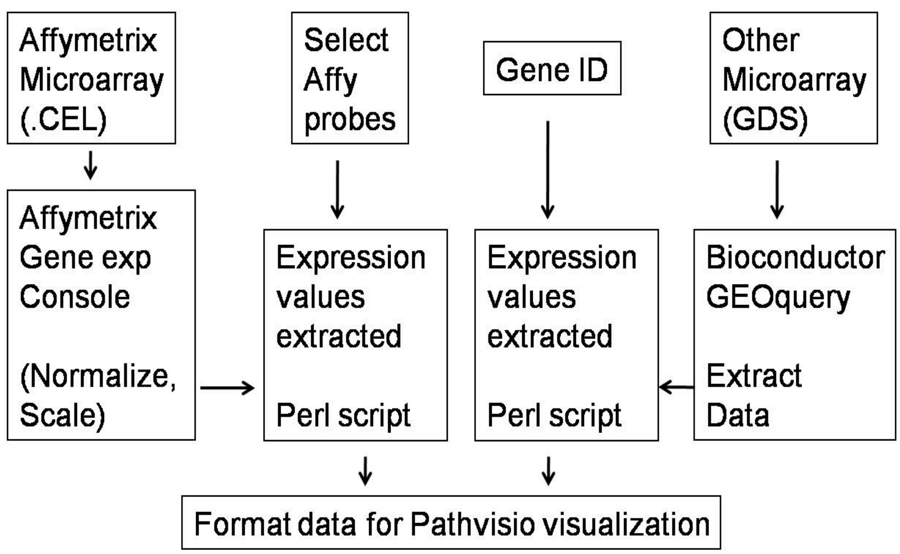
SphinGOMAP
Lycomics Ontologies Metabolomics

Prepared by Amin Momin, Hyejung Park, Brent Portz, Alfred H. Merrill, Jr.

Sphingolipid and glycosphingolipid biosynthesis pathway maps

Updated pathway maps were prepared to visualize differences in gene expression and metabolites
of the sphingolipid biosynthesis pathway using an open acess pathway browser, Pathvisio v1.1.
The modifications in the maps include newly discovered gene isoforms and metabolite brances as
reported in the current literature and summarized in BMC systems biology.

The procedure involved in the selection and extraction of gene expression datasets for the
preparation of the pathway maps are displayed in the flow diagram (Fig 1).The individual
step involved in the preparation of the pathway diagrams are elaborated in the tutorial.



The tutorial below describes the steps required to visulaize gene expression differences
using a sample microarray dataset. Details for preparation of pathway maps from raw affymetrix
datasets (.CEL files) are discussed in the later section

Begin at *Pathvisio Tutorial* if you have sphingolipid gene expression values. If not, skip to *Preparation of Expression Datasets from Raw Microarray Data File*.

PathvioTutorial

Download Pathvisio browser, and gene and metabolite databases (latest version):

The Browser Software and the Human Genome and Metabolite *Databases* should be downloaded
from www.Pathvisio.org (van Iersel et.al , BMC Bioinformatics. 2008 Sep 25;9:399).
Separate downloaders are required to be downloaded for the databases prior to thier download.
Java Virtual Machine should be installed *prior* to running Pathvisio browser and downloading
databases.

Importing Gene Expression Values:

1) Pathvisio Permits Data Application and Editing of Pathway Map Files. Such files for the Different Branches of the Sphingolipid Biosynthesis are included:

Human Pathway: click on the links below and saves the files as '.gpml' in pathways folder.

De Novo
Ganglio
Globo

2) A Sample Microarray Dataset is Provided to Prepare Pathway Maps within Pathvisio:

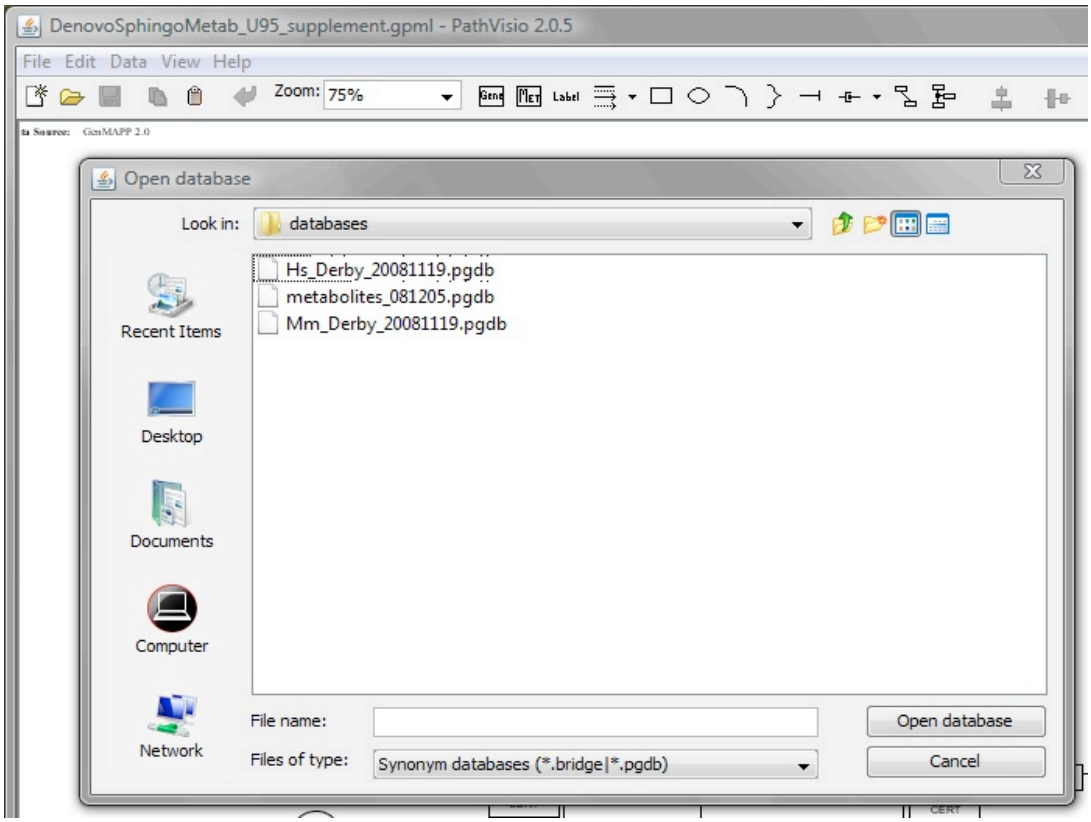
Differential gene expression between basal and luminal breast cancer cell lines (GSE12777: Hoeflich et. al, Clin Cancer Res 2009 Jul 15;15(14):4649-64.)

Download the file and save as .csv format in the pathvisio folder.

3) Pathvisio Requires the Conversion of the Microarray Dataset to a Specific File. To Create the Expression Data File:

Open the downloaded pathway map in Pathvisio browser.
From the File menu -> Open and then choose the downlaoded map (.gpml file).

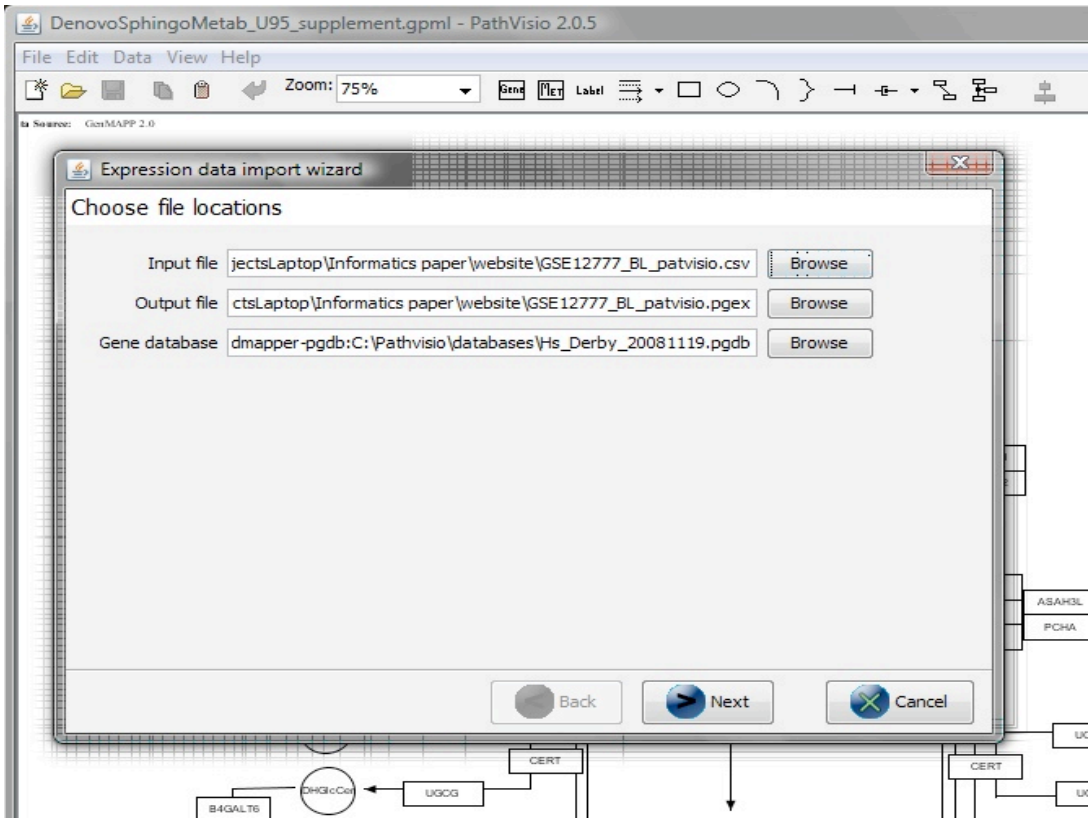
Select human gene database (The latest version)
From the Data menu -> Gene Database -> Databases (choose human Hs_Derby_20xx.pgdb).



Prepare a Pathvisio expression dataset from sample dataset:

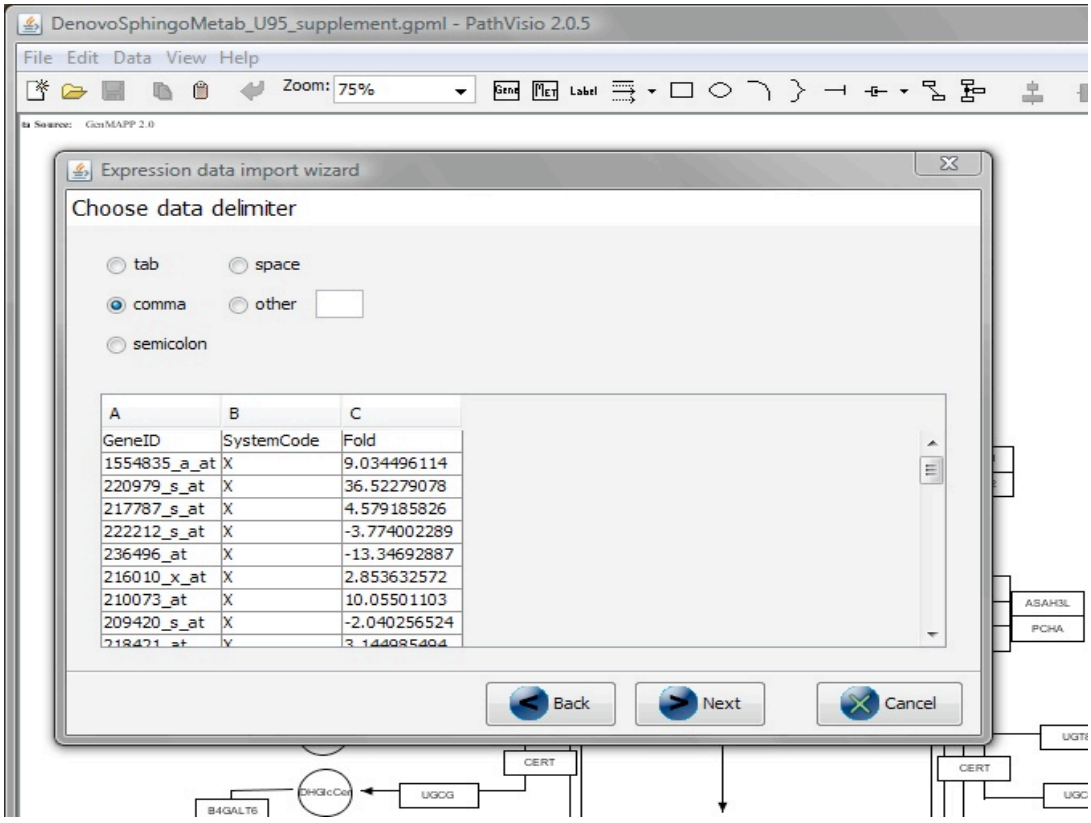
From the Data menu -> Import Expression Data

Within 'Expression data import wizard' Select the ‘Input file’ (previously prepared .csv file)
and a default output file is selected (.pgex).



Follow the steps by clicking next.

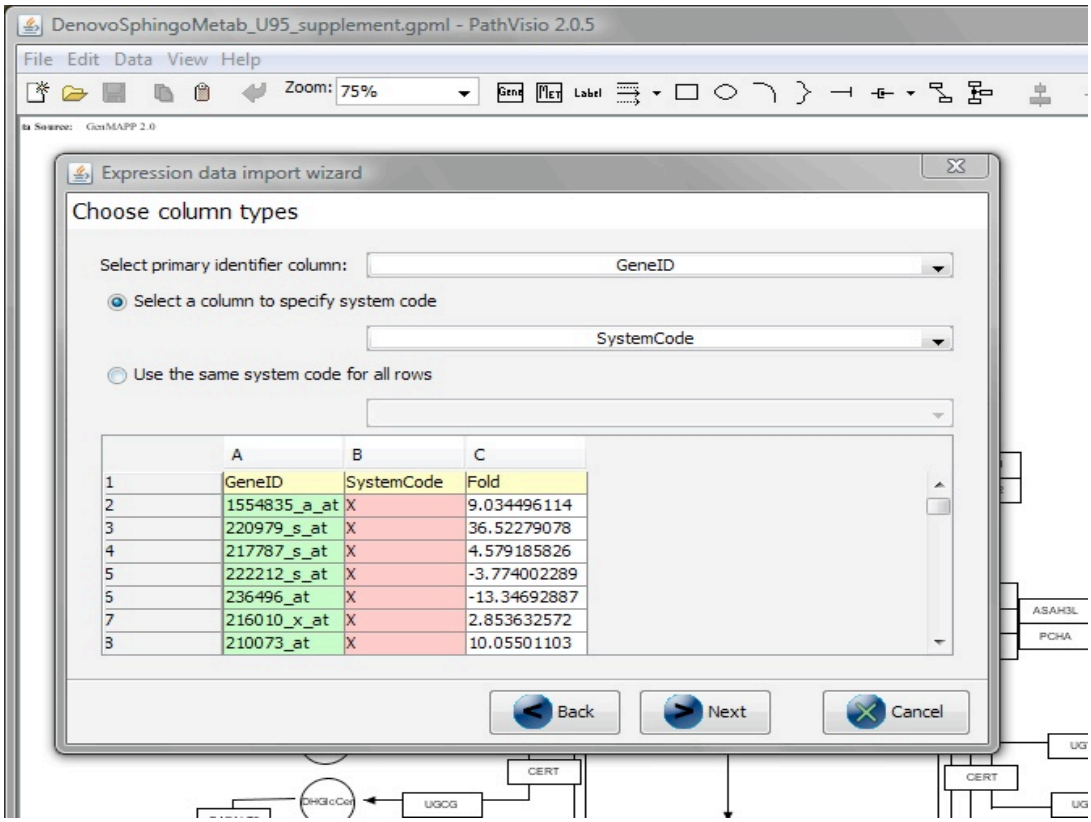
Choose Data Delimiter -> 'Comma' -> Next



Select appropiate column identifiers used:

From 'Select primary identifier column' -> GeneID.

From 'Select a column to specify system code' -> SystemCode.



Click Next

Click Finish.

Pathvisio expression file (.pgex) is created from the excel file (.csv) and is used in the next step.

4) The Created Expression File is used to Visualize Data witin Pathvisio. To Visualize
Expression Values:

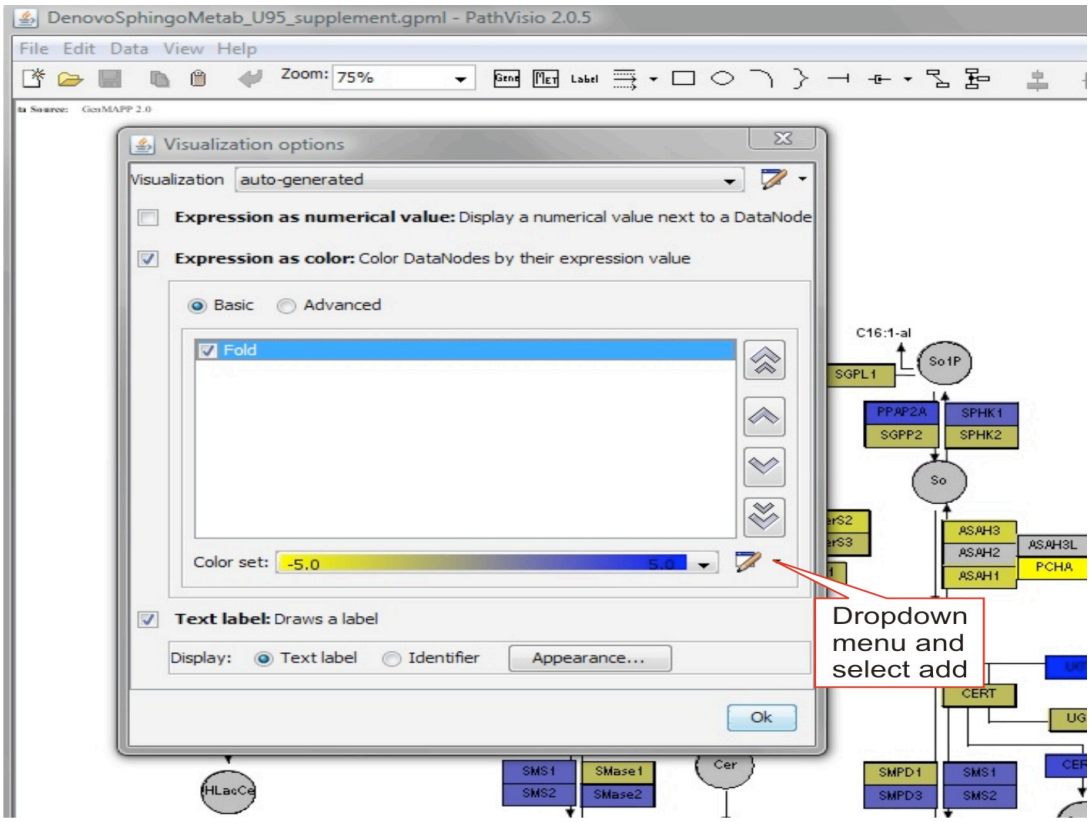
From the menu select Data -> 'Select Expression Dataset' (choose the .pgex file)

Select Data -> 'Visualization Options'

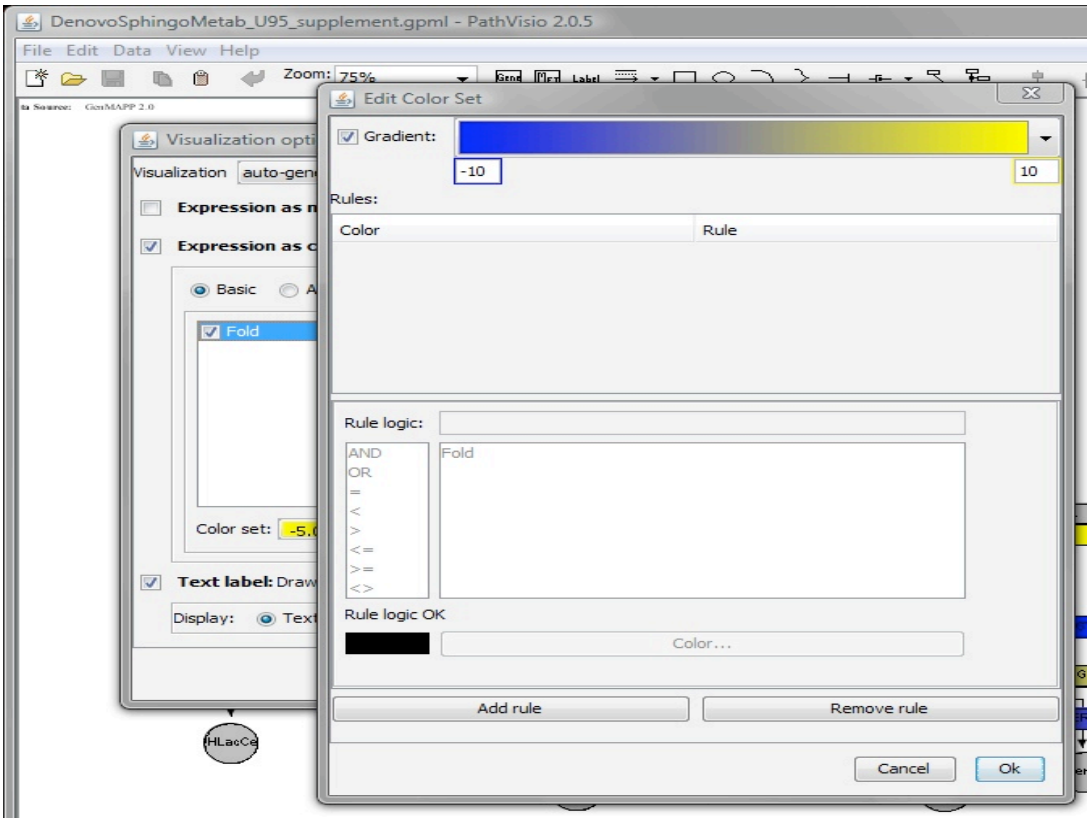
From 'Visualization' -> Auto-generated.

Select 'Expression as Color'.

To modify color gradient:
Select the drop-down menu in ' Color Set' -> 'New'

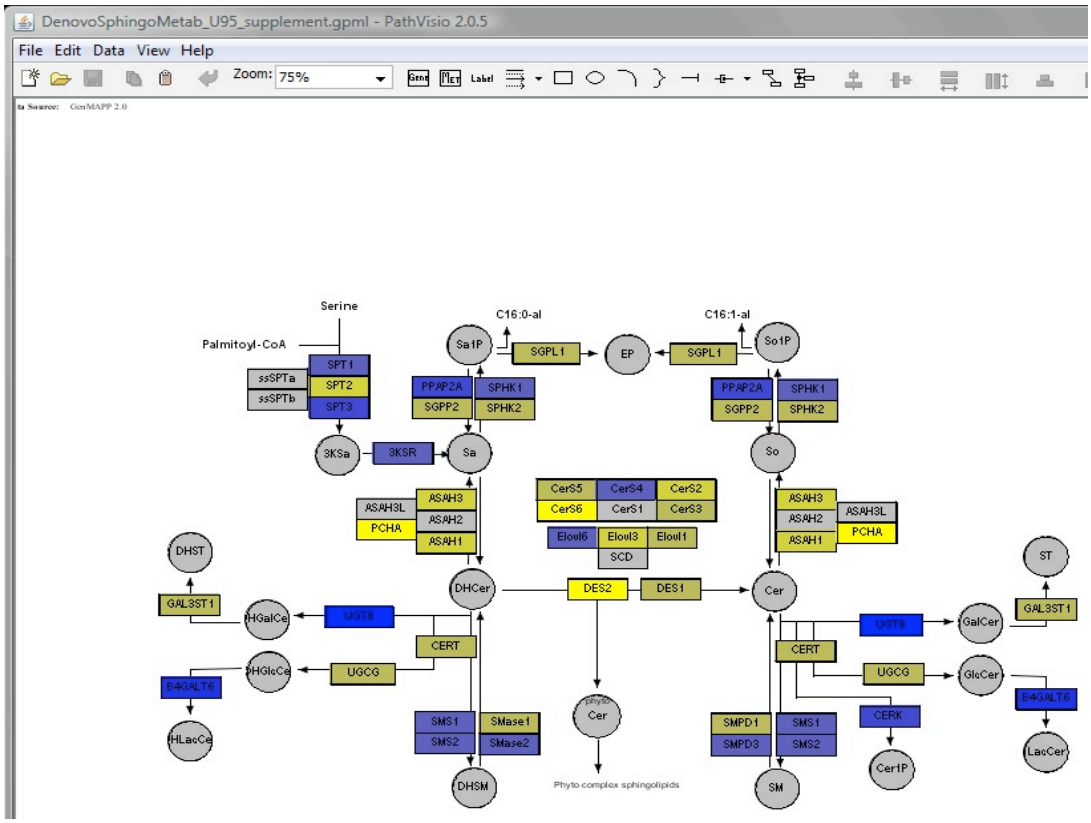


In the Edit color set dialog box, Select 'Gradient' and specify values i.e. -5 (min)
to 5.0 (max) -> Select 'OK'.



To color the map using rule, refer to the Pathvisio user tutorial at www.pathvisio.org.

Upon completion the map should be colored according to the selected criteria.



Importing Metabolite Changes as nodes in pathway maps:

1) Add metabolite differences to template file.

Open the template file using microsoft excel and fill in the available fold change values (the third column).
(Metabolite Template)

Leave the unkown values as '1' and delete the column (last) listing the metabolite annotations.

For new metabolites not included in the template make sure to assign the objects the same ID (name)
as the GeneID.

2) Copy and paste the formatted template into the gene expression file fold change file.

Select the first three columns of the template file and paste them at the bottom of the pathvisio
gene expression file (previously formated .csv file).

Save the pathvisio expression file as a new .csv file and follow the instruction as

previously
described for visulization of gene expression values (Step 4-*Importing Gene Expression Values*).

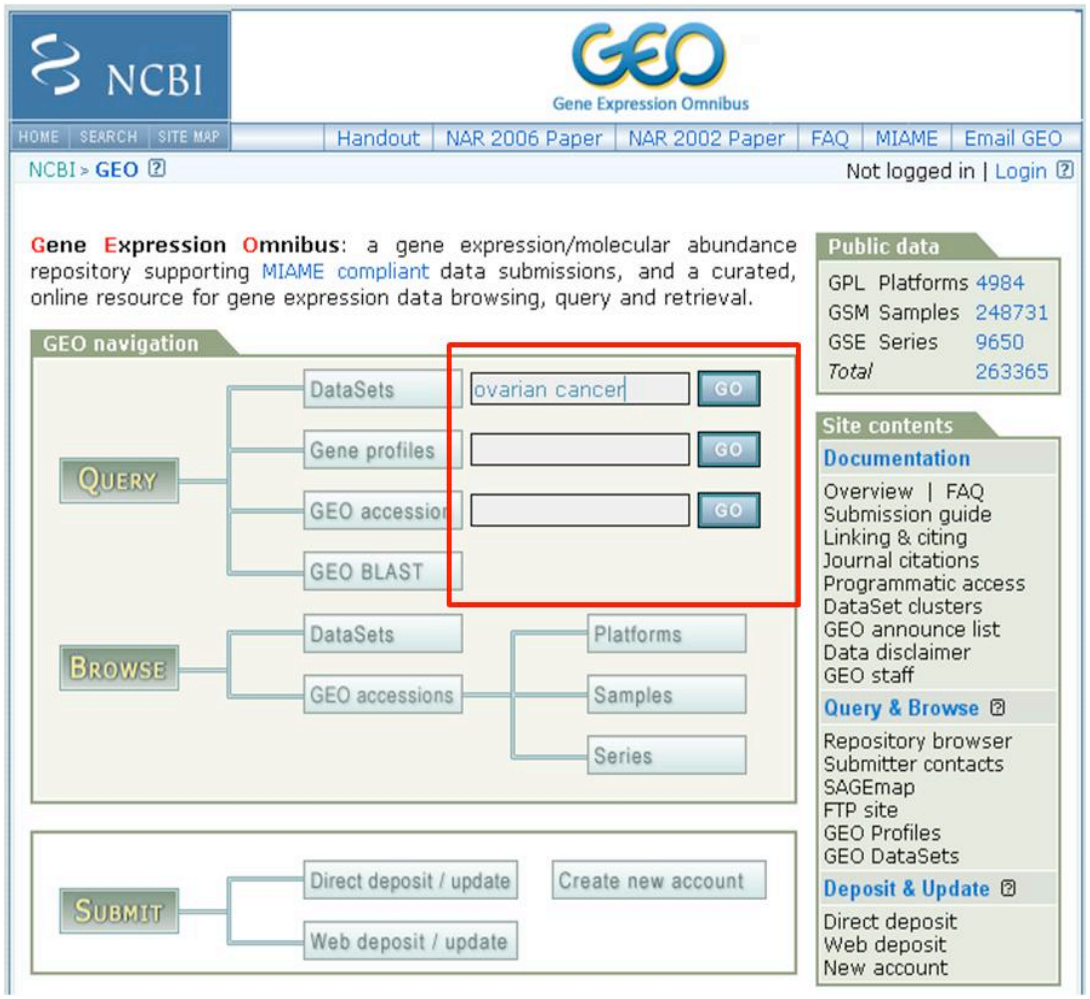
Preparation of Expression Datasets from Raw Microarray Data File

Steps 1-4 describe the process involved in preparation of pathvisio espression file from affymetrix .CEL file obtained from published datasets or in house studies. To prepare
expression files from previously normalized datasets obtained as text or .xls files, skip to Sphingolipid Gene Extraction

1) Expression Datasets from Major Repositories can be used within Pathvisio. To Obtain
Public Gene Expression Datasets, Search:

NCBI GEO (Note: Download http)
ArrayExpress
Oncomine (Note: Free for academic use)

Perform a keyword search for the appropriate gene expression dataset in NCBI GEO
either with disease/cancer type or the experiemnt ID provided in a publication. Download the apprpriate .CEL file (affymetrix experiments) or the GDS file
for other platforms or cDNA array datasets.



2) Data Obtained from Affymatrix Chips Must be Normalized Before Importing to Pathvisio
To Normalize Raw Gene Expression files:

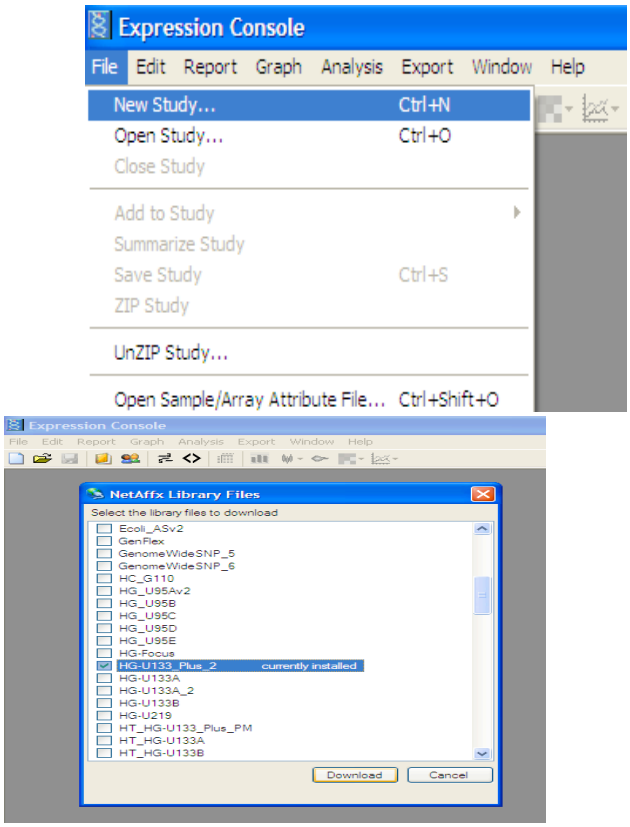
Unzip/Extract and Store Affymatrix .CEL file

Download Affymatrix Gene Expression Console to normalize Affymatrix expression values:

Create free user account (Note: Compatible with PC only)

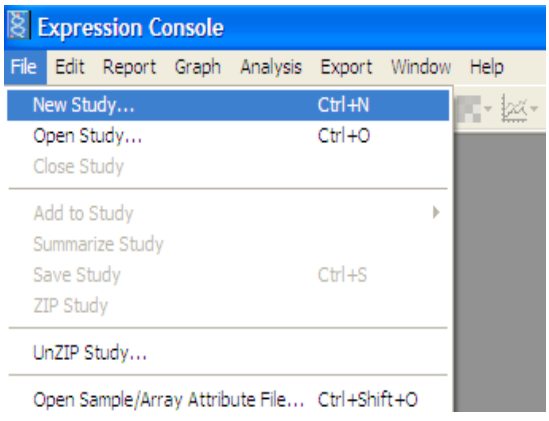
Download Library file for Affymatrix chip:

From the File menu -> "Download library files…"

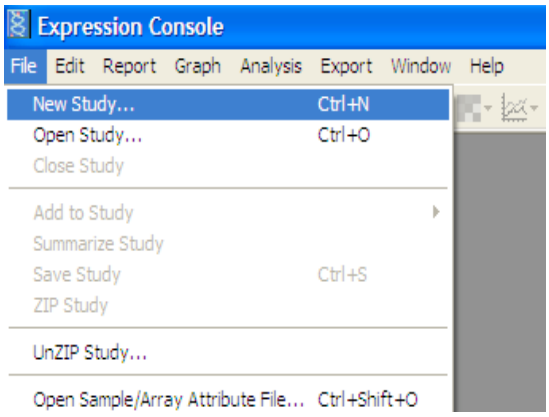


Select appropriate affymatrix chip and Download

Create new study to analyze CEL files:
From File menu -> "New Study"



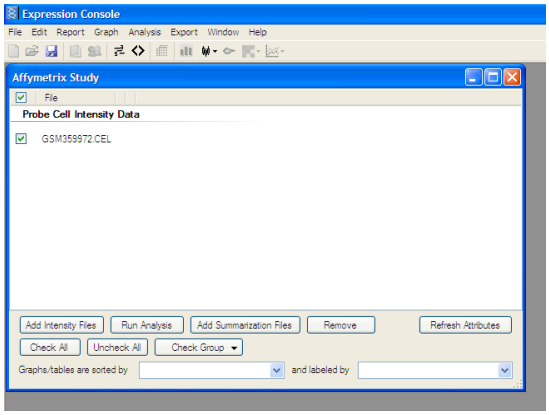
Add extracted .CEL files from data set -> Add Intensity Files button:
Add all files to be analyzed.

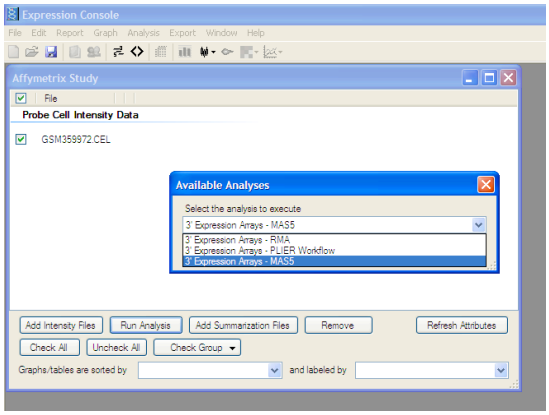


Analyze Selected File:

Click "Run Analysis" button and Select appropriate 3' Expression Array (MAS5, RMA, or PLIER)

as described in study





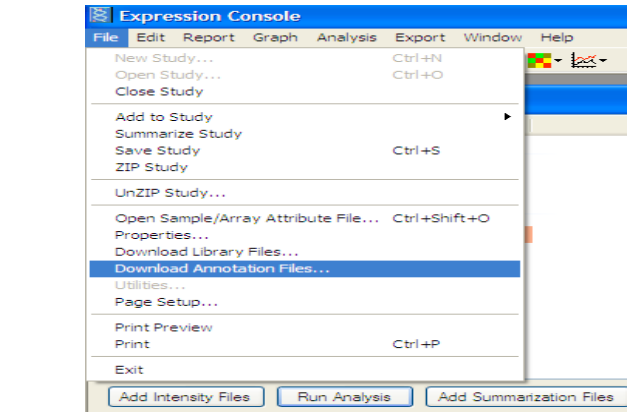
Note: If error message appears, select 'ok'

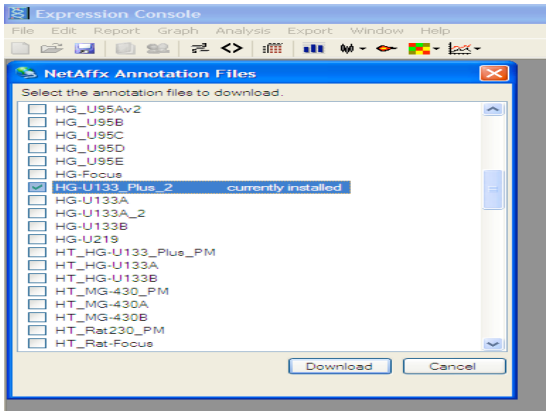
Affymatrix chip has been normalized and dataset is ready to export to external file

3) Results from Affymatrix Data Normalization can be Exported to an External File (.cvs)
used by Pathvisio. In Affymatrix Gene Expression Console:

Download Gene Annotation file corresponding to Affymatrix chip used:

From File Menu -> "Download Annotation Files…"

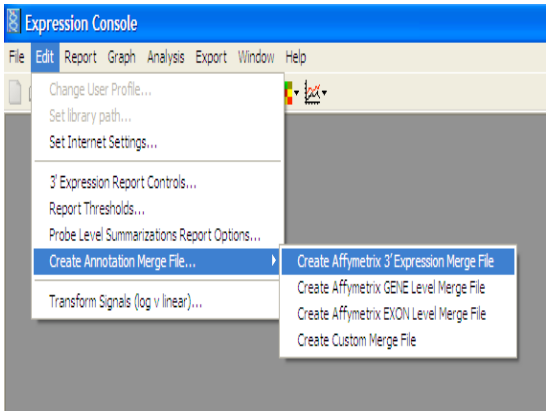




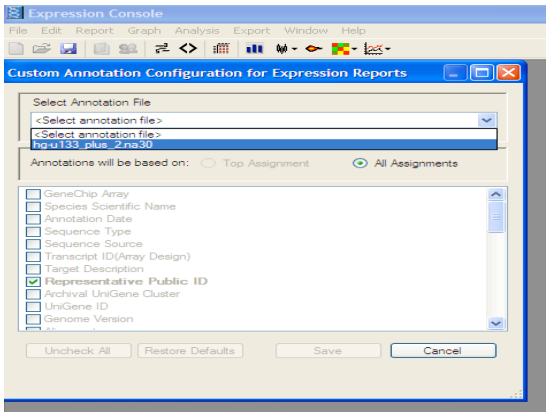
Select appropriate chip and Download

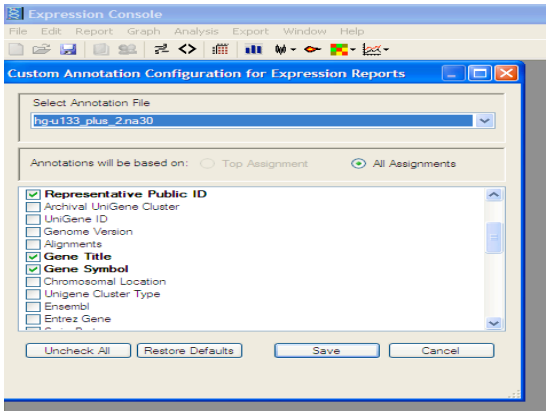
Merge Annotation file with expression results:

From the Edit menu -> "Create Annotation Merge File" -> "Create Affymatrix 3' Expression Merge File"



From drop-list, Select appropriate annotation file

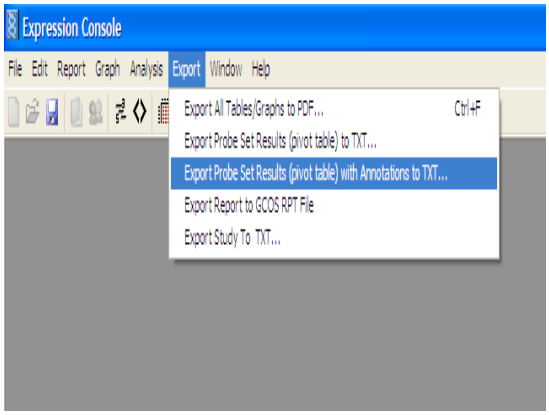




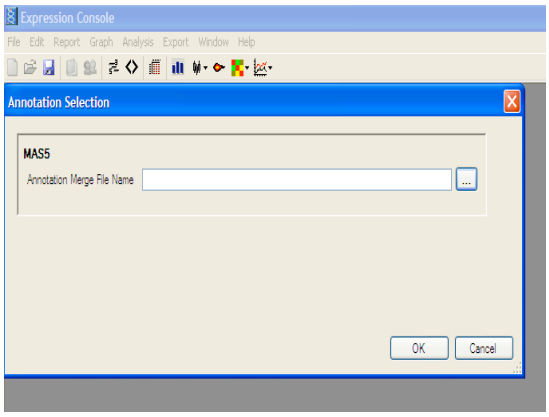
Select annotations to be included in results report and Save

Export Results to Comma Separated Value (.CSV) File:

From the Export Menu-> "Export Probe Set Results (pivot table) with Annotations to TXT"



"Annotation Selection Window" Appears, Browse for created Annotation File



4) .CSV File has been created for Pathvisio

Extraction of gene expression values pertinent to the sphingolipid biosynthesis pathway

Download the script for extraction of expression values corressponding to probes specific to
the sphingolipid biosysnthesis pathway and save it as a perl (.pl) file.

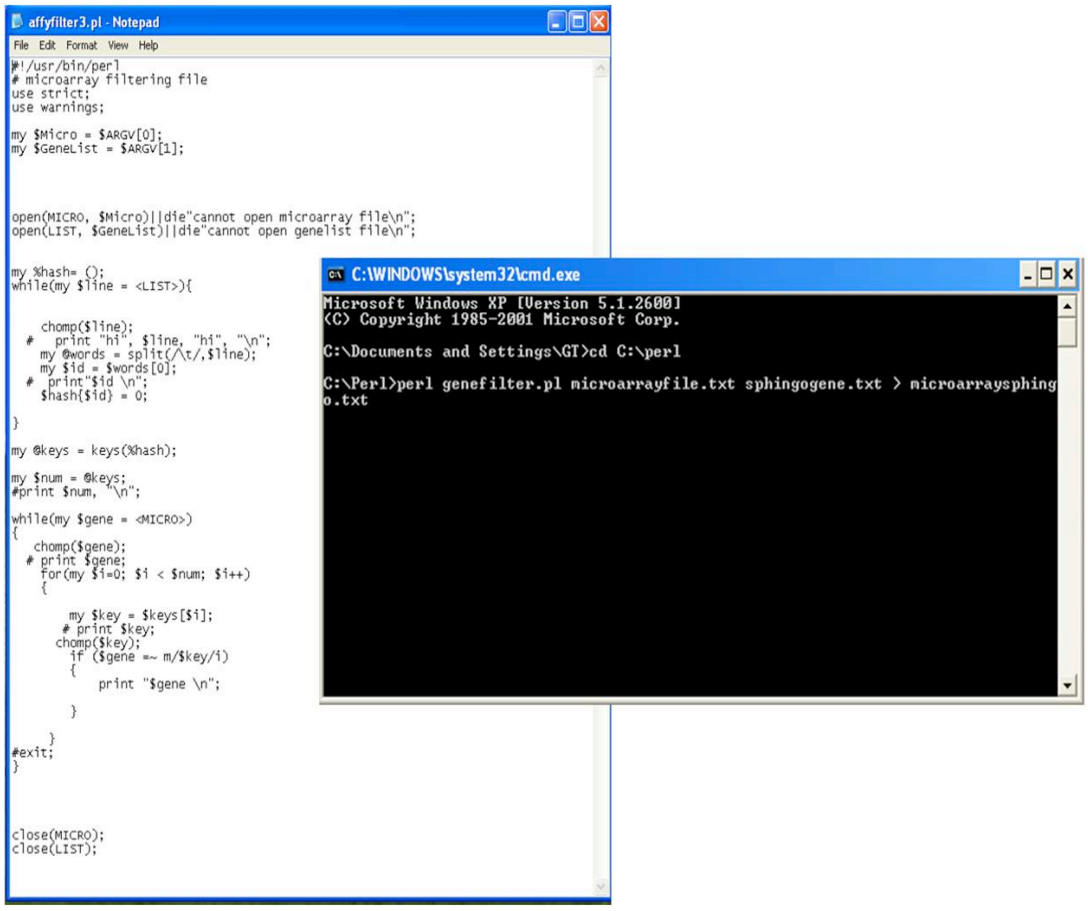
Perl script to extract expression values

The list of gene ID's or their preselected probes for affymetrix HG-U133 Plus2 or HG-U95
chipset can be obtained from the link below.

GENE ID List
Affy HG-U133 Plus2
Affy HG-U95

On windows PC install the Activeperl package from www.Activestate.com. (Perl is already installed on
Mac OS in the X-terminal). Copy and paste the above script into a text editor (notepad) and save
it as 'genefilter.pl' in a separate folder. Next copy and paste the list of gene IDs and probe IDs
in another separate text file and save them as 'shingogene.txt' or 'sphingoprobe.txt' in the same
folder as the Perl script. Save the normalized microarray dataset with gene expression values and
gene ID (or affymetrix probe IDs) in a tab delineated text file in the folder along with the gene/probe
list and the Perl script files. To filter the sphingolipid related gene expression values from all
the microarray gene probes run the Perl script on the command line (as shown in the figure below
for windows PC).

'c:\perl> perl genefilter.pl microarrayfile.txt sphingogene.txt > microarraysphingo.txt'



The script generates an output text file 'microarraysphingo.txt' with the selected sphingolipid
gene expression values, which is used to prepare pathvisio input dataset file.

Formatting the expression values into a expression file

Gene expression data for sphingolipid specific genes extracted with the perl Script should be
formatted into a pathvisio input file with three essential columns and saved as a comma
separated file (.csv).

An example is given below

GeneID SystemCode Fold
1552833_at X -1.143967291
1552903_at X 1.010328449
1552965_a_at X 1.422851697
1553046_s_at X 1.220136212
1553257_at X 1.113669687
1553727_at X 4.407868628
1553959_a_at X 1.83540546
1554053_at X -2.338778491

1554253_a_at X 1.068452133
1554383_a_at X 1.139011527
1554835_a_at X 2.045004684
1555123_at X 2.645276048
1555171_at X -1.123596112
1555419_a_at X -2.284137975

GeneID - is the gene identifier or probe ID
SystemCode - type of gene identifier
Fold – the calculated fold change for the specific gene probe.

Further information about the tile format can be obtained from www.pathvisio.org.

The file can be used to prepare pathvisio expression dataset as described in the previous section.

Last modified on 5/15/08

Supplementary Figure 1 for

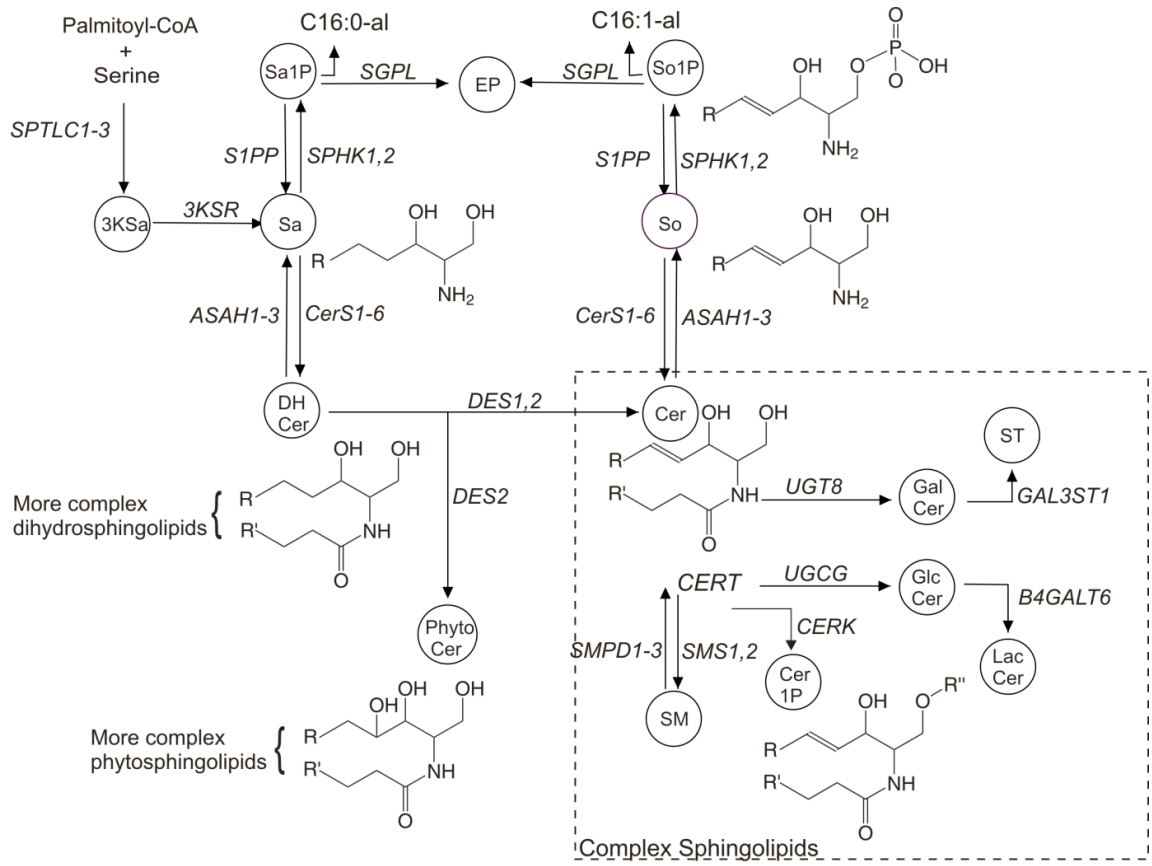
A simple method for visualization of sphingolipid and glycosphingolipid pathway transcriptomic data to predict metabolomic differences: Application to cancer

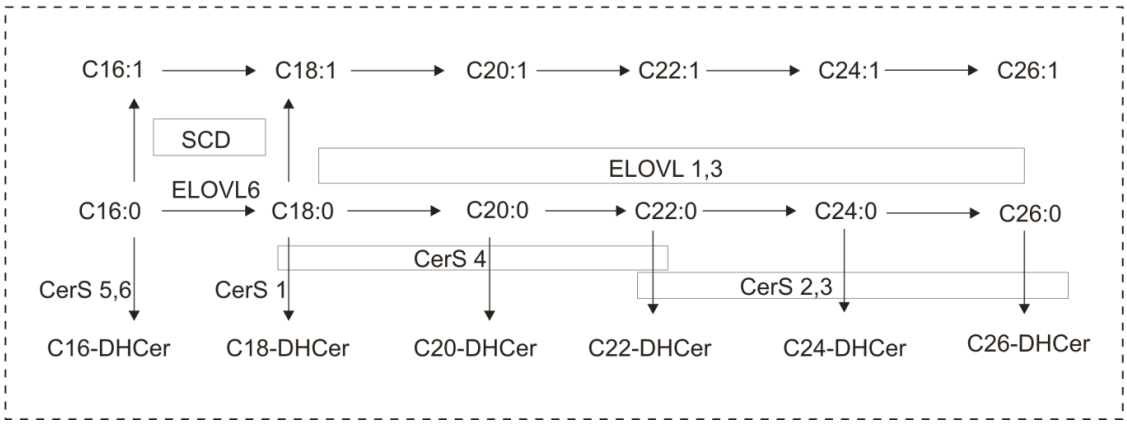
Amin A. Momin[a], Hyejung Park[a], Brent J. Portz[a], Christopher A. Haynes[a],

Rebecca L. Shaner[b], Samuel L. Kelly[a], I. King Jordan[a] and Alfred H. Merrill, Jr.* [a,b]

[a]School of Biology, [b]School of Chemistry and Biochemistry and the Petit Institute for Bioengineering and Bioscience, Georgia Institute of Technology, Atlanta, GA 30332, USA

Legend for supplementary Fig 1. Early steps of sphingolipids biosynthesis The pathway begins with the condensation of serine and palmitoyl-CoA by serine palmitoyltransferase (SPTLC) to produce 3-ketosphinganine (3KSa), which is reduced (by 3KSa reductase, 3KSR) to sphinganine (Sa). Dihydroceramide (DHCer) synthases (CerS 1-6) N-acylate Sa with different fatty acyl-CoA (R') to produce DHCers, which are converted to ceramides (Cer) or Phyto-Cer by DHCer desaturases (DES1,2). Substitution of the 1-OH with different head groups (R") produces Cer 1-phosphate (Cer1P by Cer kinase, CERK), sphingomyelin (SM by SM synthase, SMS1,2), galactosylceramide (GalCer by GalCer synthase, UGT8) and glucosylceramide (GluCer by GluCer synthase, UGCG), which can be further metabolized to sulfatide (ST by ST transferase, GAL3ST1) and lactosylceramide (LacCer by LacCer synthase, B4GALT6), respectively. CERT is a transporter of Cer from the ER to Golgi and is thought to play a role in the synthesis of SM, Cer1P and GluCer. These headgroup modifications are shown for Cer in the area circumscribed by a dashed line (complex sphingolipids) and also pertain to DHCer, Phyto-Cer and other backbones. Also shown is the catabolism of Cer to sphingosine (So) (and analogous DHCer to Sa) by ceramidase (ASAH1-3), phosphorylation by So (Sa) kinases (SPHK1,2) to So 1-phosphate (S1P) (and Sa to Sa1P), and cleavage by S1P lyase (SGPL1) to ethanolamine phosphate (EP) and hexadecanal (C16:0al for Sa1P) and hexadecenal (C16:1al for S1P). The lower panel illustrates the synthesis of the precursor fatty acyl-CoA by a combination of fatty acid elongases (ELOVL 1-7) and stearoyl CoA desaturase (SCD), and their utilization for the N-acylation of sphingoid bases by different CerS isoforms (CerS1-7).





Supplemental Fig. 1

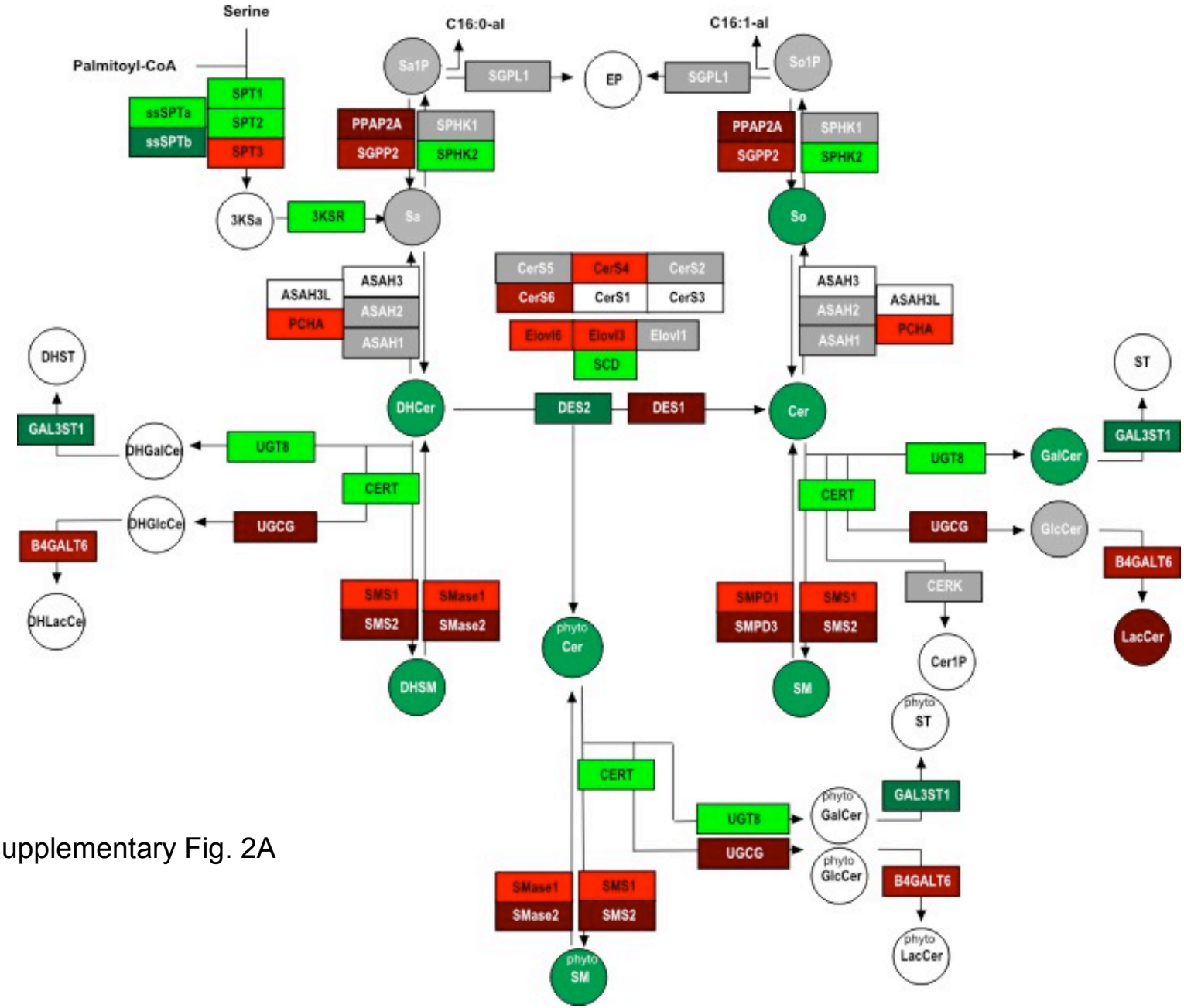
Supplementary Figure 2 for

A method for visualization of "omic" datasets for sphingolipid metabolism to predict potentially interesting differences

Amin A. Momin[a], Hyejung Park[a], Brent J. Portz[a], Christopher A. Haynes[a],
Rebecca L. Shaner[b], Samuel L. Kelly[a], I. King Jordan[a] and Alfred H. Merrill, Jr[* a,b]
[a]School of Biology, [b]School of Chemistry and Biochemistry and the Petit Institute for
Bioengineering and Bioscience, Georgia Institute of Technology, Atlanta, GA 30332, USA

Legend for supplementary Fig 2. Comprehensive illustration of transcription and metabolite changes in backbone sphingolipids biosynthesis between MCF7 and MDA-MB-231 cells using modified KEGG pathway maps using Pathvisio v2. The figure depicts sphingolipid genes (indicated as rectangles) that participate in the biosynthesis of backbone sphingolipids (including dihydro and phyto sphingolipids), along with corresponding metabolites (depicted as circles).

Supplementary Figure 2A. Sphingolipid biosynthesis begins with the condensation of serine and palmitoyl-CoA to produce 3-ketosphinganine (3KSR), sphinganine (Sa), sphinganine 1-phosphate(Sa1P), dihydroceramide (DHCer), phytoceramides (phyto Cer), ceramides (Cer), ceramide 1-phosphate (CerP), sphingomyelin (SM) , galactosylceramide (GalCer), sulfatide (ST), glucosylceramide (GluCer) and lactosylcermide (LacCer). The metabolites with 'DH' and 'phyto' possess a Sa and phyto-Sa base. Also shown is the catabolism of Cer to sphingosine (So), sphingosine 1-phsphate (So1P), ethanolamine phosphate (EP), hexadecanal (C16:0al) and hexadecenal (C16:1al). The layout of the modified pathway maps is based on KEGG (Kyoto Encyclopedia of Genes and Genomes, map00600) pathway diagram (1), using Pathvisio v2 (2) . The shades of the rectangles and circles represents the degree of up and down regulation as indicated by the color scale, were prepared using metabolite data from the current study and mRNA abundance measurement from the NCI60 study (3), and visualized on pathway maps by Pathvisio v2 (2). References given in the legend for Fig. 2B.

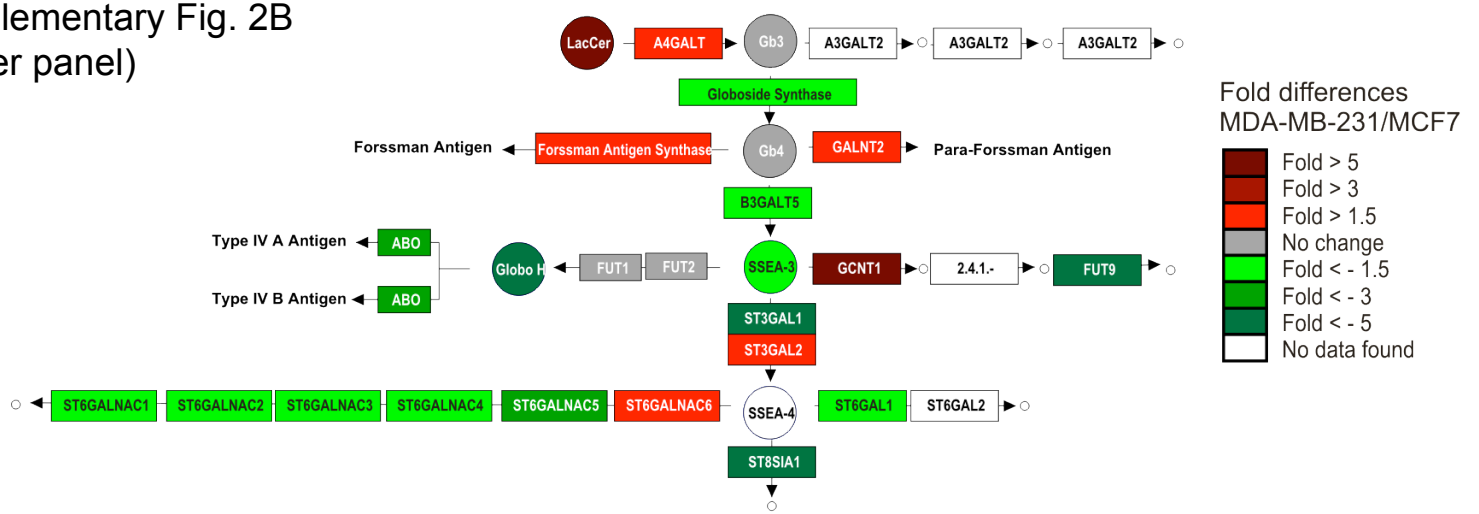


Supplementary Fig. 2A

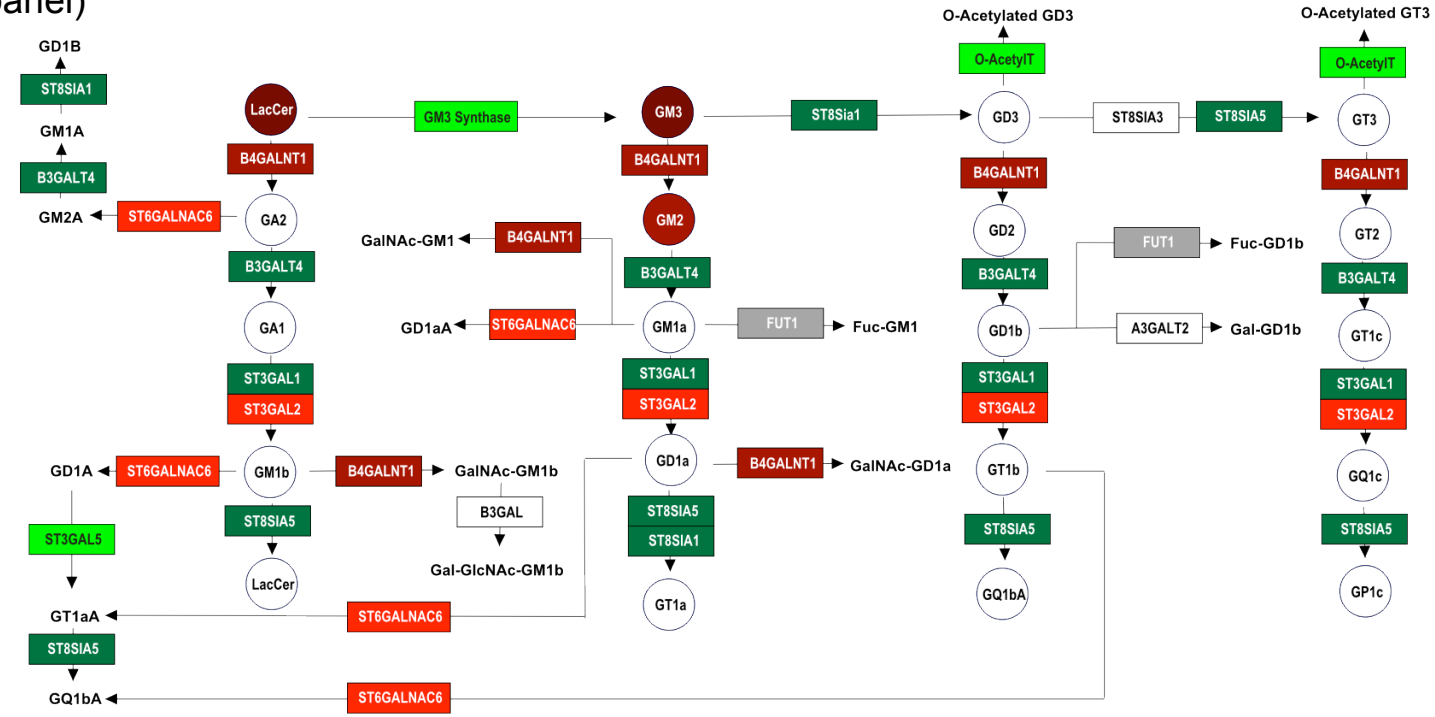
Supplement Fig 2B. Depiction of genes and metabolites differences in complex sphingolipids metabolism between MCF7 and MDA-MB-231 cells using modified KEGG pathway maps using Pathvisio v2.
The upper panel shows the metabolism of LacCer to globoseries glycolipids; ceramidetrihexoside (Gb3), globoside (Gb4), Forssman and para-forssman antigen, stage specific embryonic antigen-3 (SSEA-3), Globo-H antigen, Type IV A and B antigens, stage specific embryonic antigen-4 (SSEA-4) and disialyl-Gb5. The lower panelillustrates ganglioside biosynthesis that results in the formation of asilo-ganglioside (GA2 and GA1), monosialyl-ganglioside(GM3, GM2, GM1), disialyl-ganglioside (GD3, GD2, GD1 and O-acetyl GD3), trisialyl-ganglioside (GT3, GT2, GT1 and O-acetyl GT3) and more complex GQ1 and GP1. Structural isomers belonging to A, B or C series are indicated with an 'a', 'b' and 'c'. The layout of the modified pathway maps is based on KEGG (Kyoto Encyclopedia of Genes and Genomes, map00603 and map00604) pathway diagram (1), which were prepared using Pathvisio v2 (2). For this figure gene expression ratios between MCF7 and MDA-MB-231 cells were compared using data from a previous study (3), and visualized on pathway maps by Pathvisio v2. Metabolic data for GSL was obtained from a previous study (4, 5) .

1. Kanehisa, M., and S. Goto. 2000. KEGG: kyoto encyclopedia of genes and genomes. Nucleic Acids Res 28: 27-30.
2. van Iersel, M. P., T. Kelder, A. R. Pico, K. Hanspers, S. Coort, B. R. Conklin, and C. Evelo. 2008. Presenting and exploring biological pathways with PathVisio. BMC Bioinformatics 9: 399.
3. Shankavaram, U. T., W. C. Reinhold, S. Nishizuka, S. Major, D. Morita, K. K. Chary, M. A. Reimers, U. Scherf, A. Kahn, D. Dolginow, J. Cossman, E. P. Kaldjian, D. A. Scudiero, E. Petricoin, L. Liotta, J. K. Lee, and J. N. Weinstein. 2007. Transcript and protein expression profiles of the NCI-60 cancer cell panel: an integromic microarray study. Mol Cancer Ther 6: 820-832.
4. Schrump, D. S., K. Furukawa, H. Yamaguchi, K. O. Lloyd, and L. J. Old. 1988. Recognition of galactosylgloboside by monoclonal antibodies derived from patients with primary lung cancer. Proc Natl Acad Sci U S A 85: 4441-4445.
5. Nohara, K., F. Wang, and S. Spiegel. 1998. Glycosphingolipid composition of MDA-MB-231 and MCF-7 human breast cancer cell lines. Breast Cancer Res Treat 48: 149-157.

Supplementary Fig. 2B
(upper panel)



(lower panel)



Supplement #3

for

A simple method for visualization of sphingolipid and glycosphingolipid pathway transcriptomic data to predict metabolomic differences: Application to cancer

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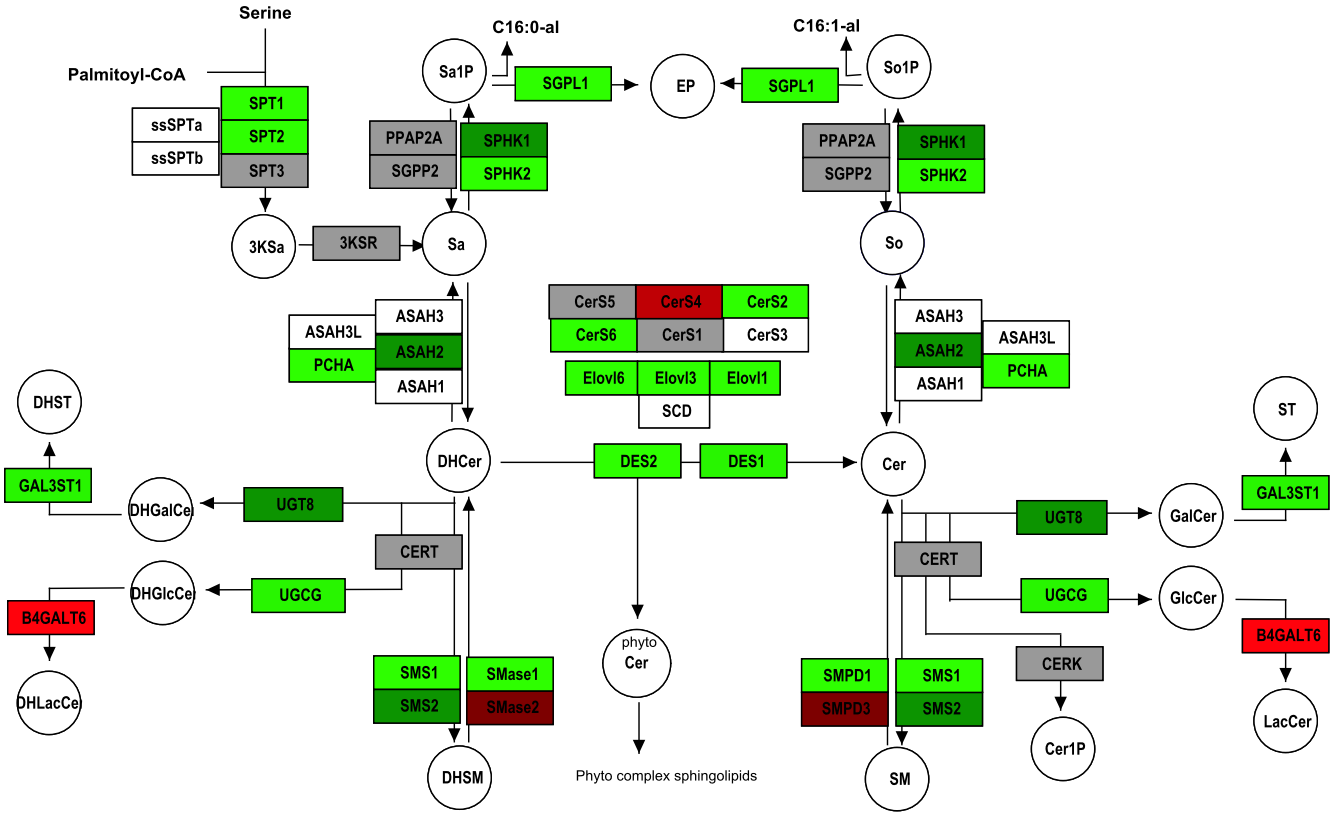
Legend. Comparison of the expression of the sphingolipid pathway genes for specific cell lines in the NCI 60-cell line screen versus the average for all (59) cell lines.

Previously published gene expression data (1) for the 59 cancer cells lines in the NCI 60-cell line screen (NCI-60) was obtained from Cellminer (2). The raw affymetrix genechip data (U133-A and B) was normalized by the MAS5 algorithm using the Affymetrix gene expression console. Shown is the fold difference between a particular cancer cell line versus the average for all 59 cell lines. Expression changes associated with the sphingolipid biosynthetic pathway were visualized using the updated pathway described in the text maps.

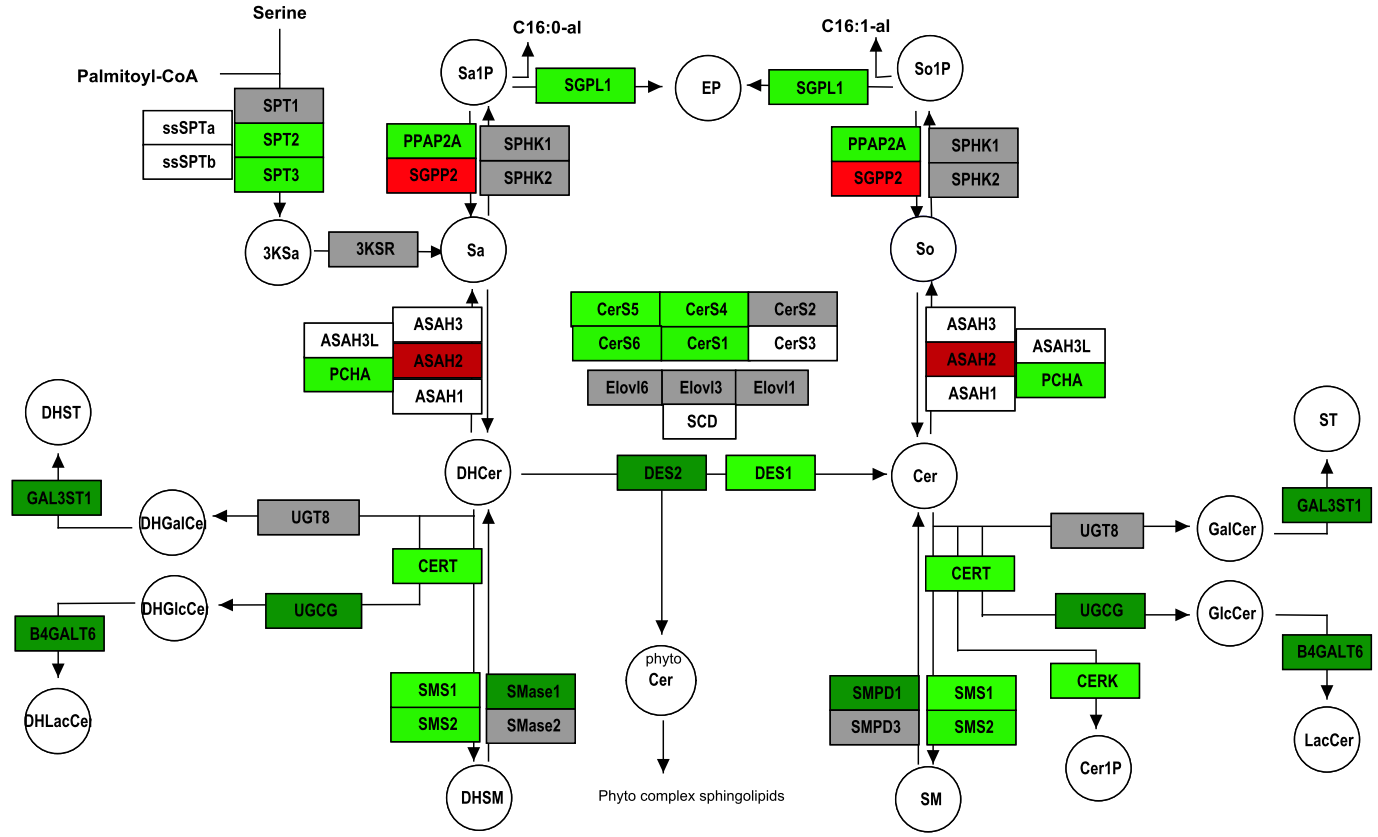
1. Shankavaram UT, Reinhold WC, Nishizuka S, et al. Transcript and protein expression profiles of the NCI-60 cancer cell panel: an integromic microarray study. Mol Cancer Ther 2007; 6: 820-32.

2. Shankavaram UT, Varma S, Kane D, et al. CellMiner: a relational database and query tool for the NCI-60 cancer cell lines. BMC Genomics 2009; 10: 277.

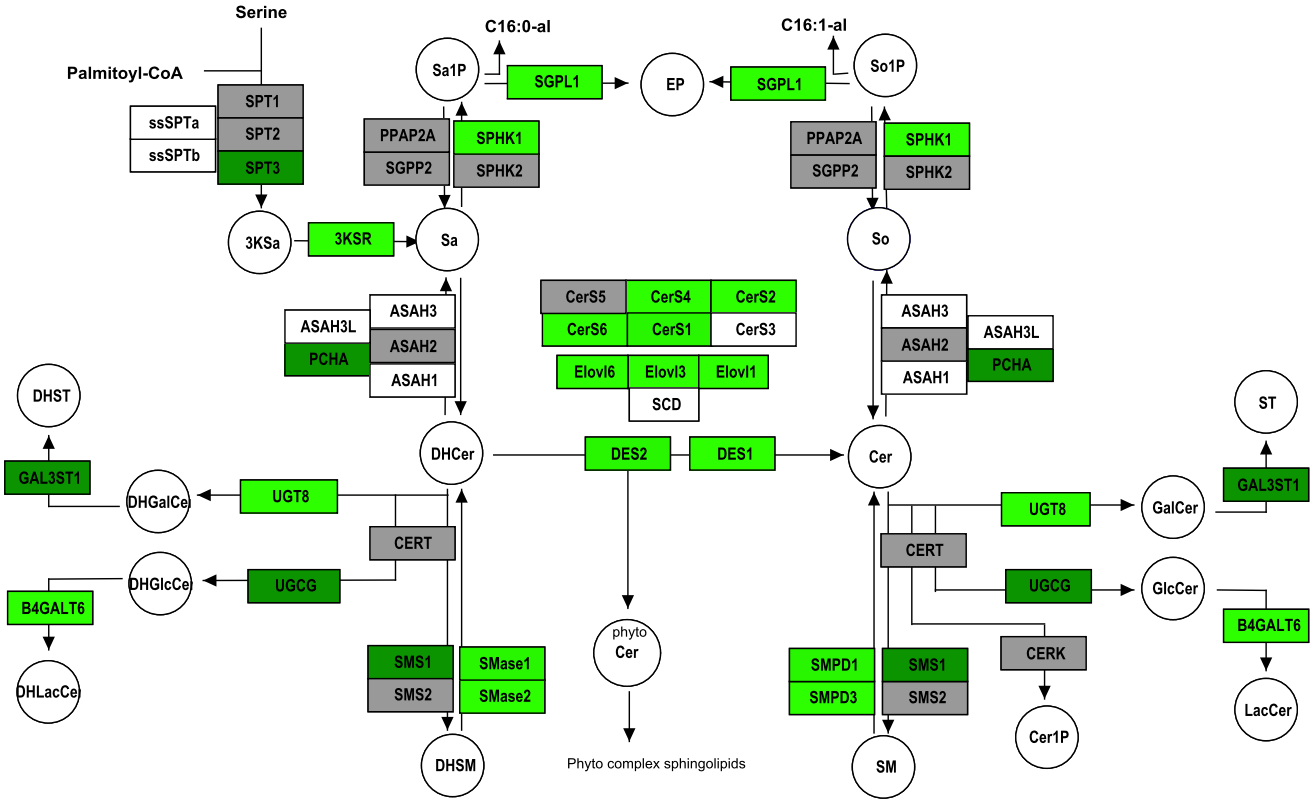
Supplement 3. Sphingolipid backbone biosynthesis pathway maps for NCI60 cell lines. The heat maps were made by dividing the gene expression value for the line of interest by the average for all of the cell lines of the NCI60 cell line screen.



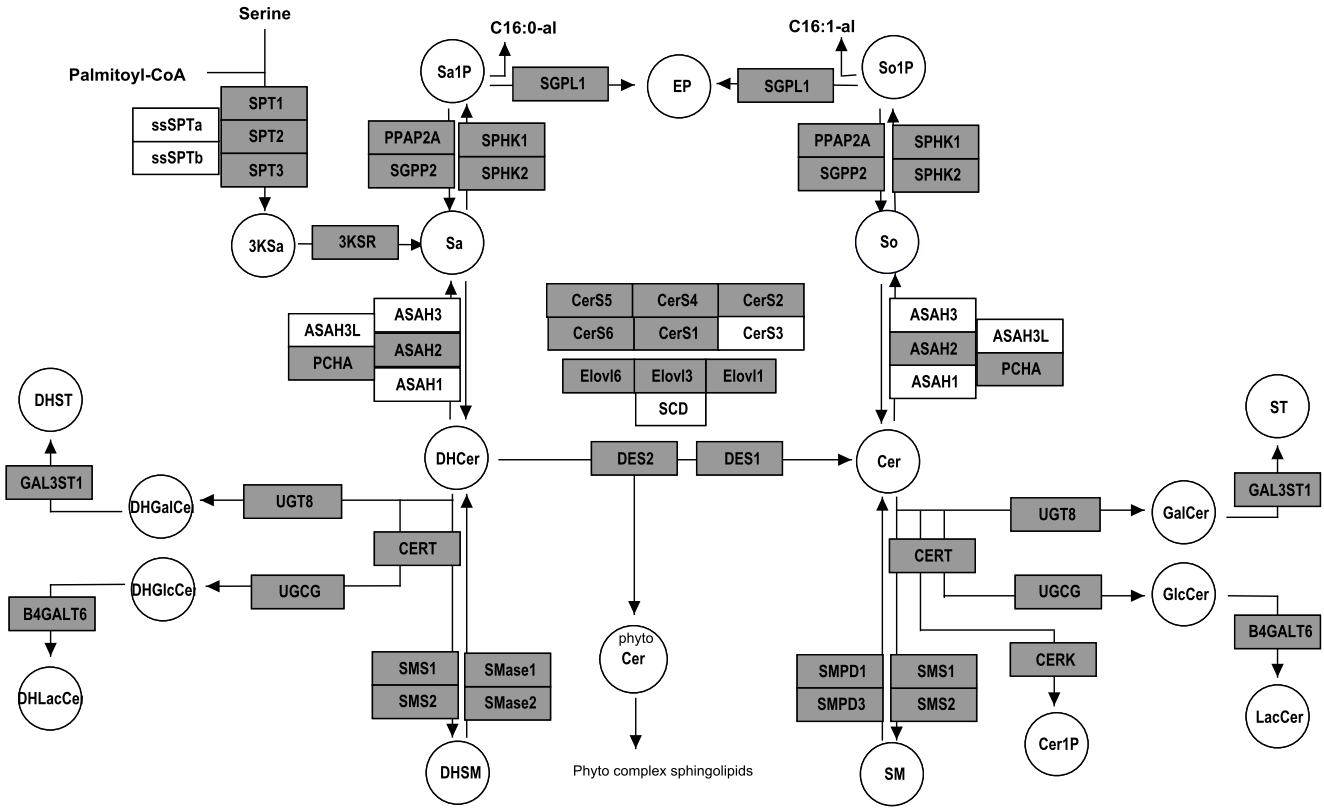
1: CCRF_CEM



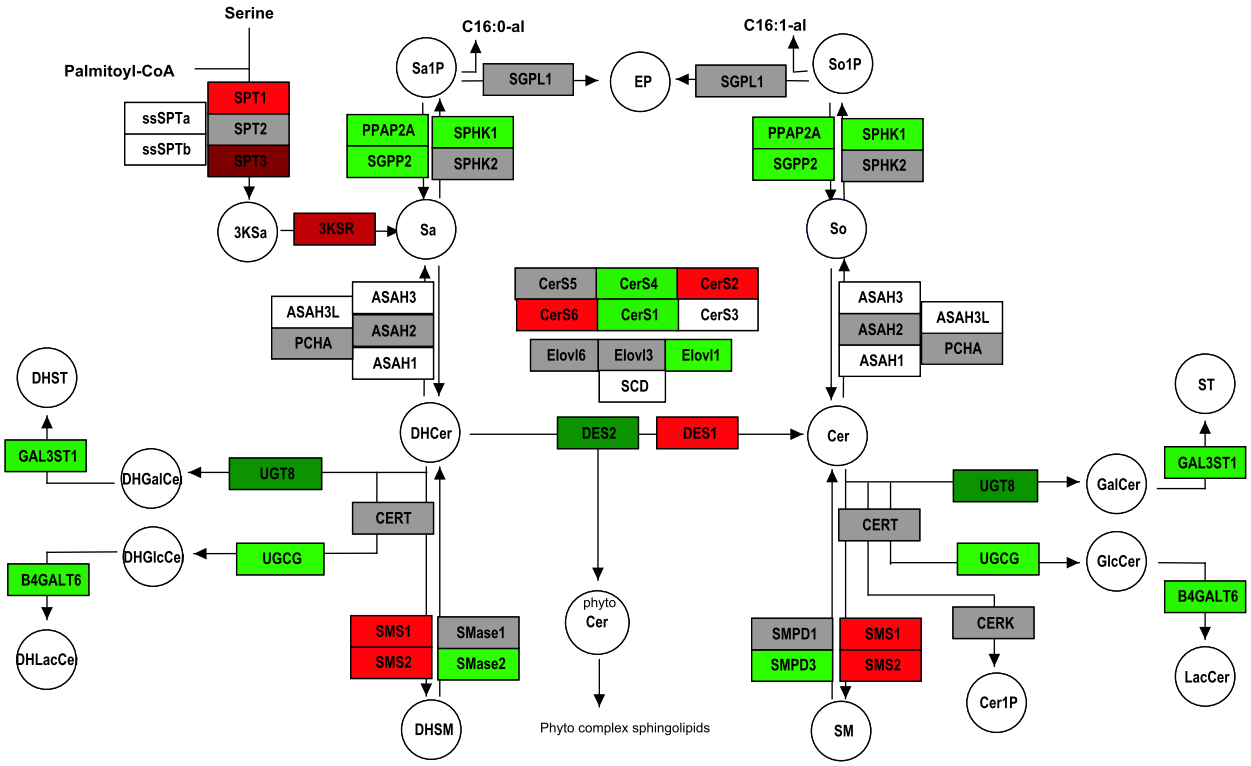
2: HL_60



Sf 3: MOLT_4



Sf 4: RPMI_8226



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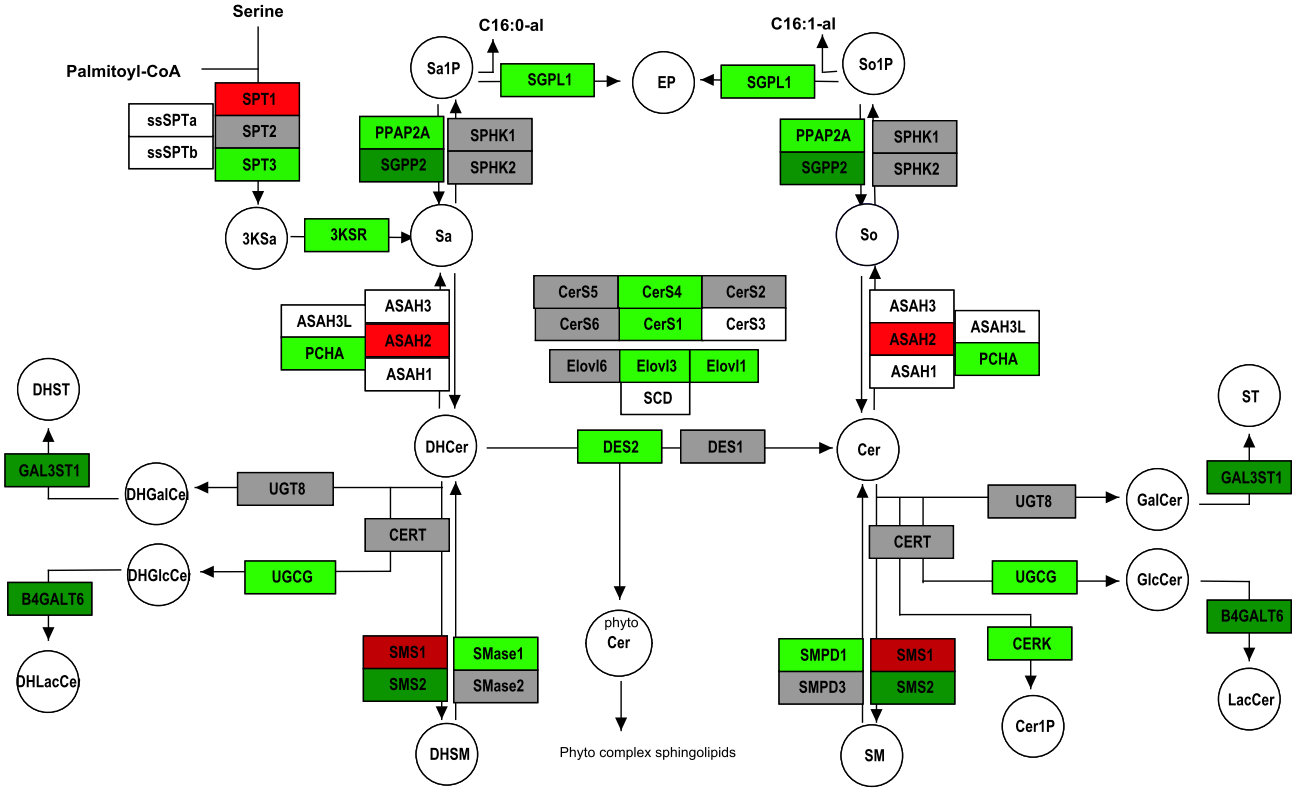
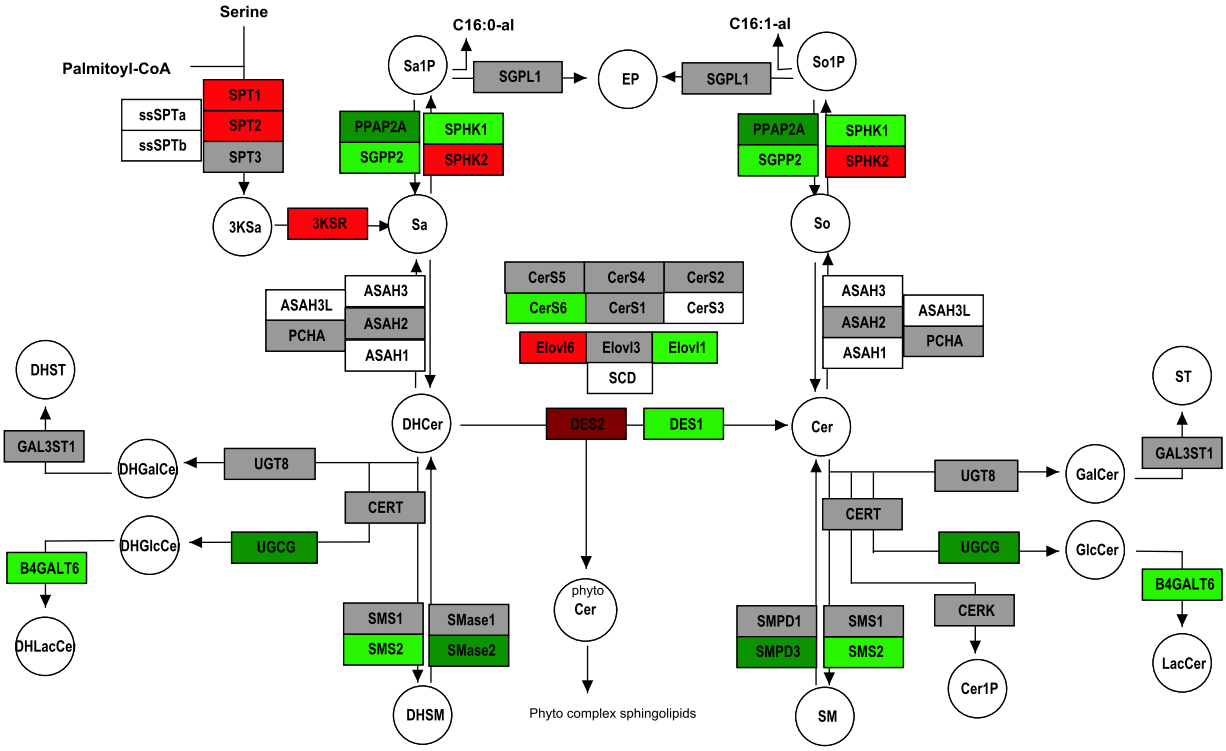


Sf 6: K_562



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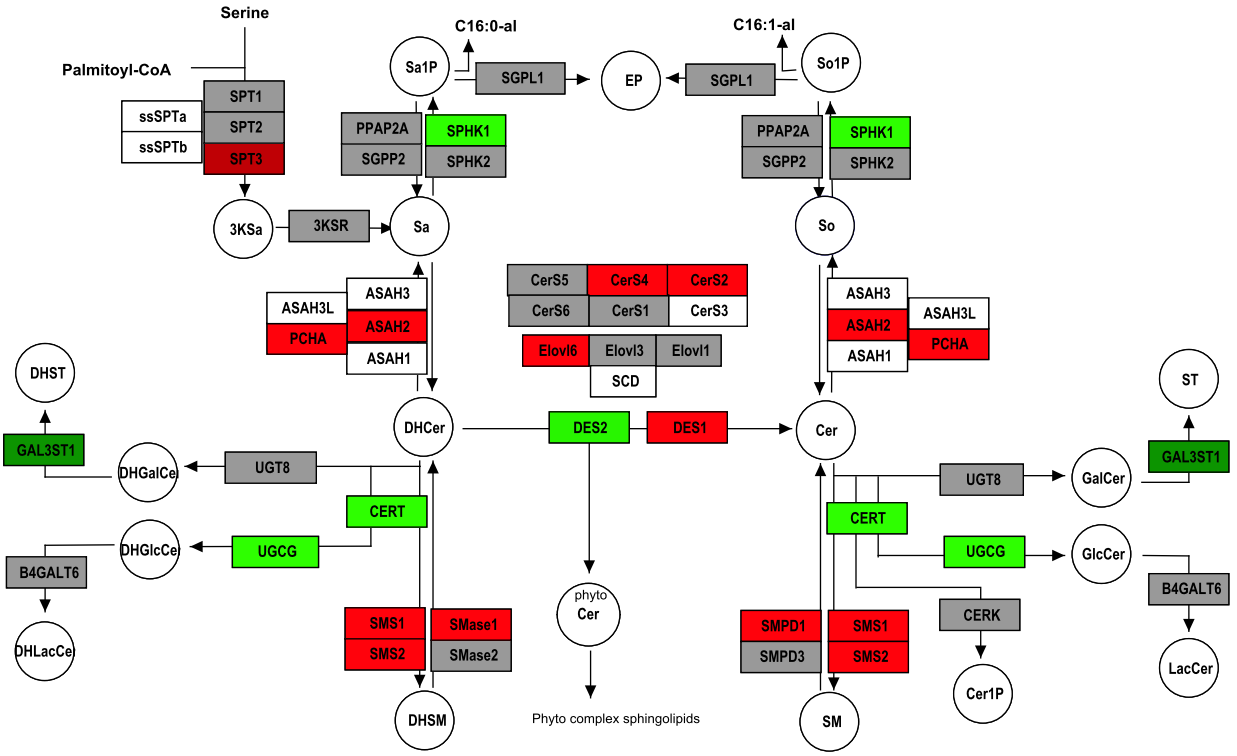

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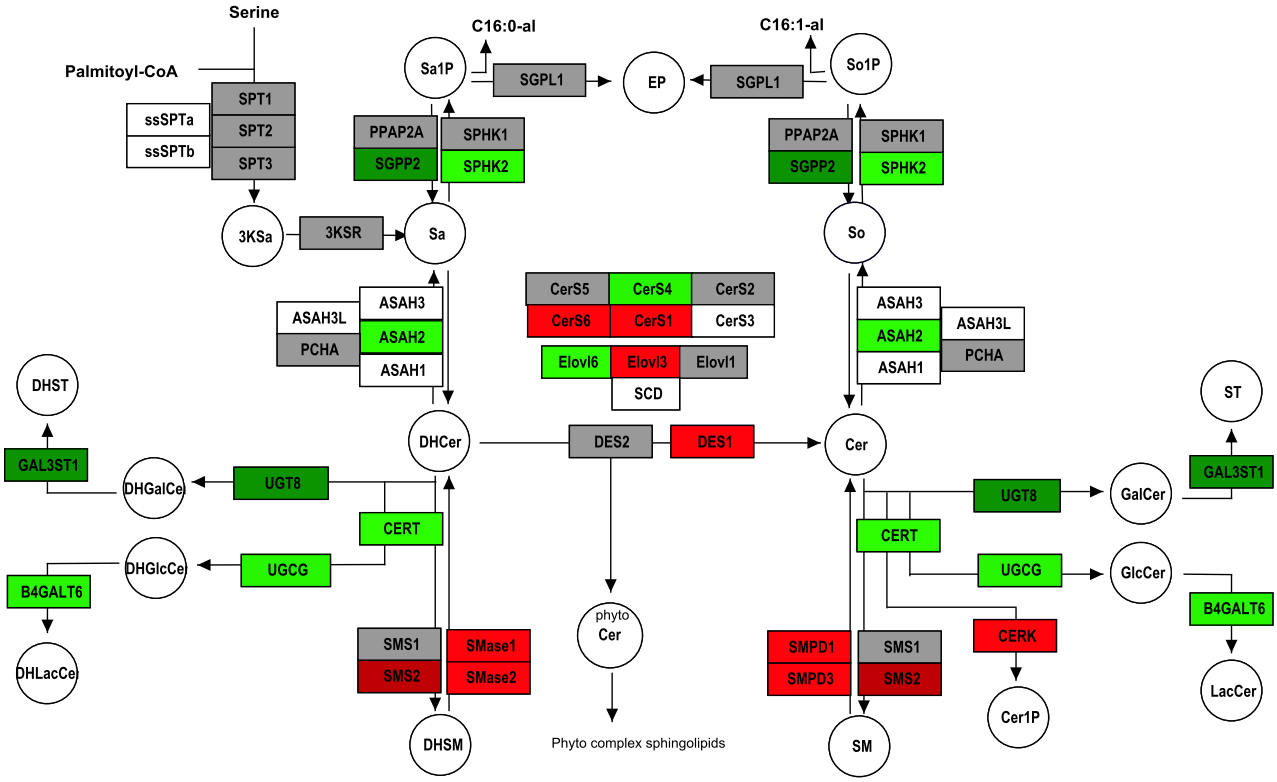

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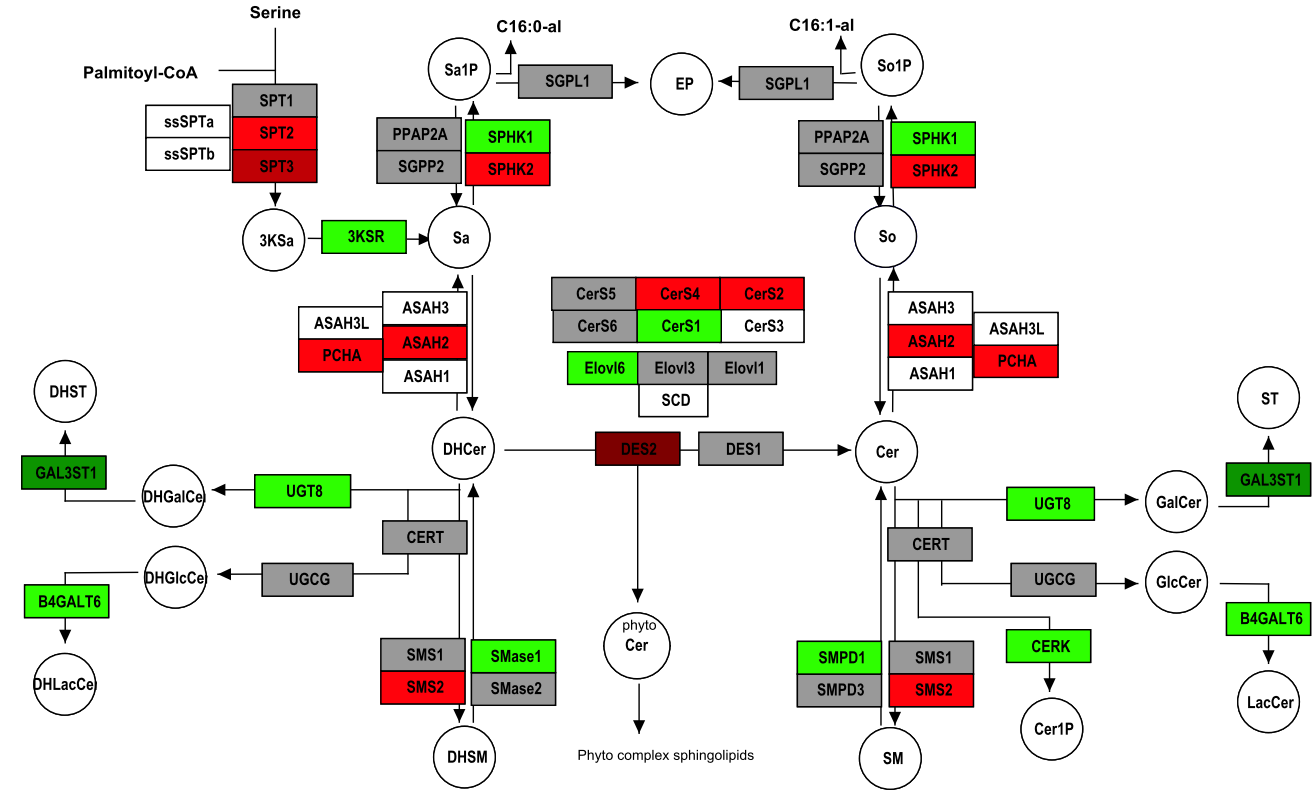

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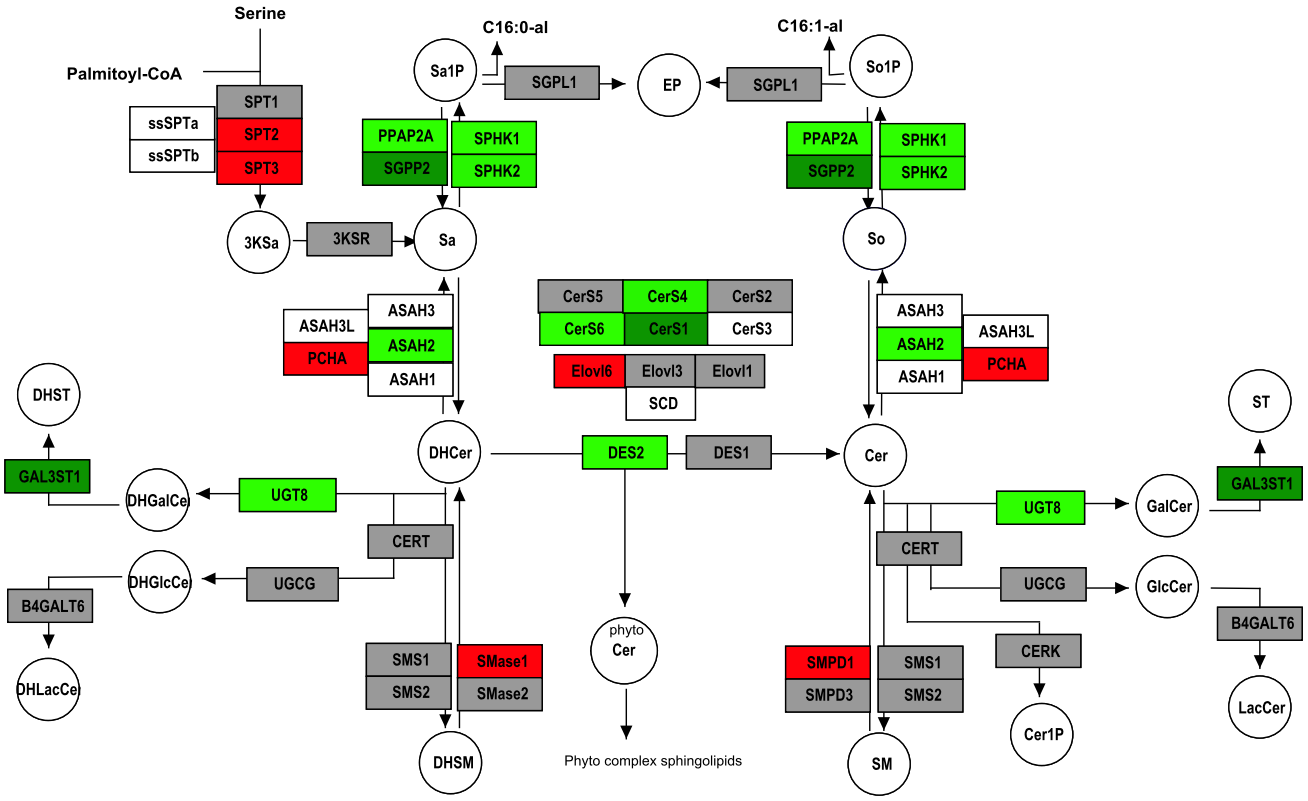

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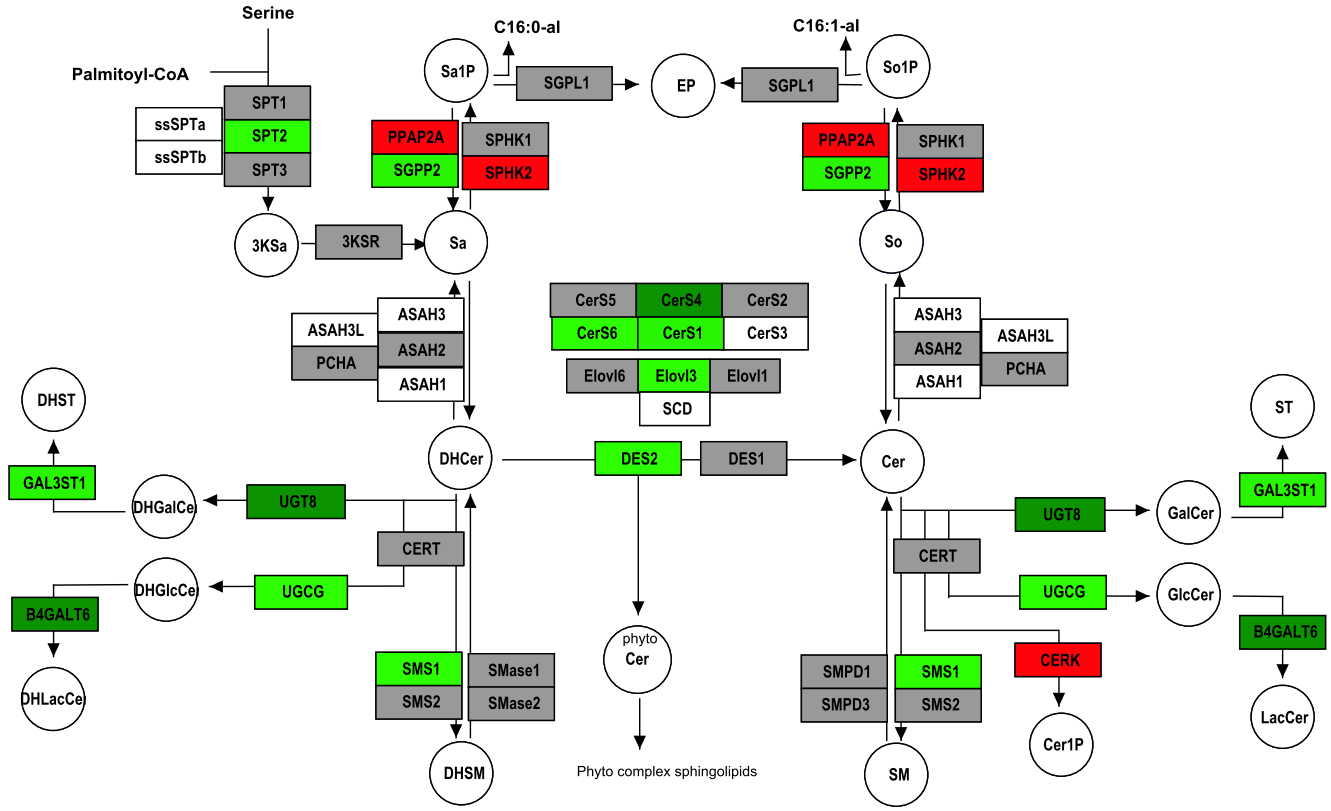

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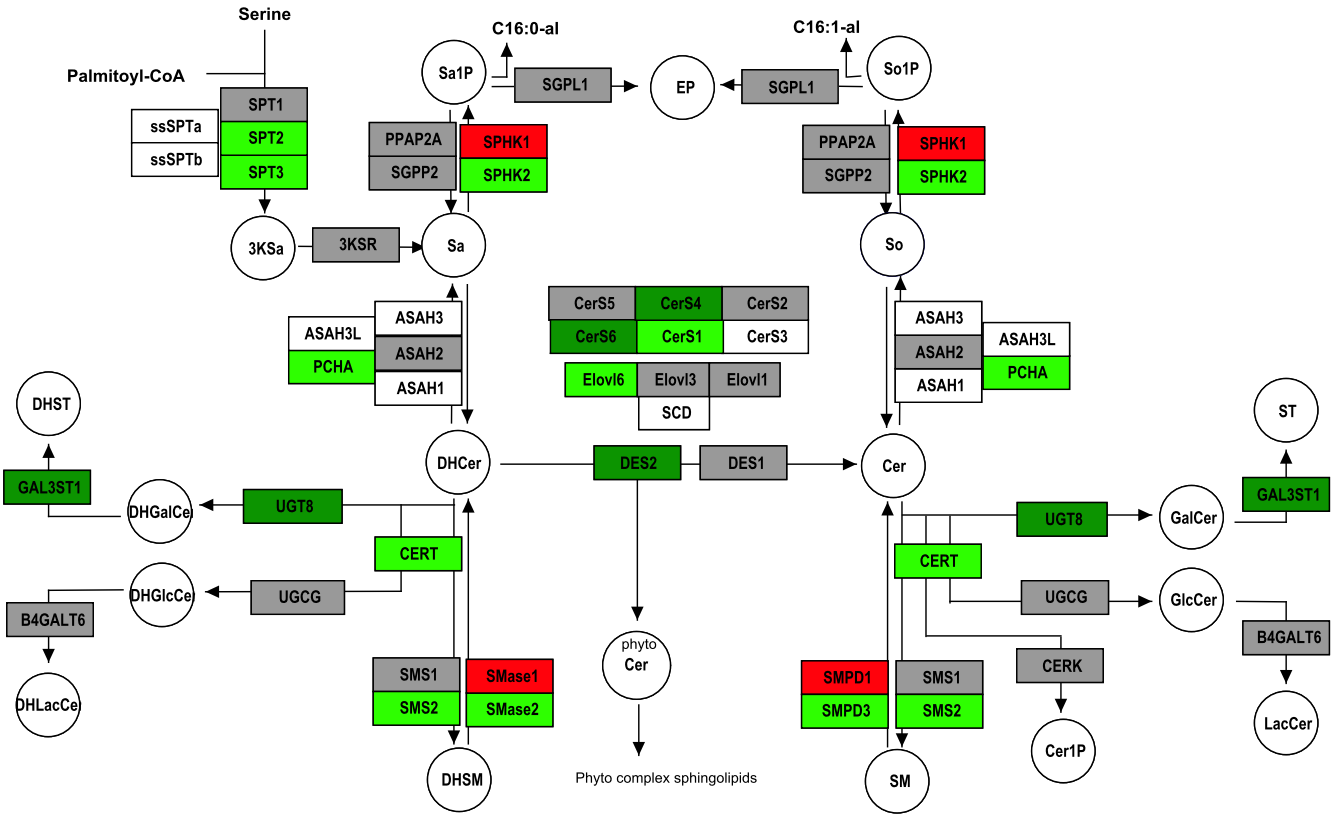

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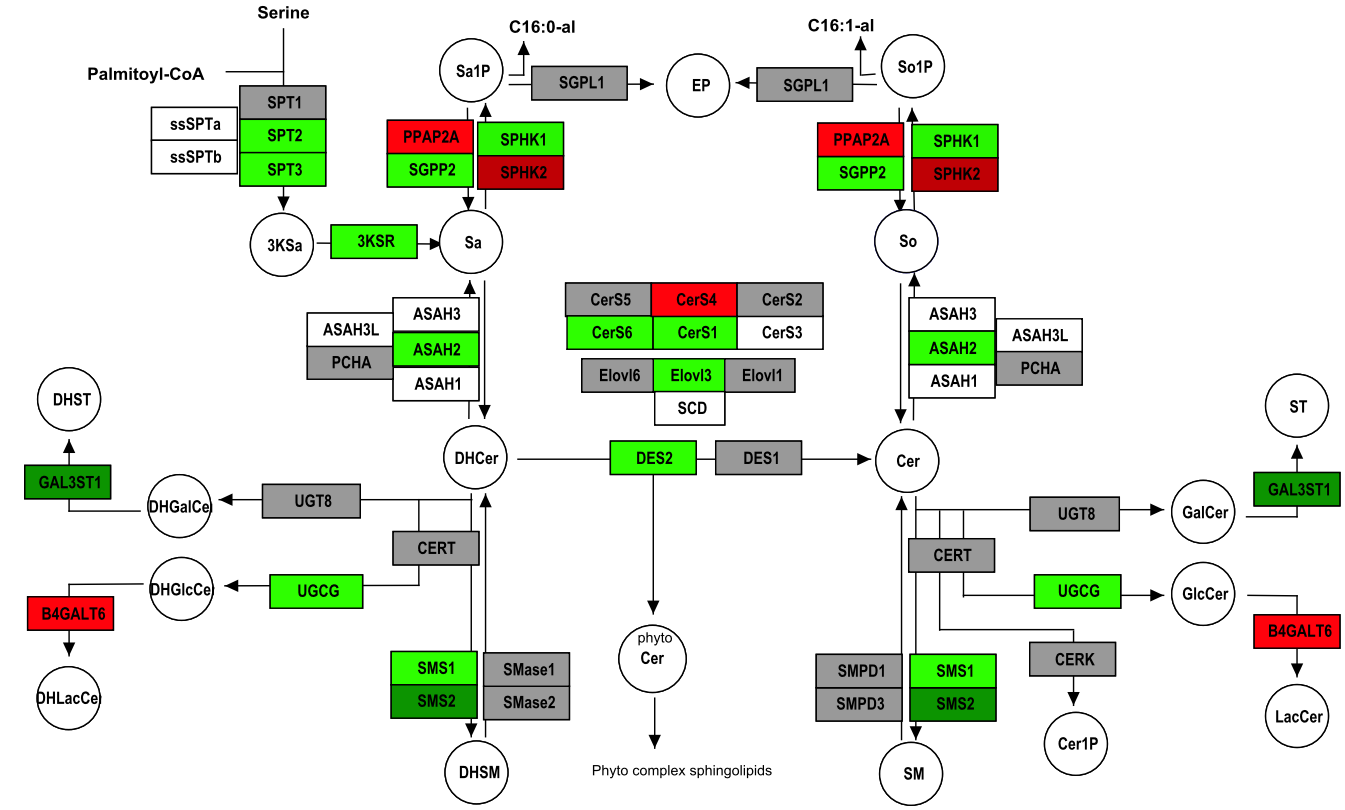

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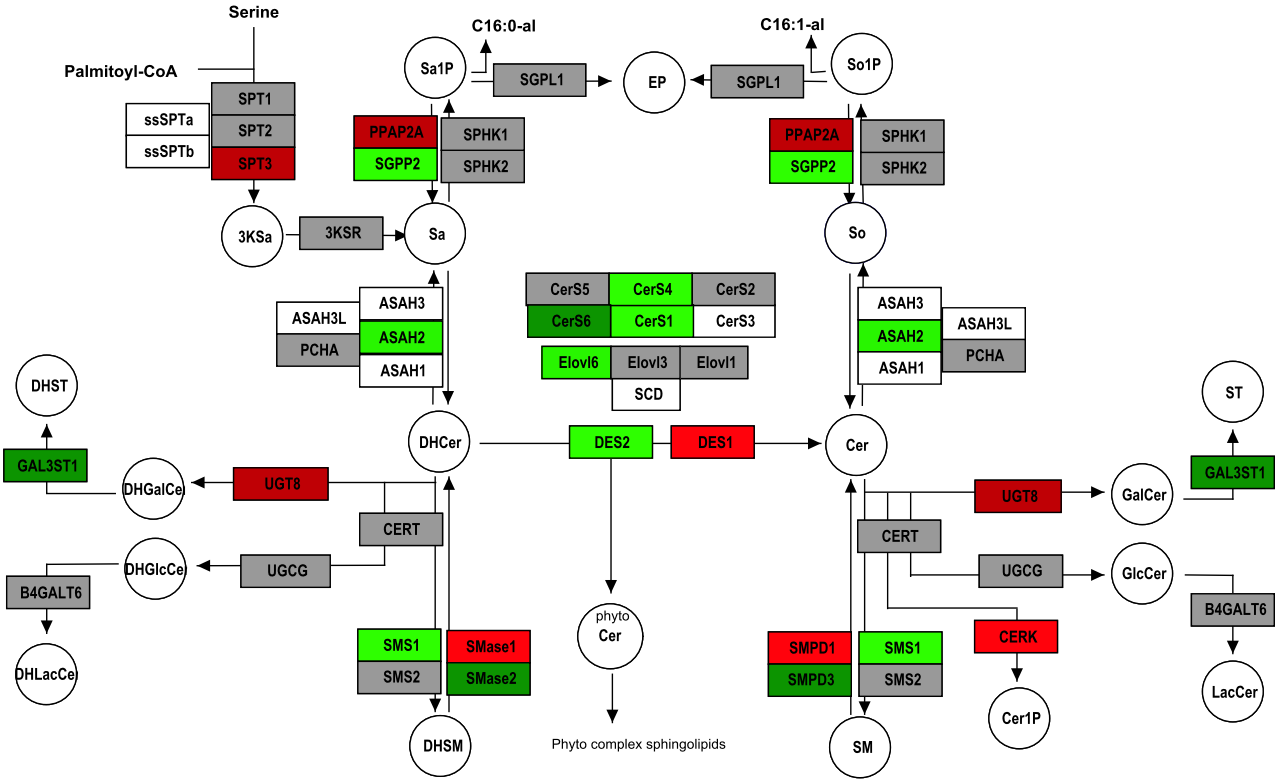

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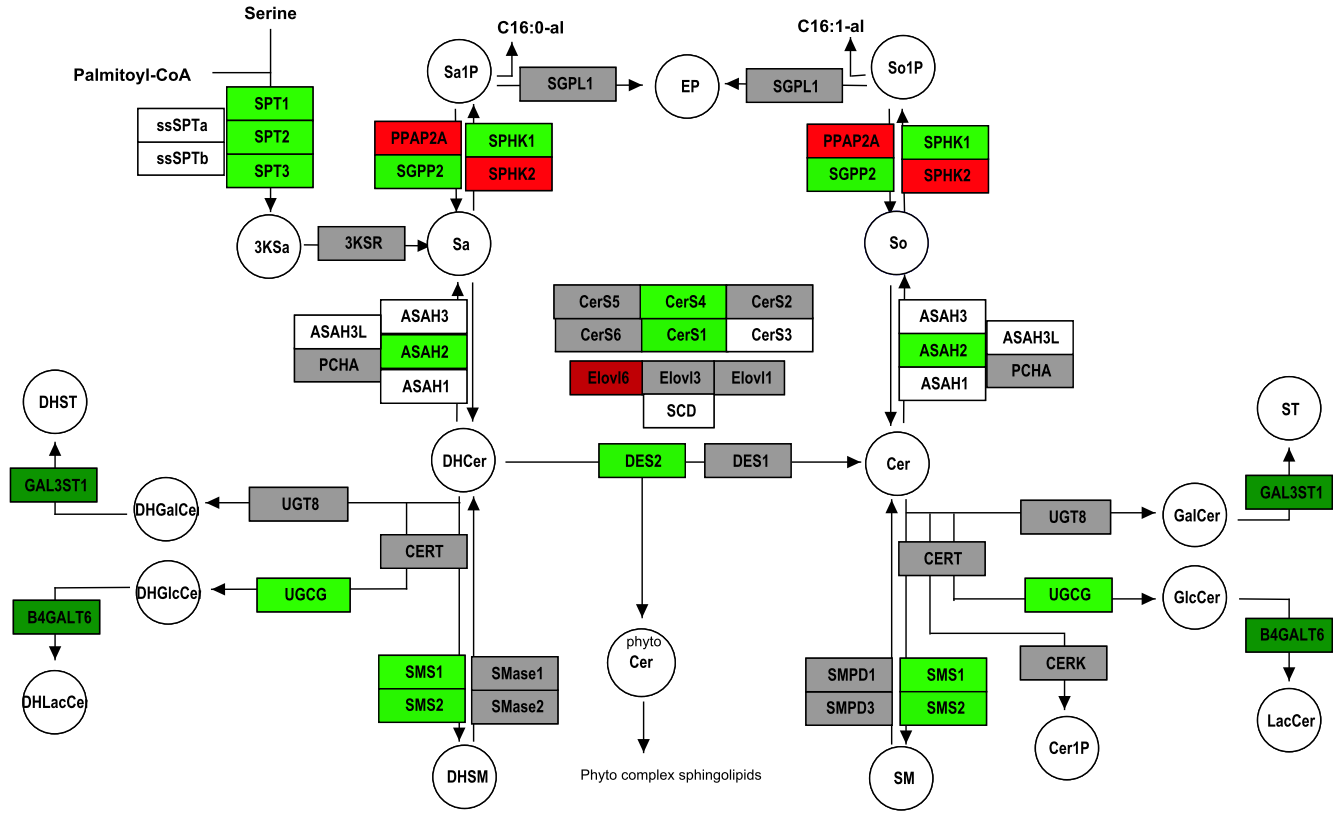

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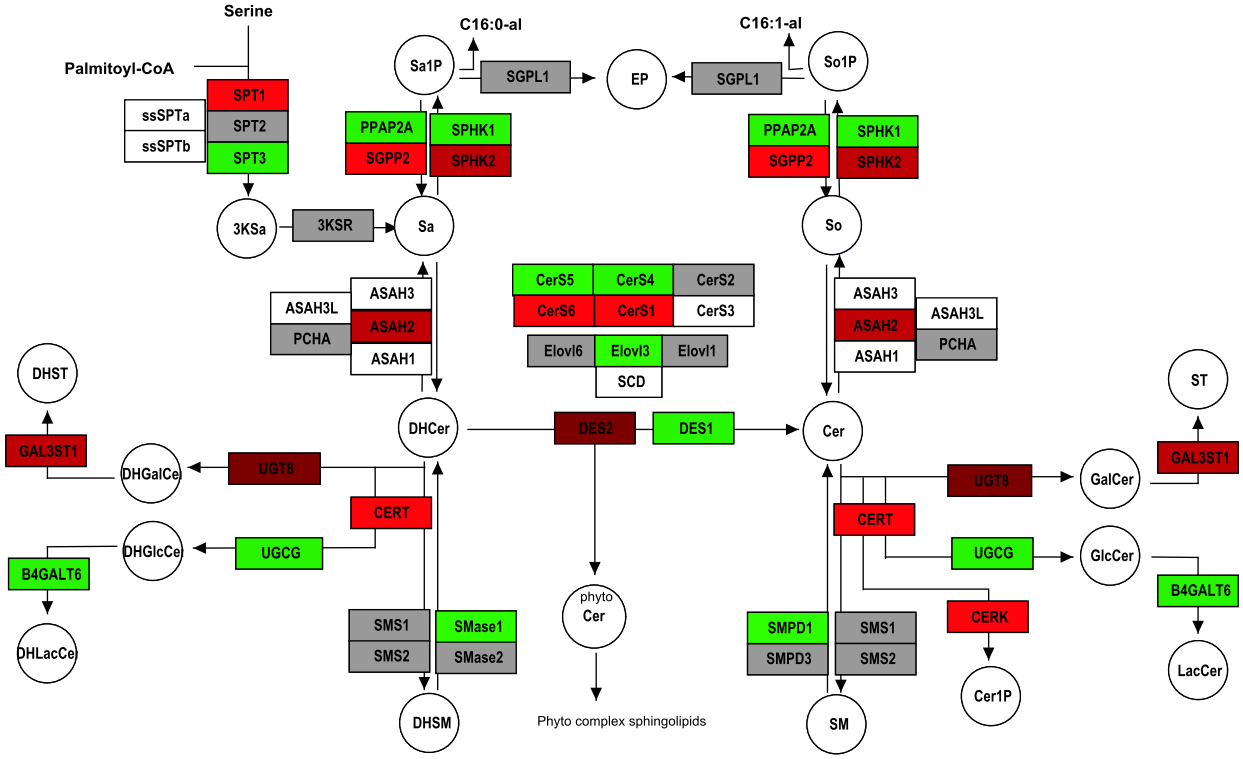

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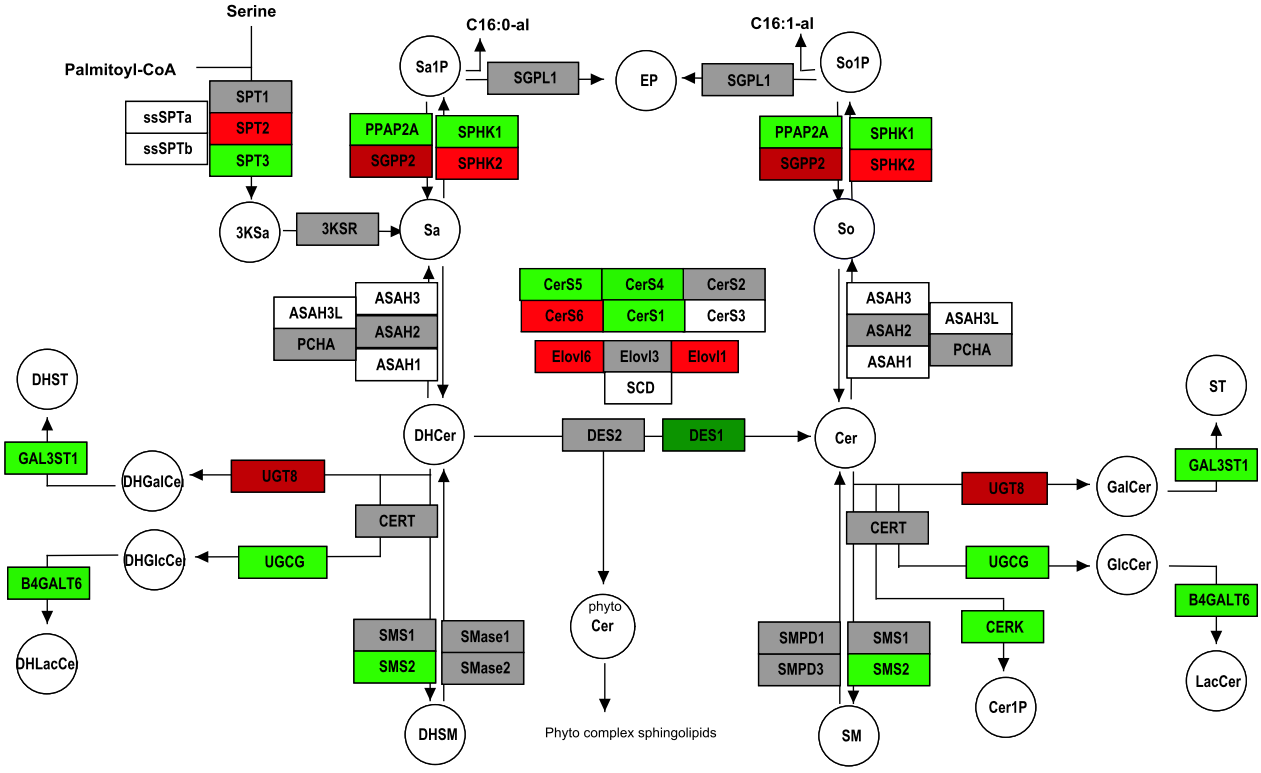

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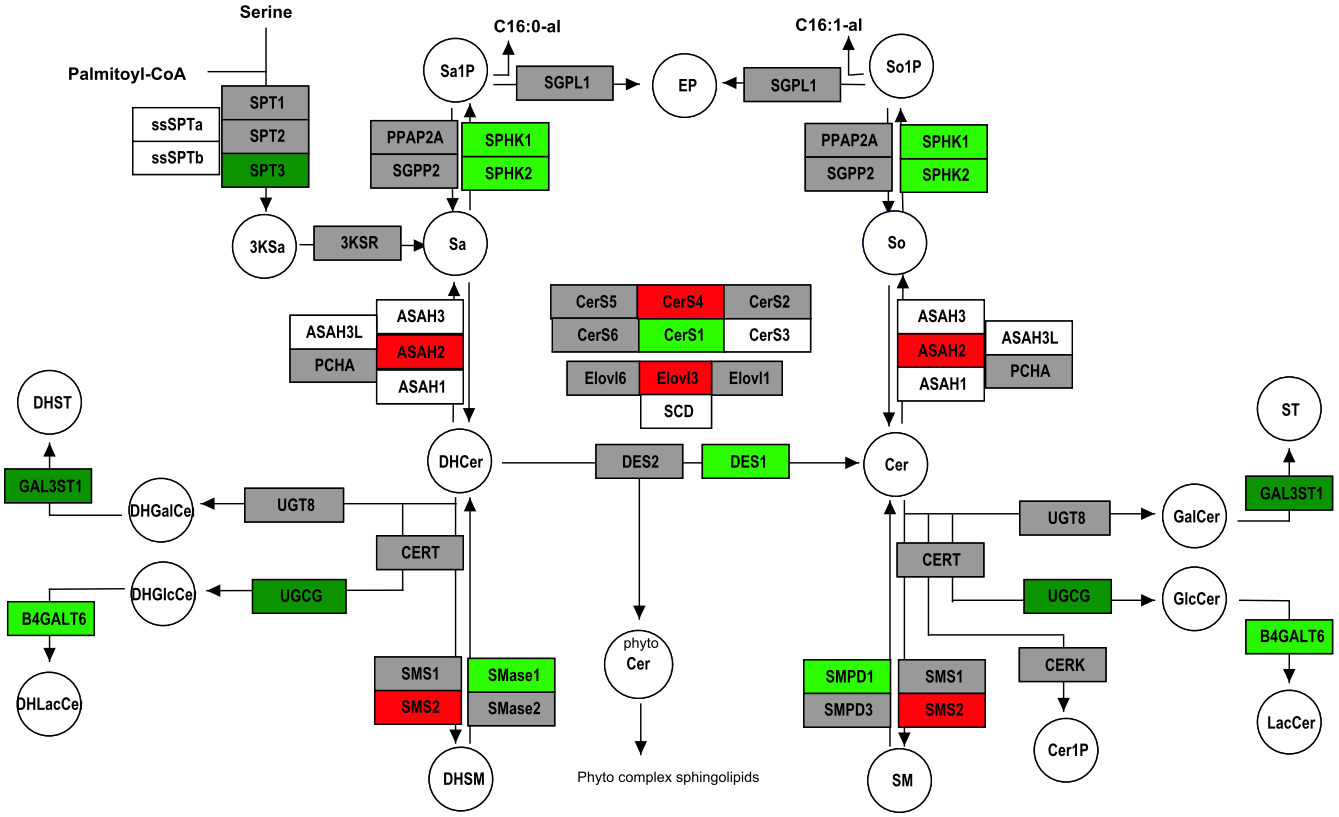

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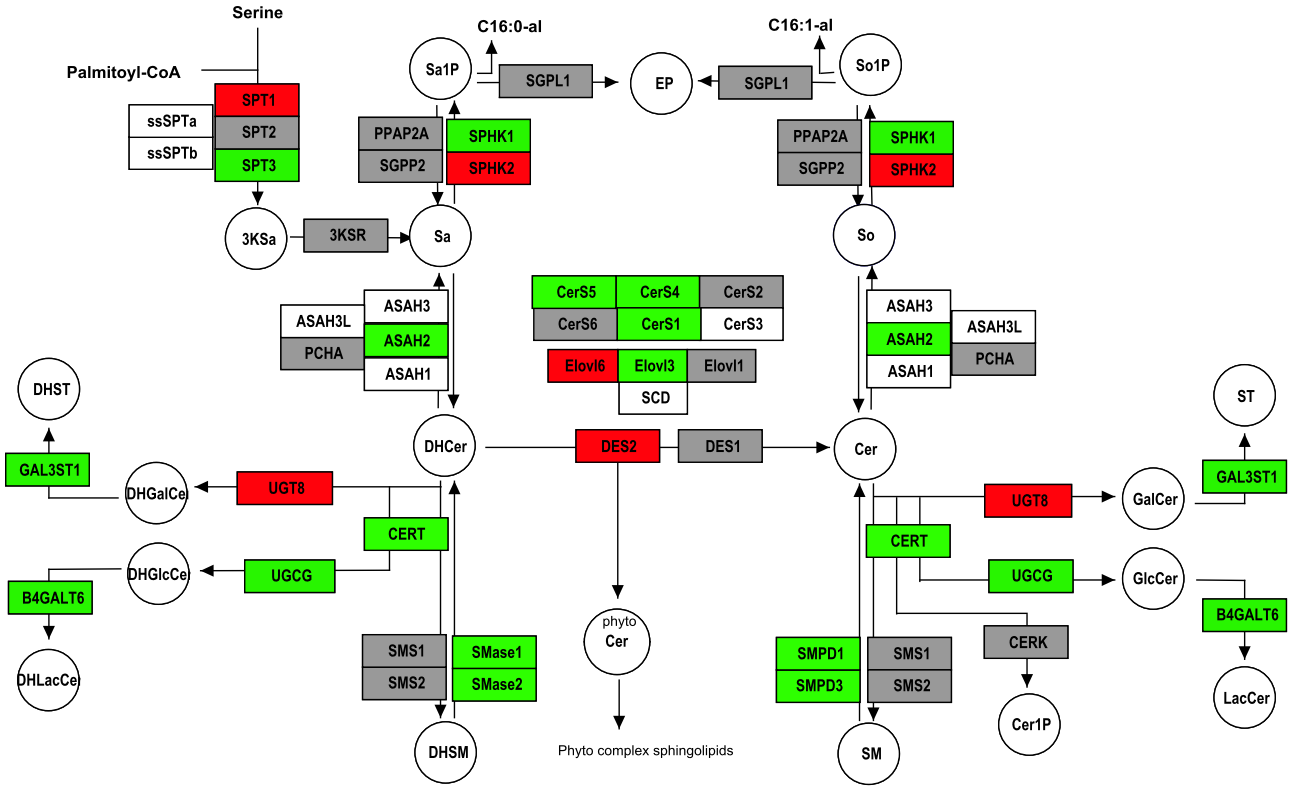

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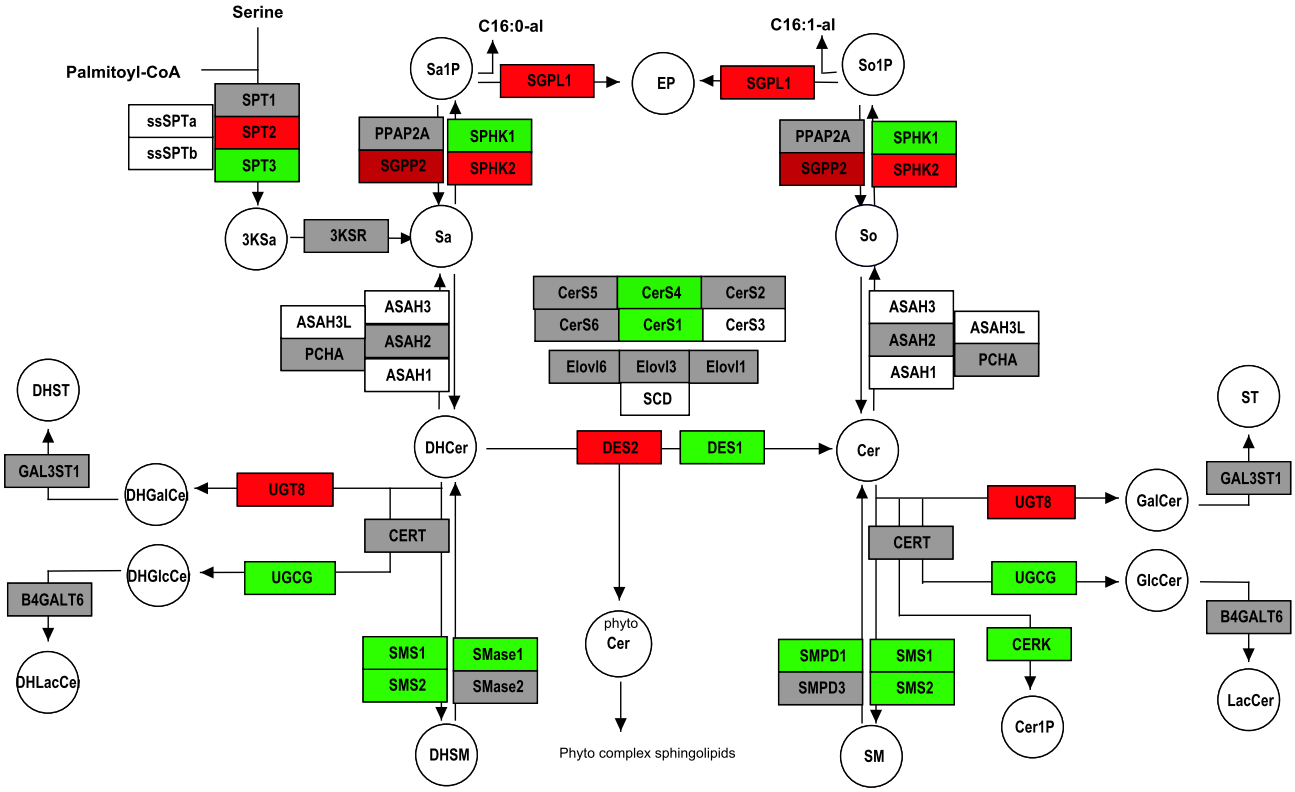

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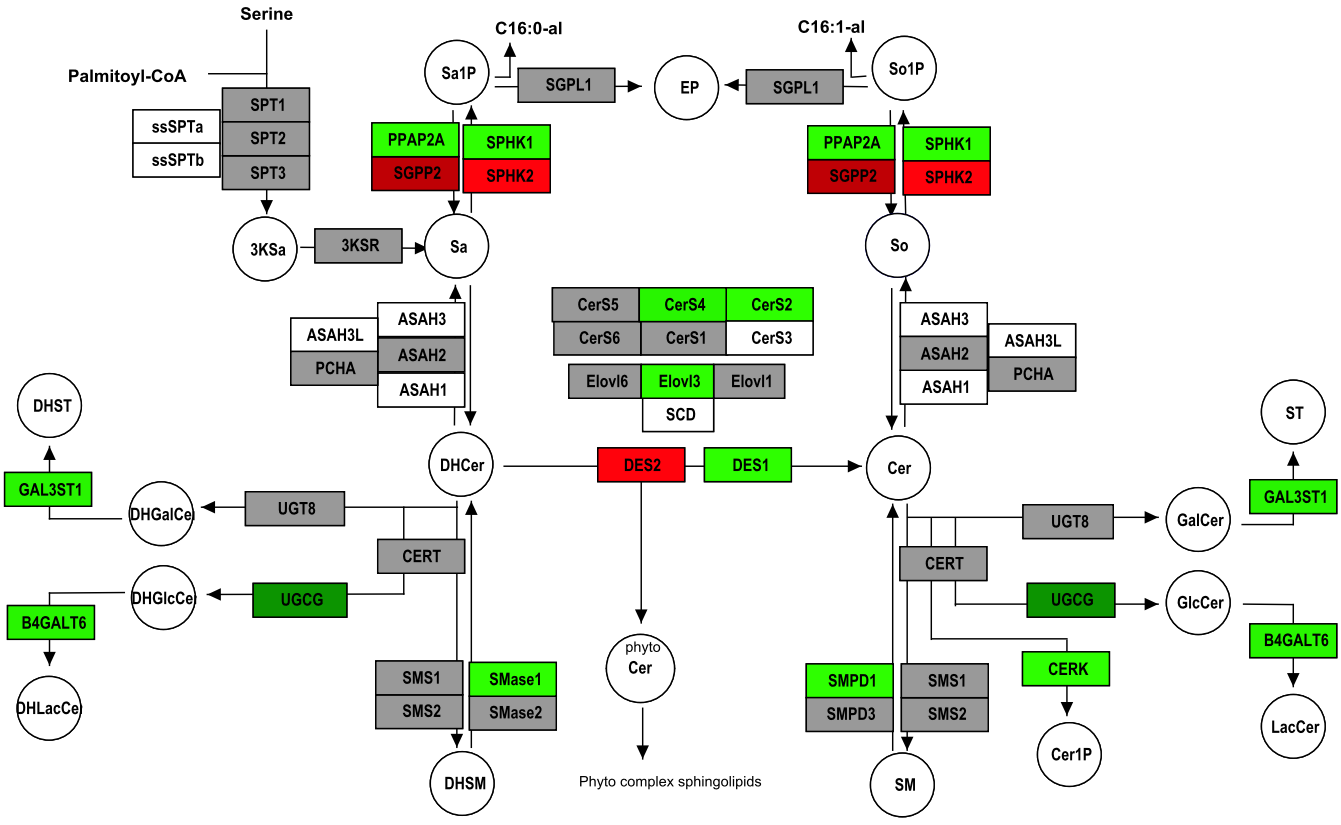

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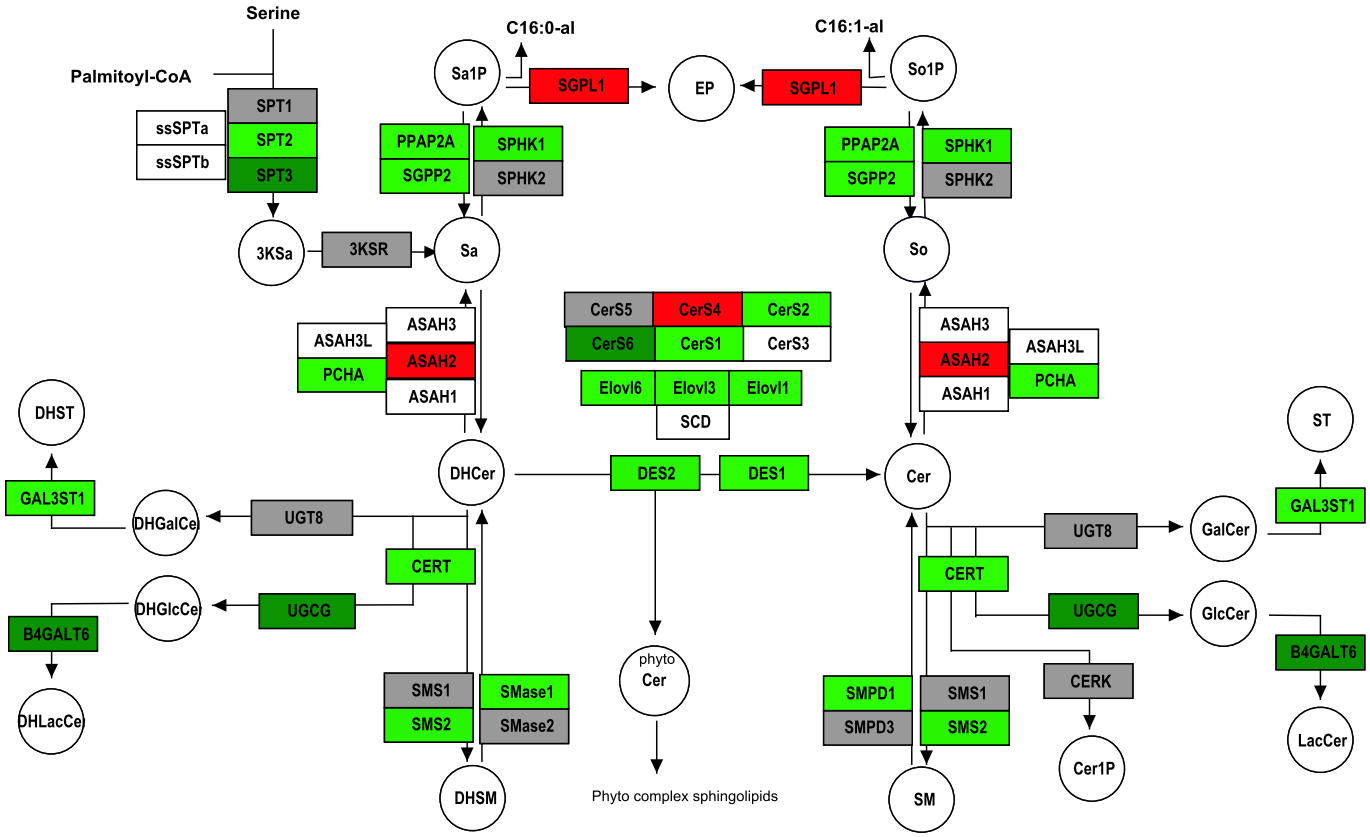

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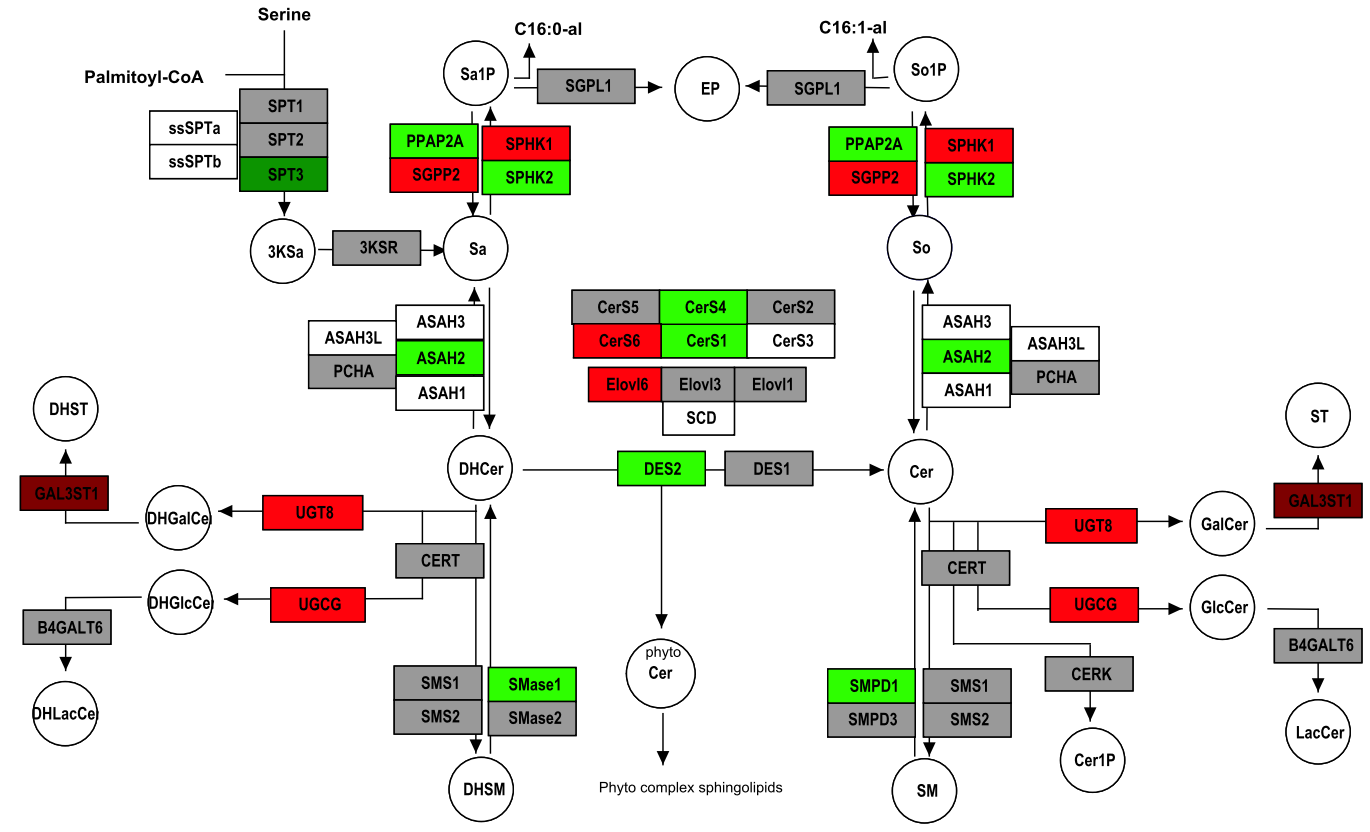

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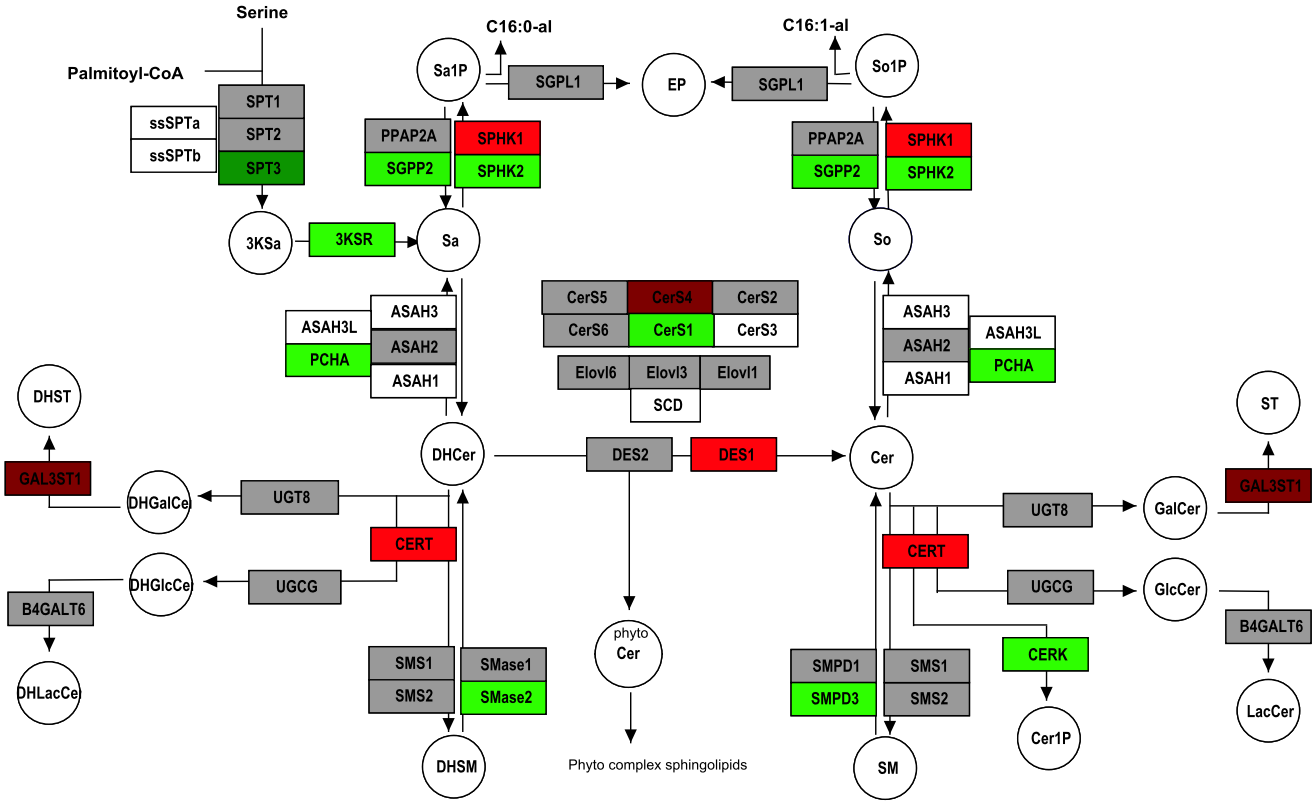

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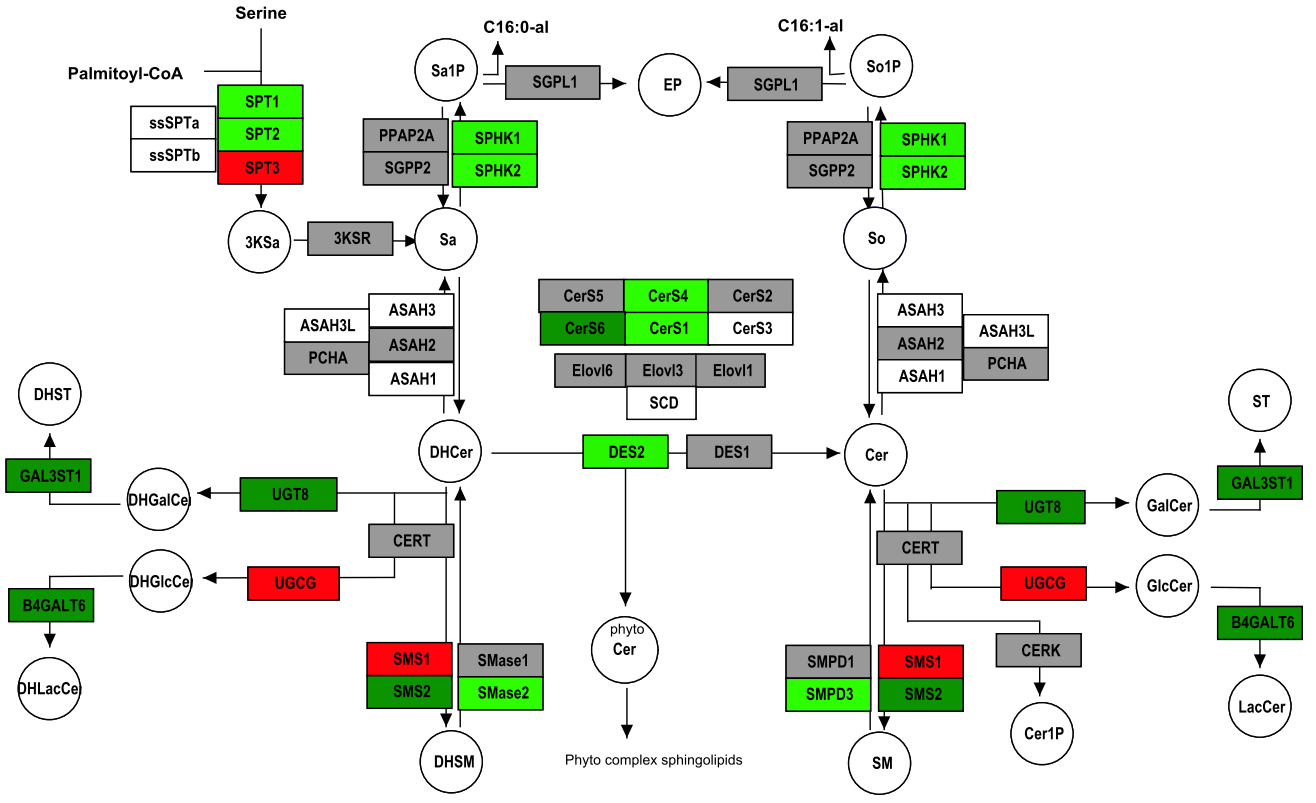

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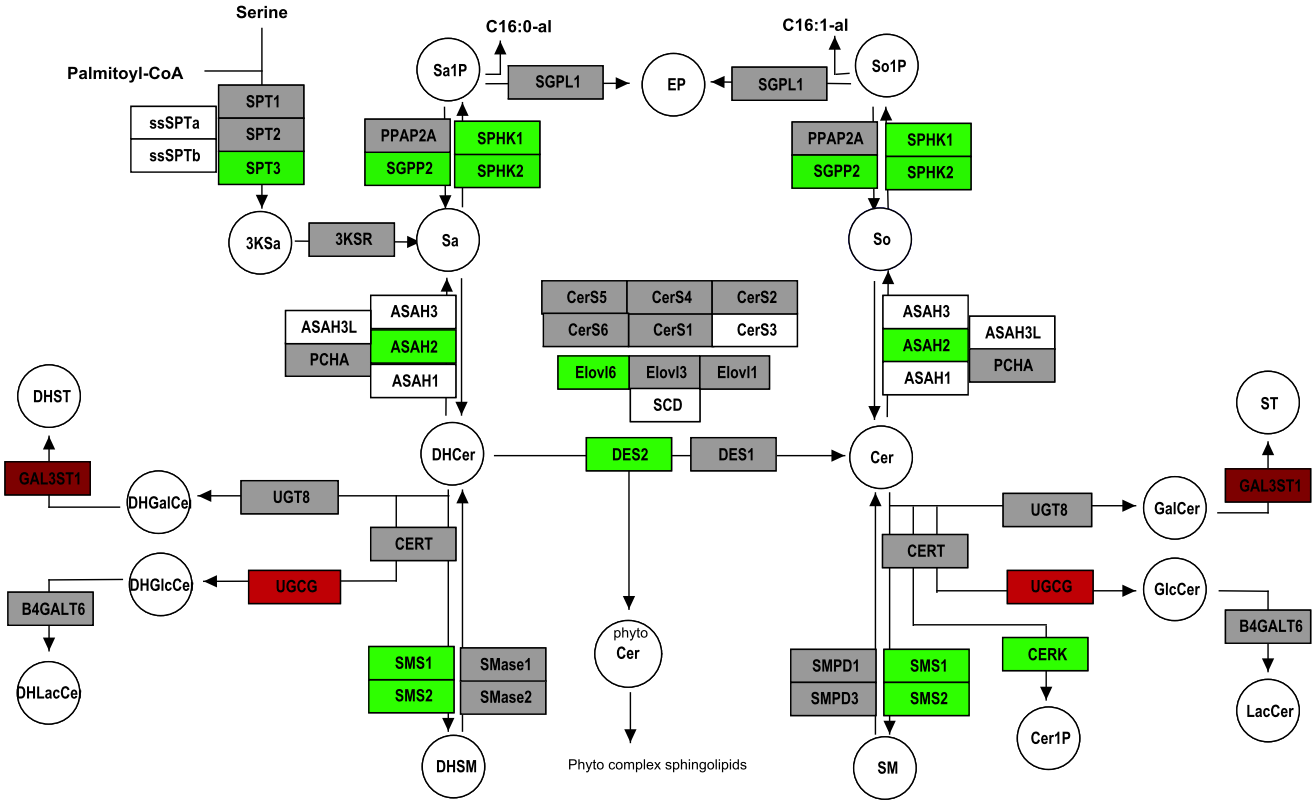

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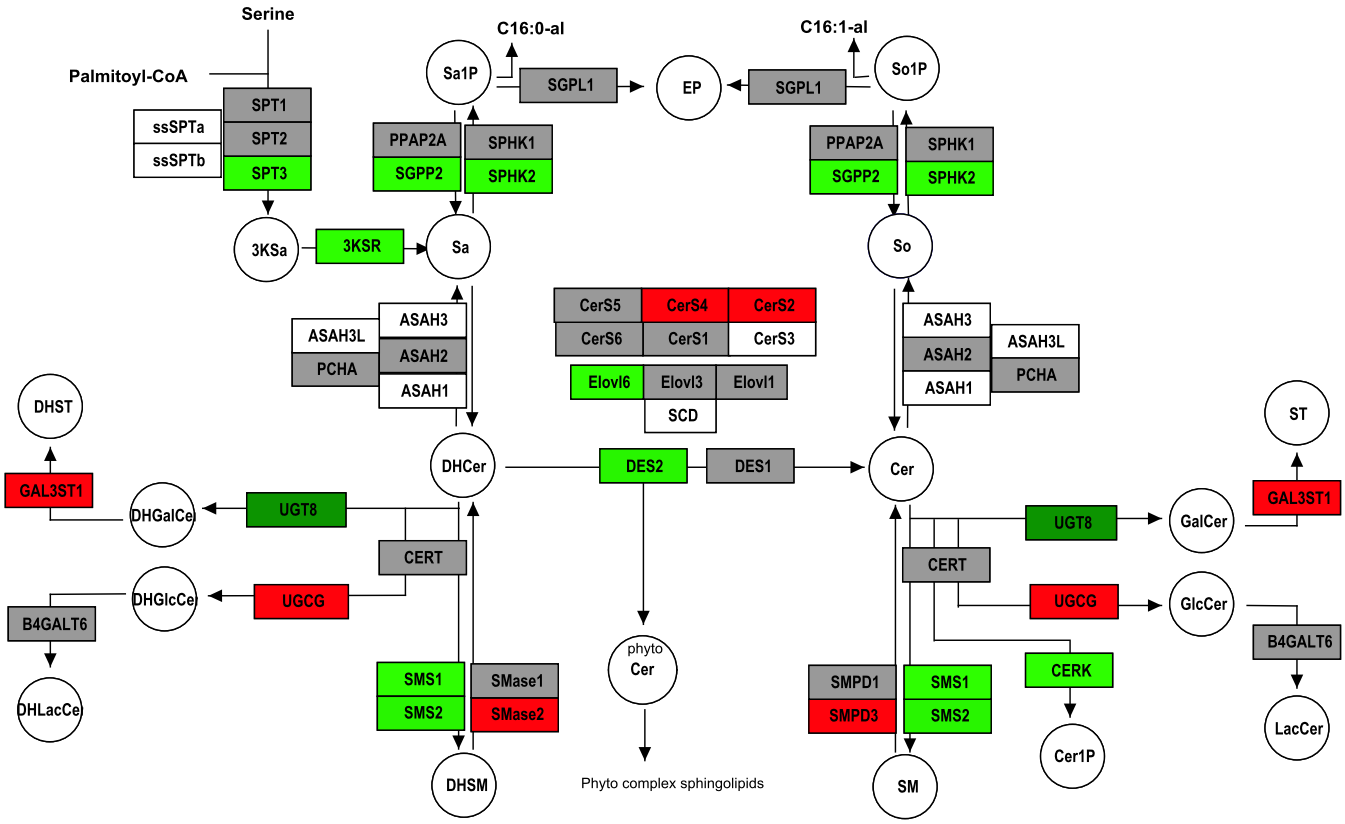

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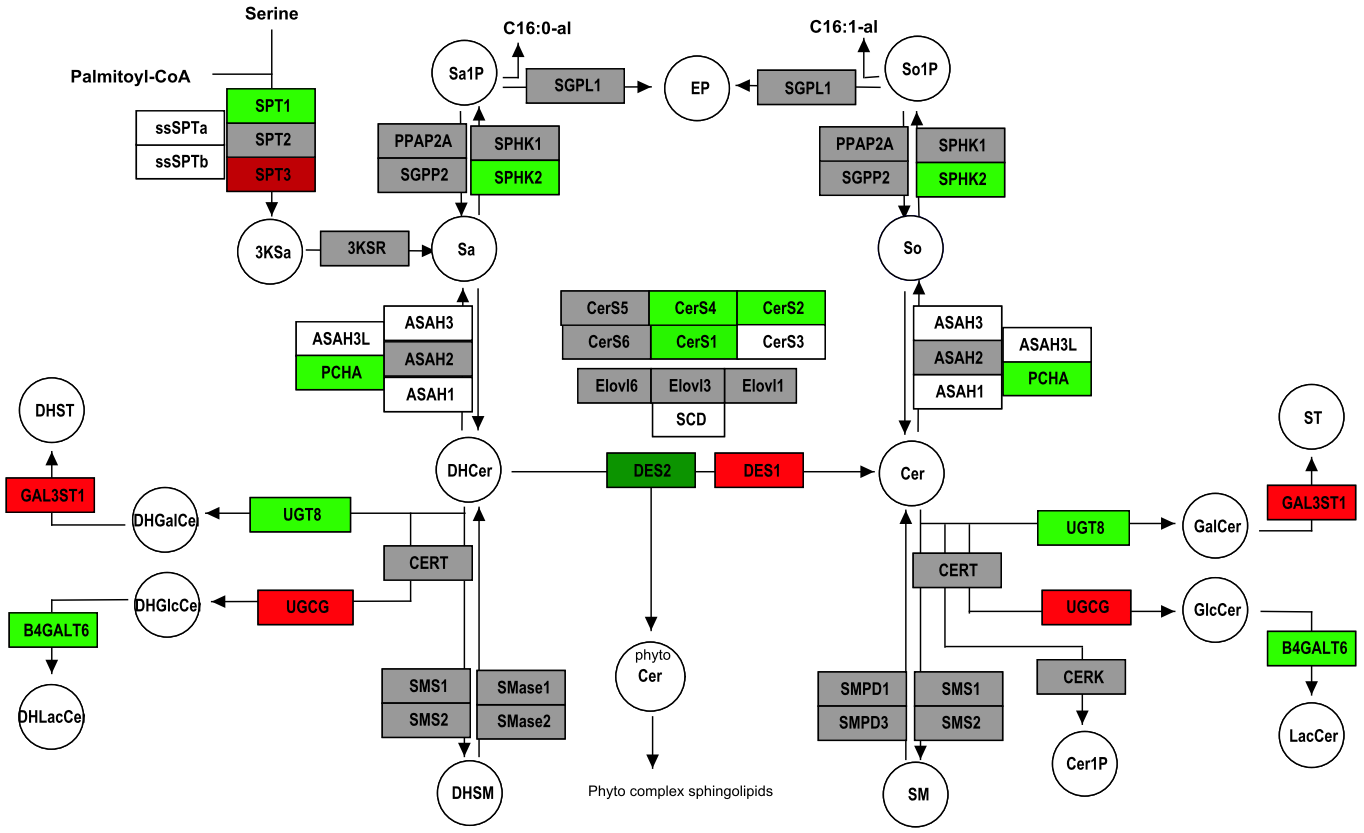

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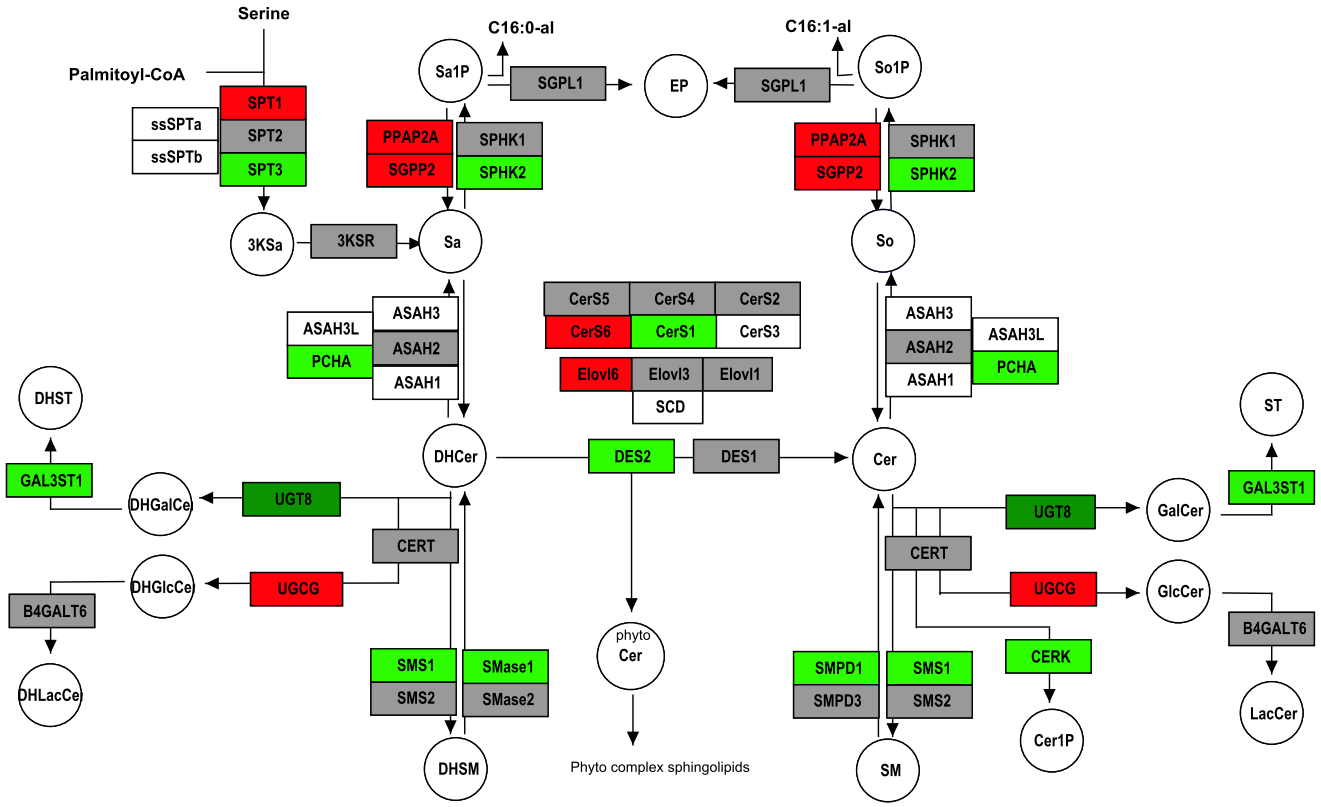

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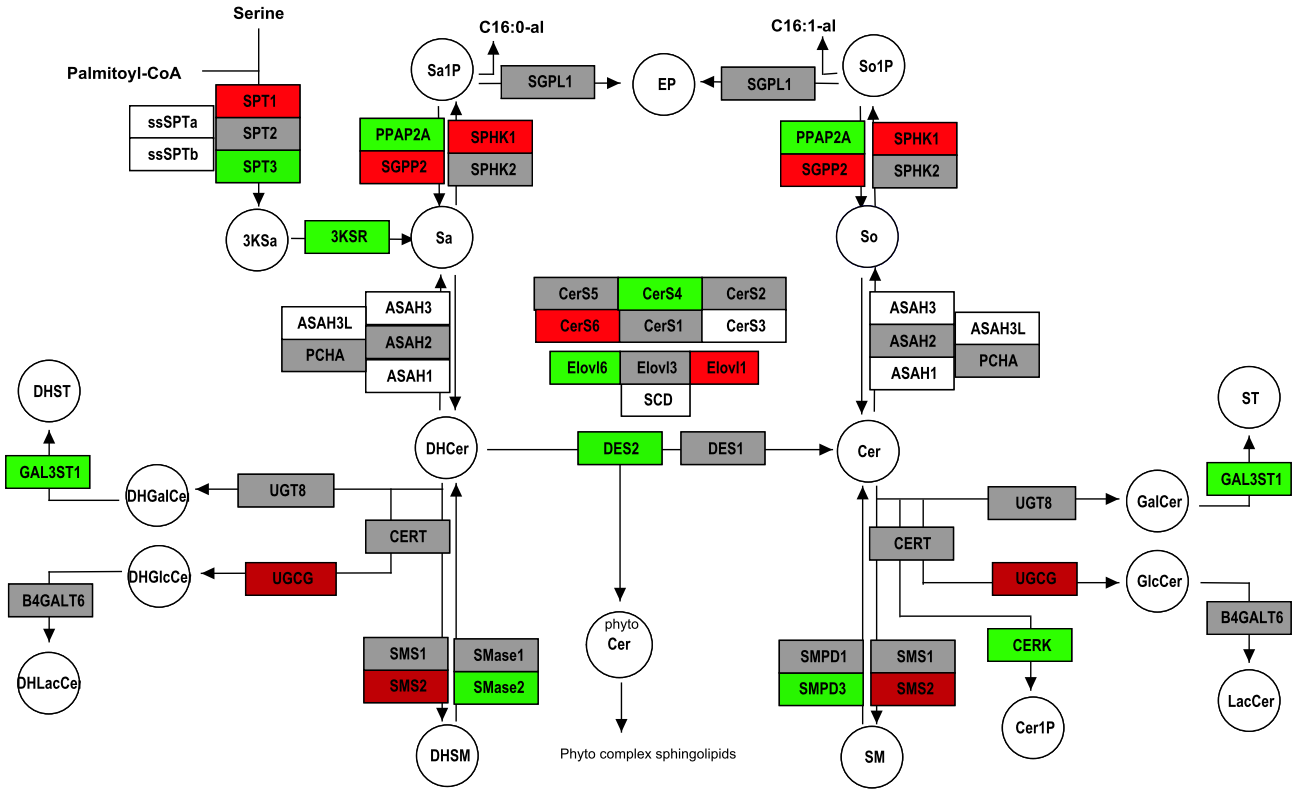

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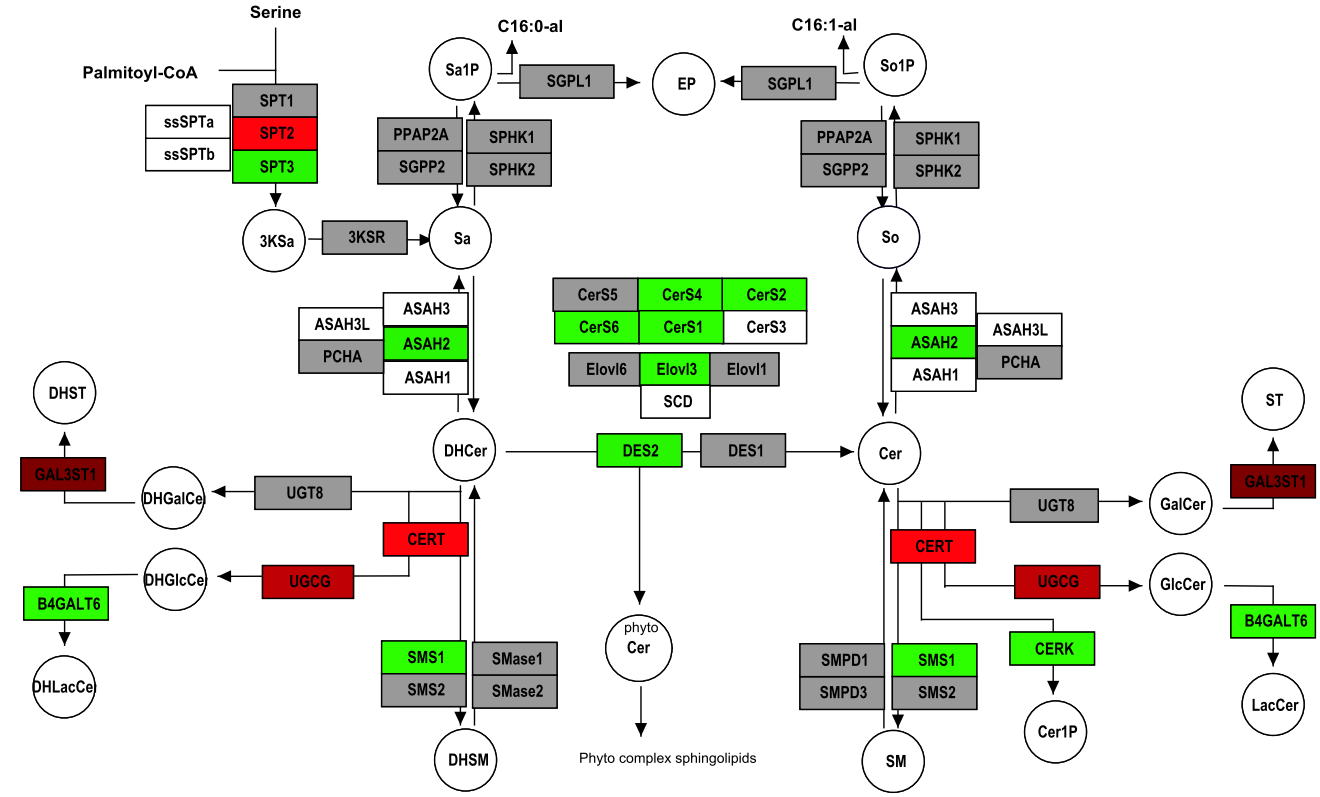
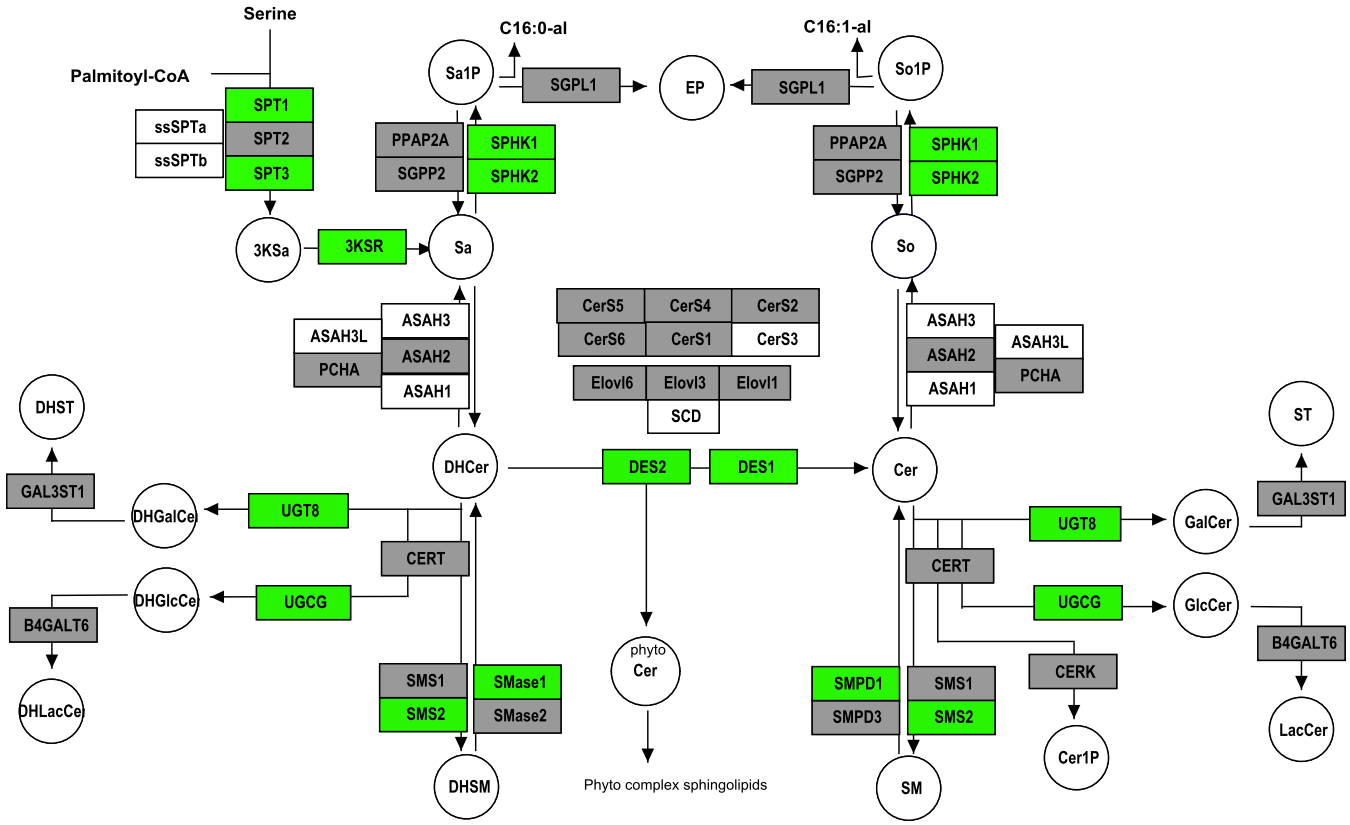
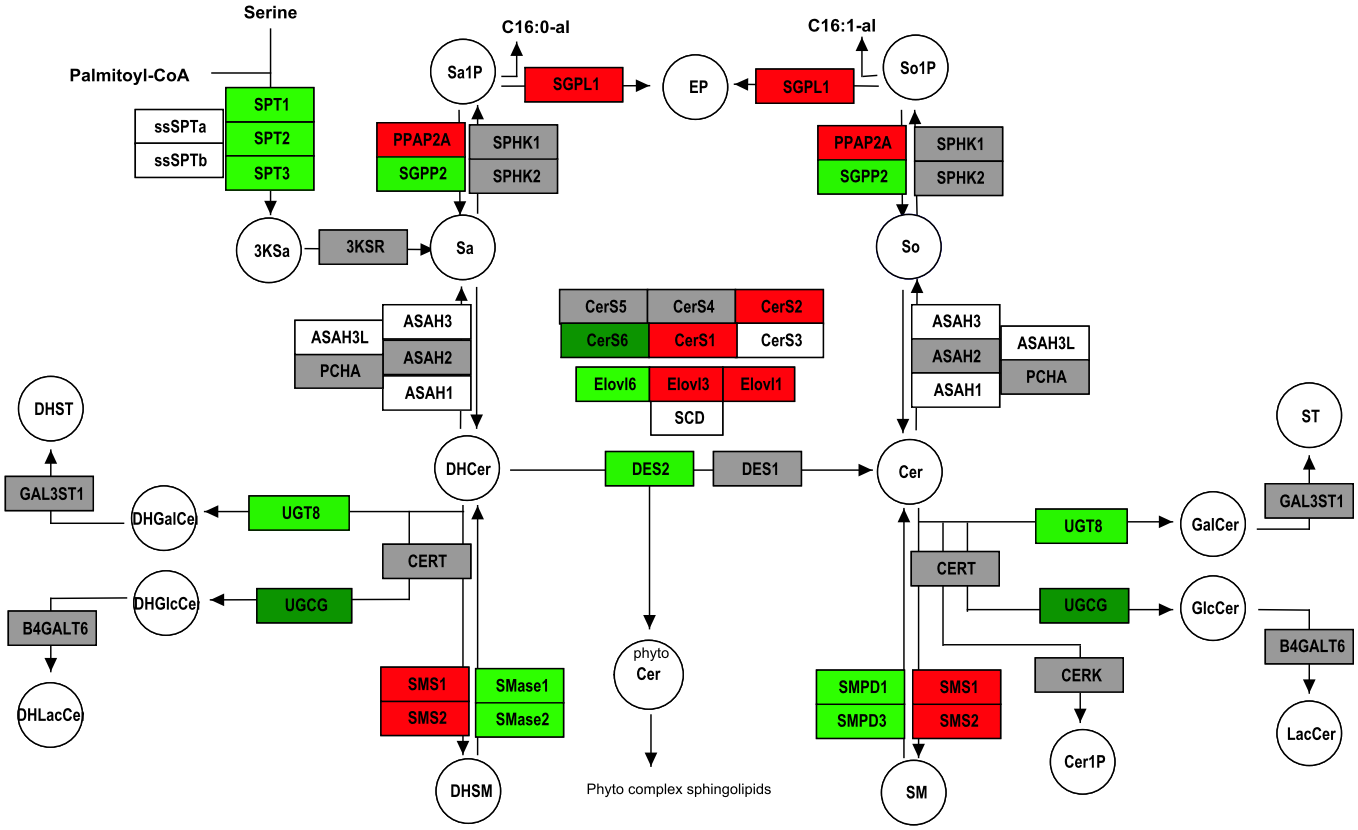


Sf 32: UO_31



Sf 33: A549



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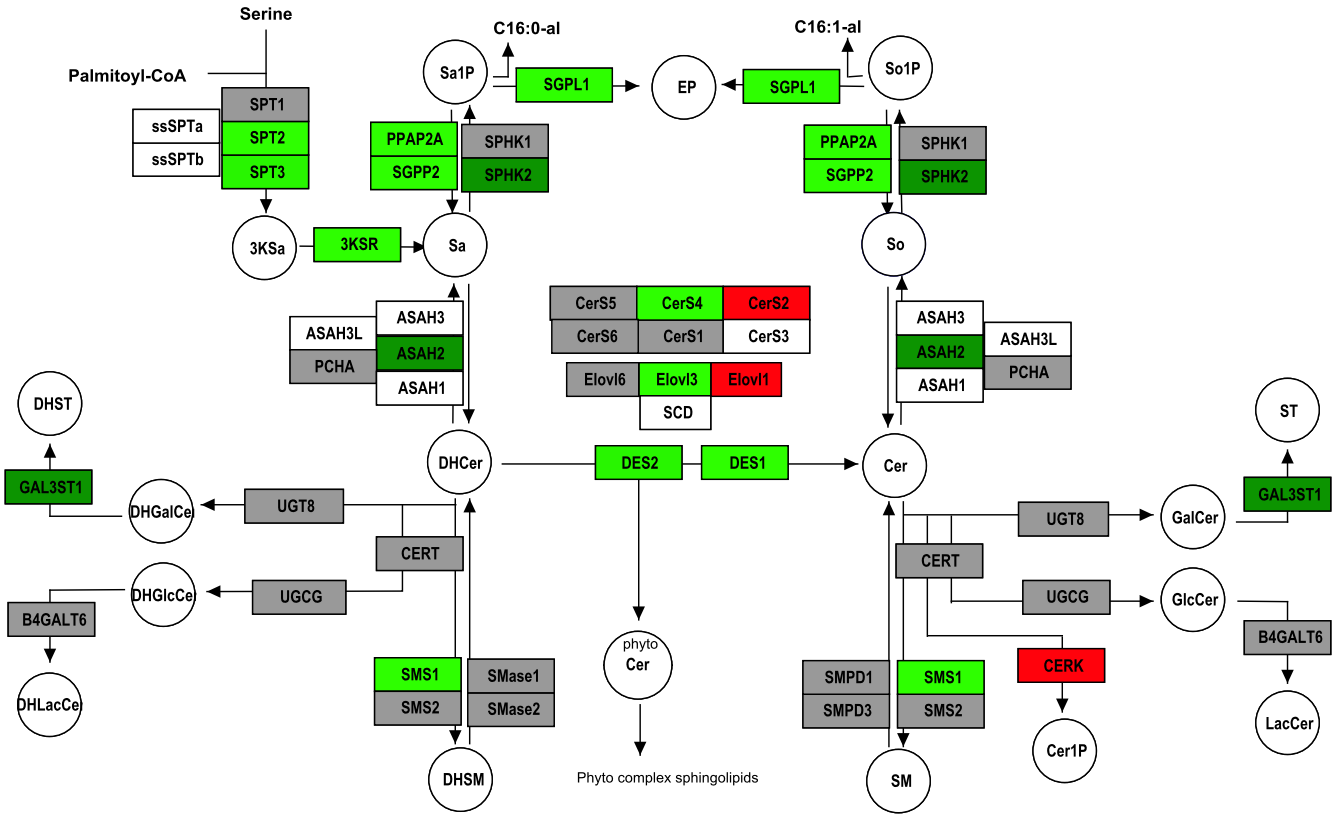

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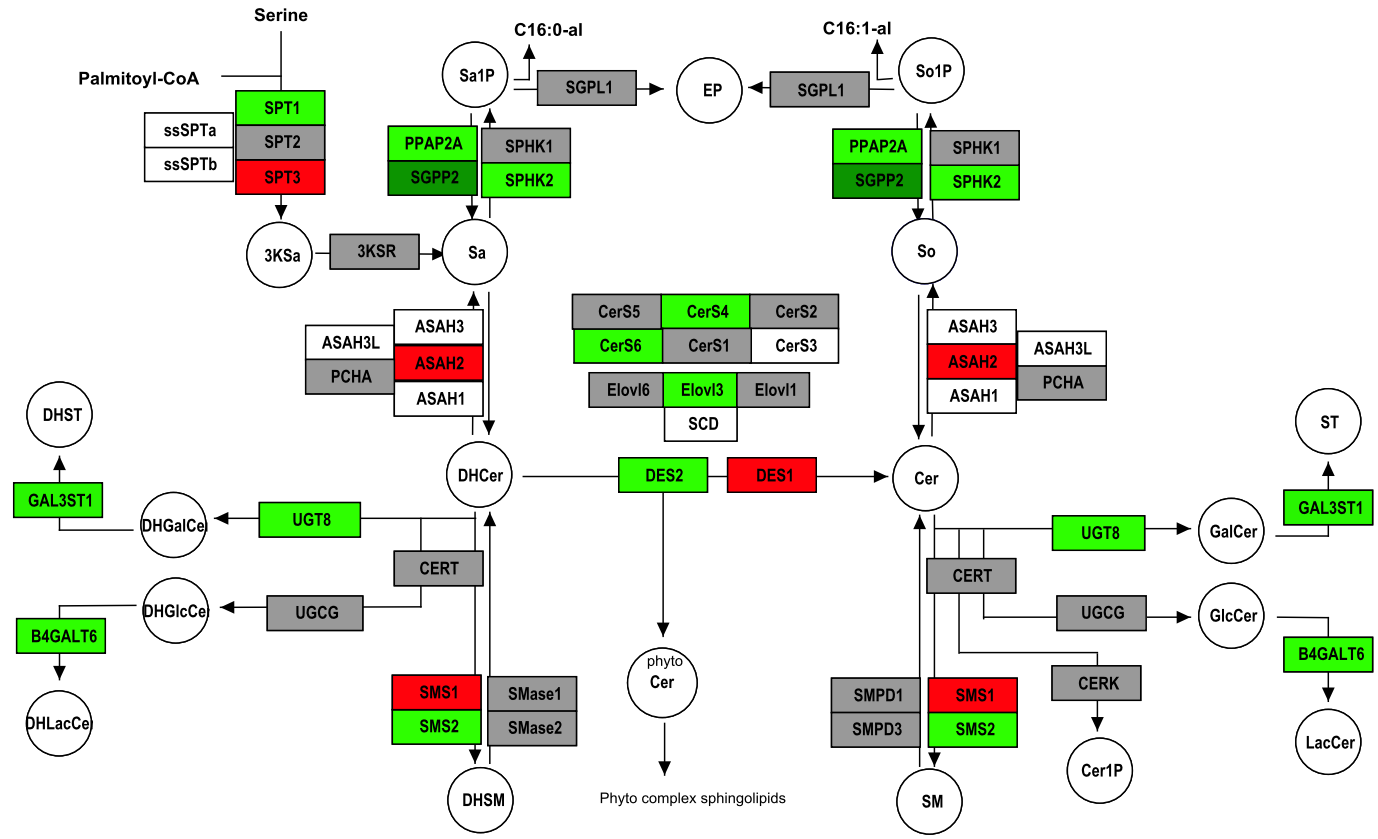

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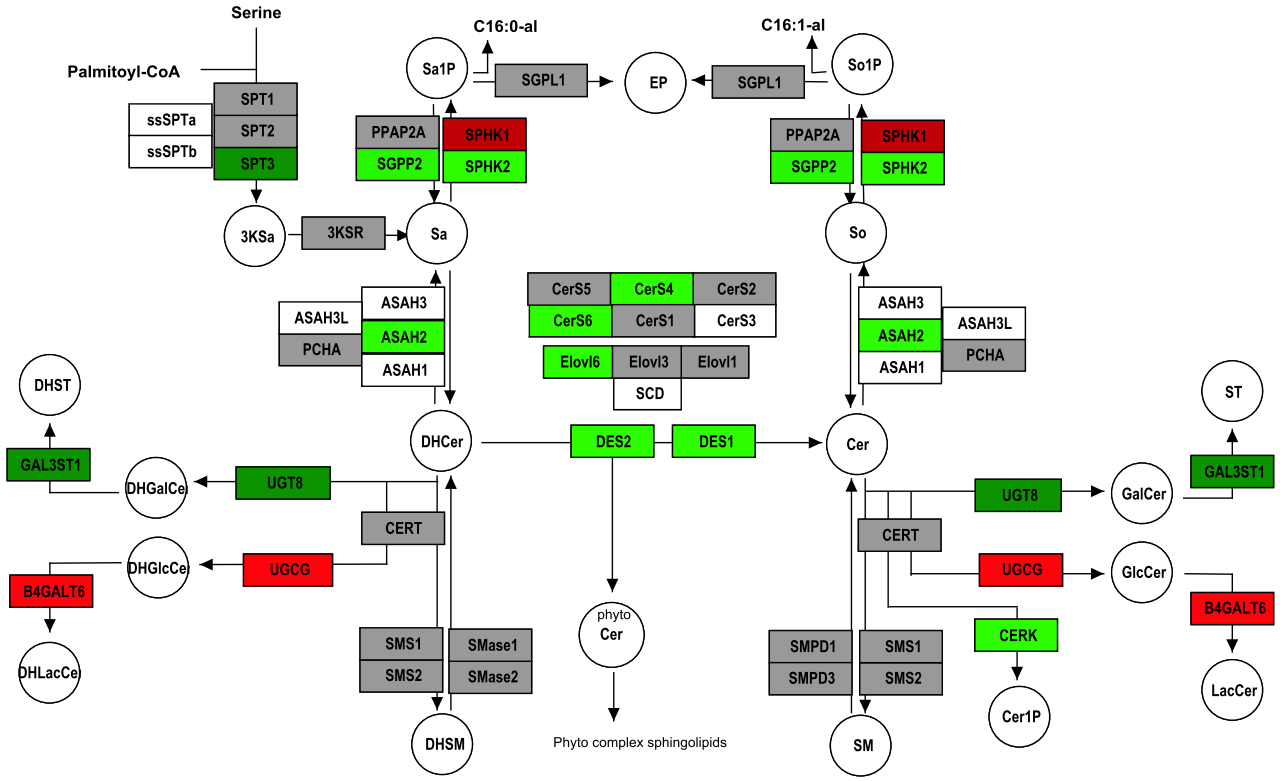
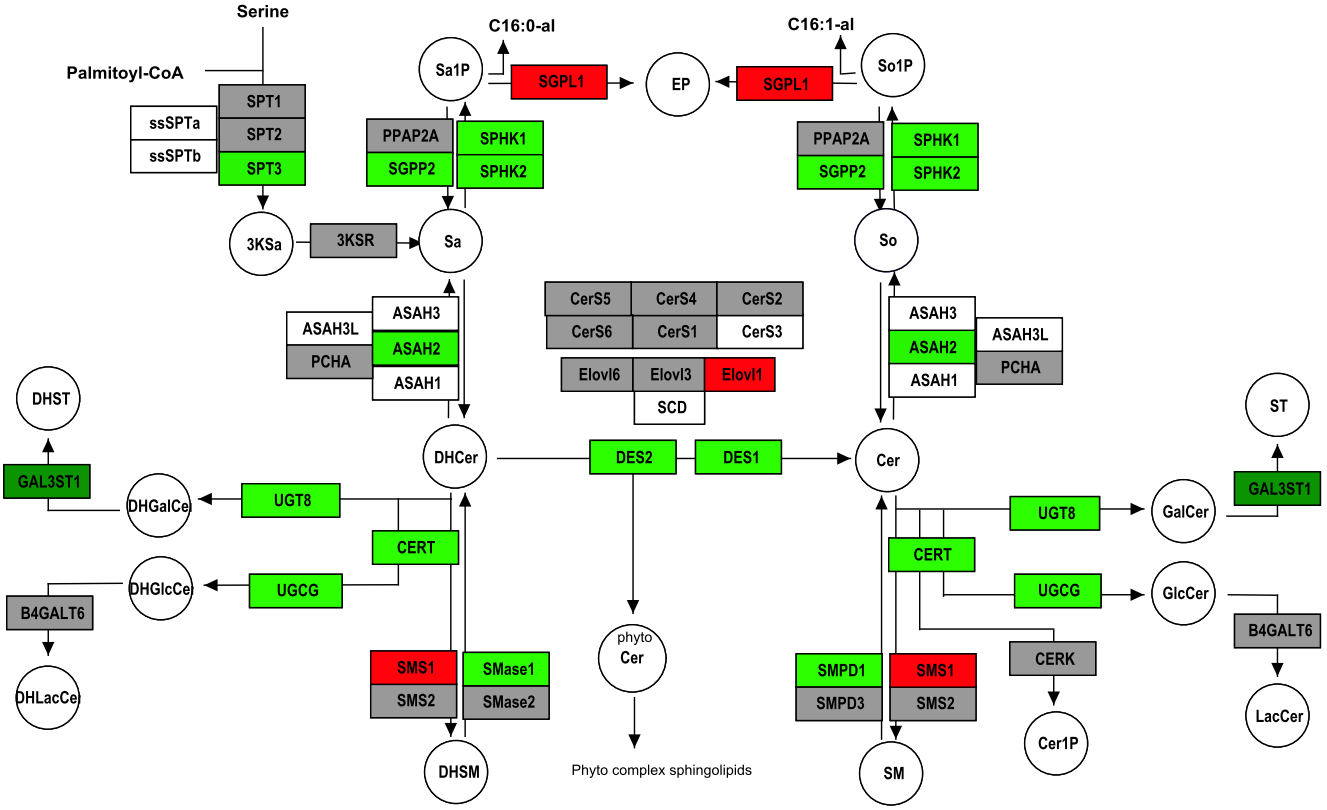


Sf 37: NCI_H226



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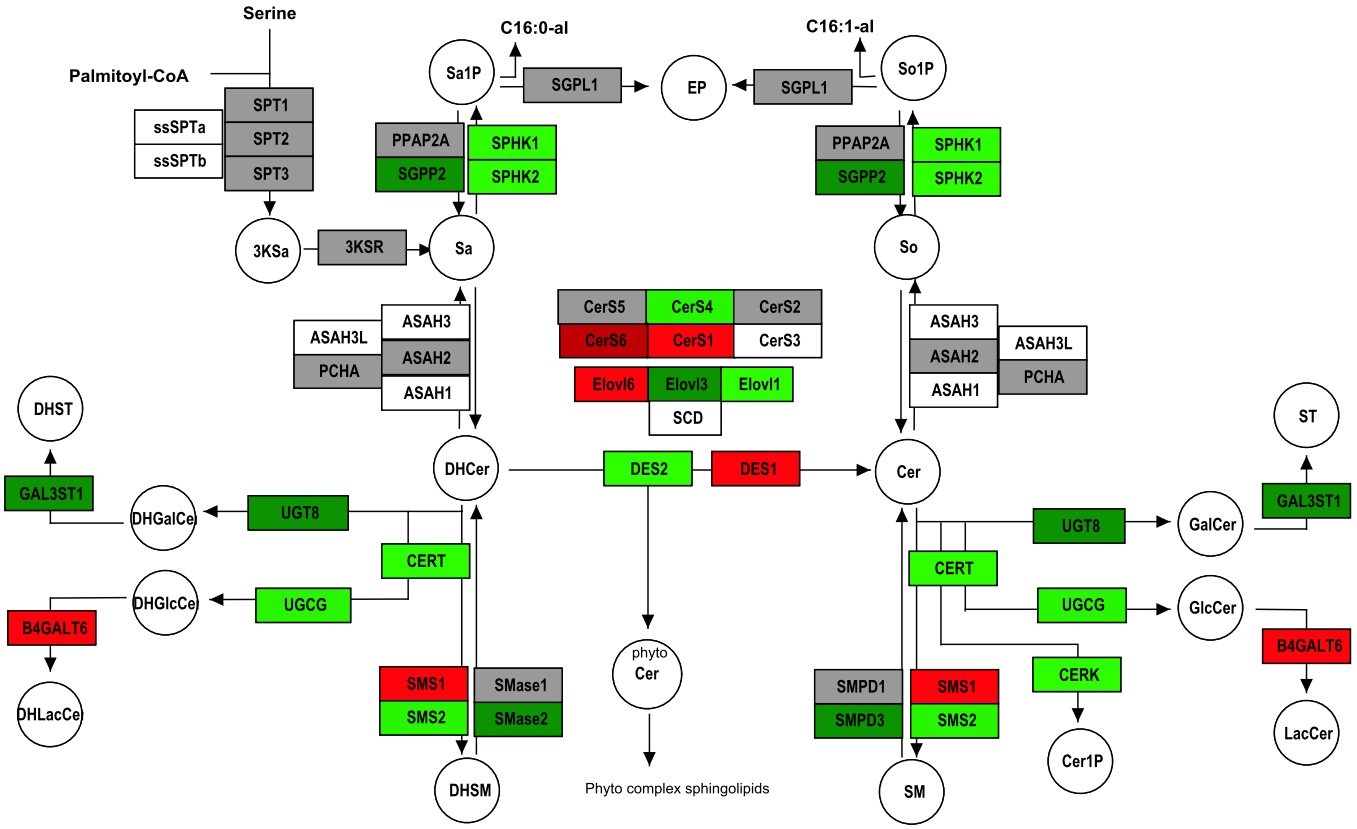

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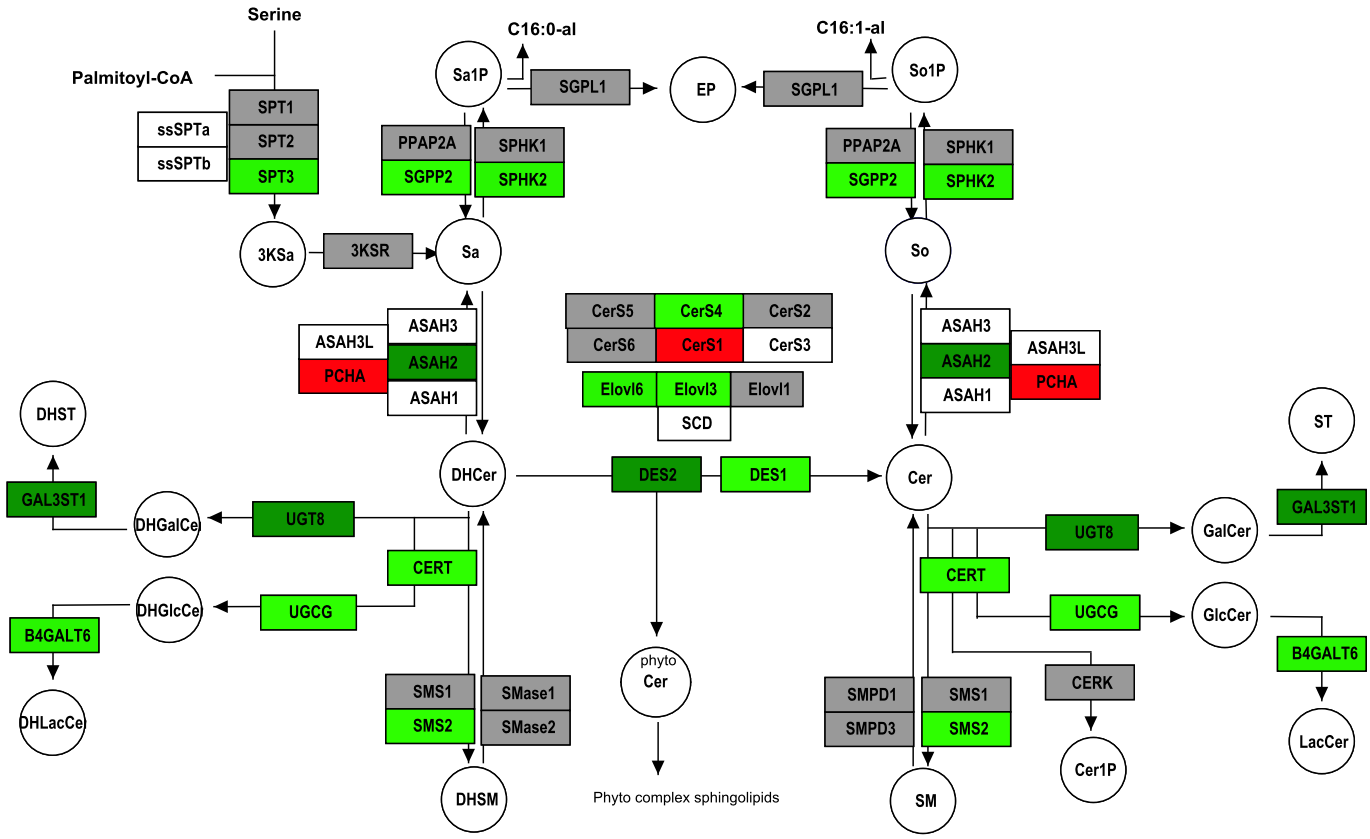

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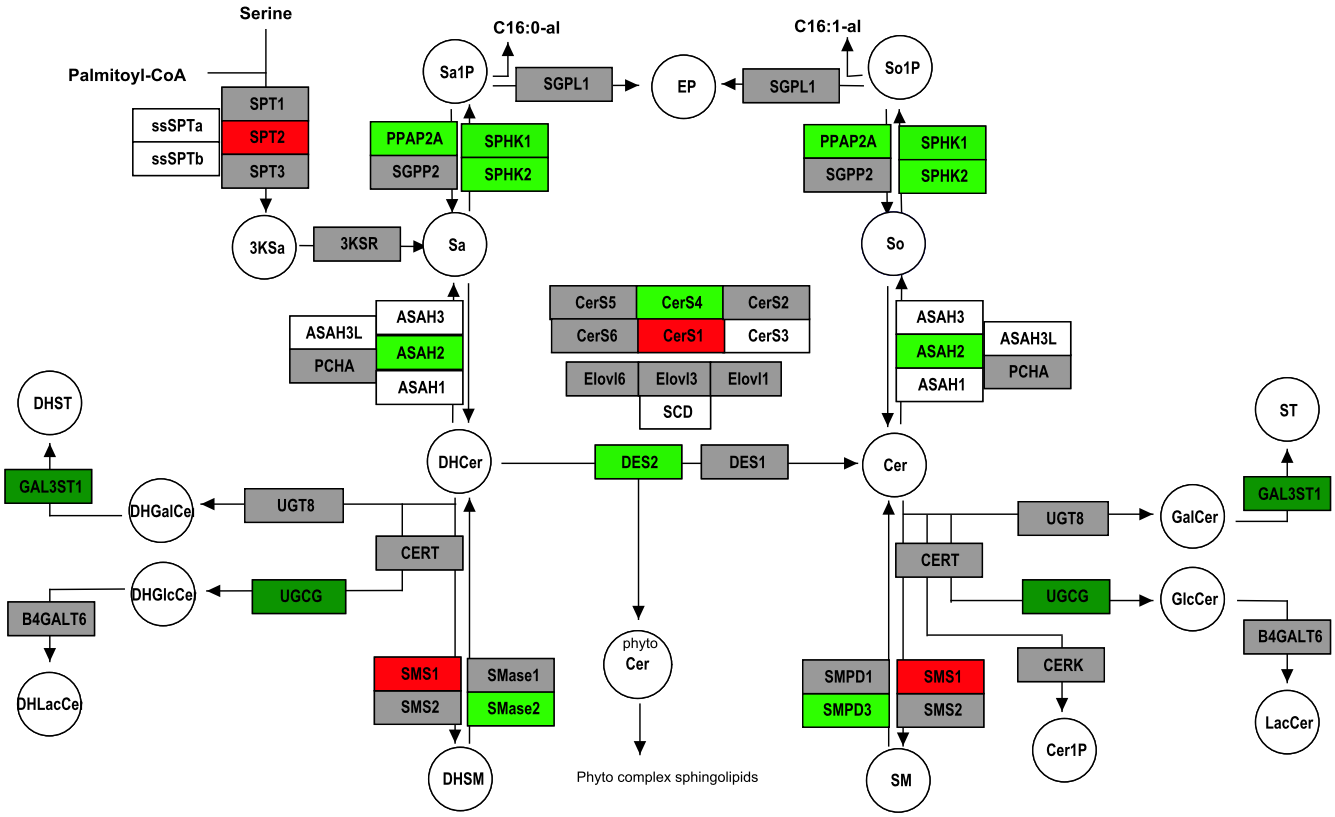
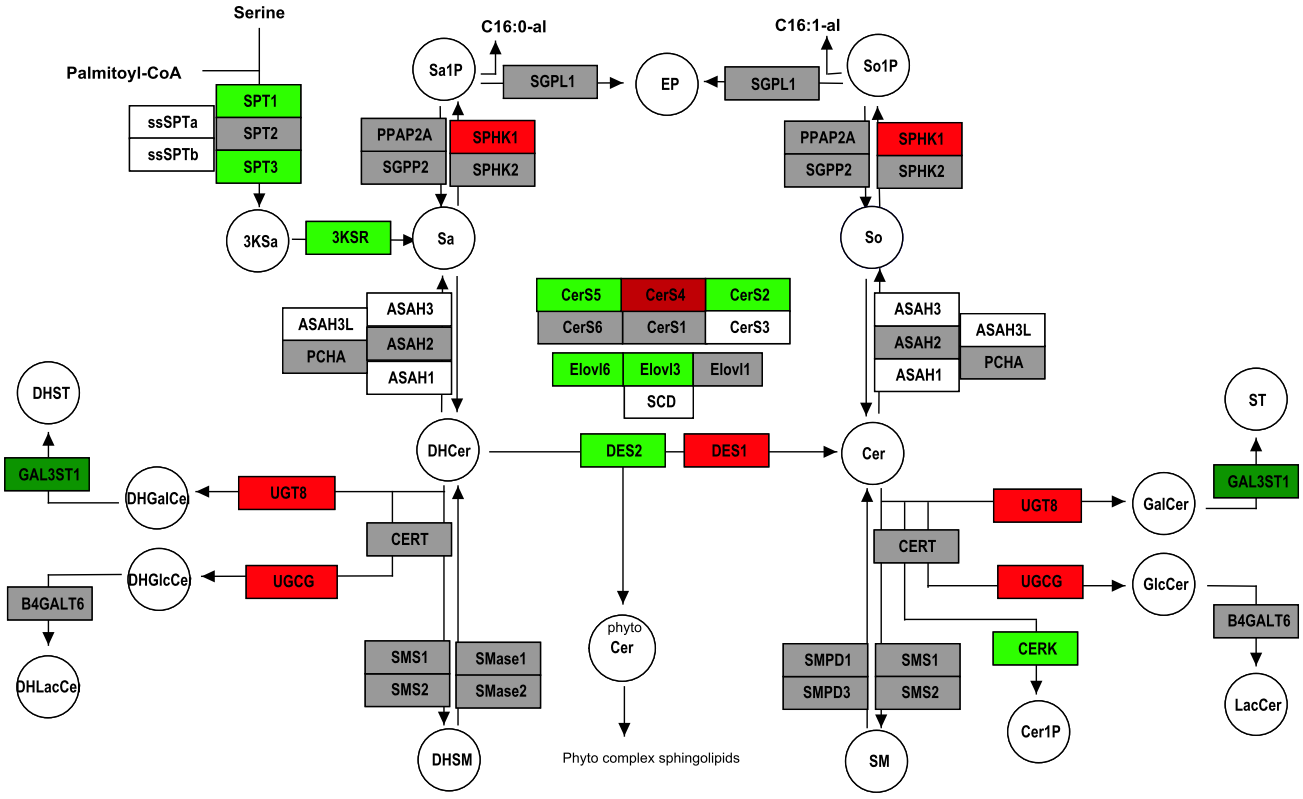


Sf 41: IGROV1



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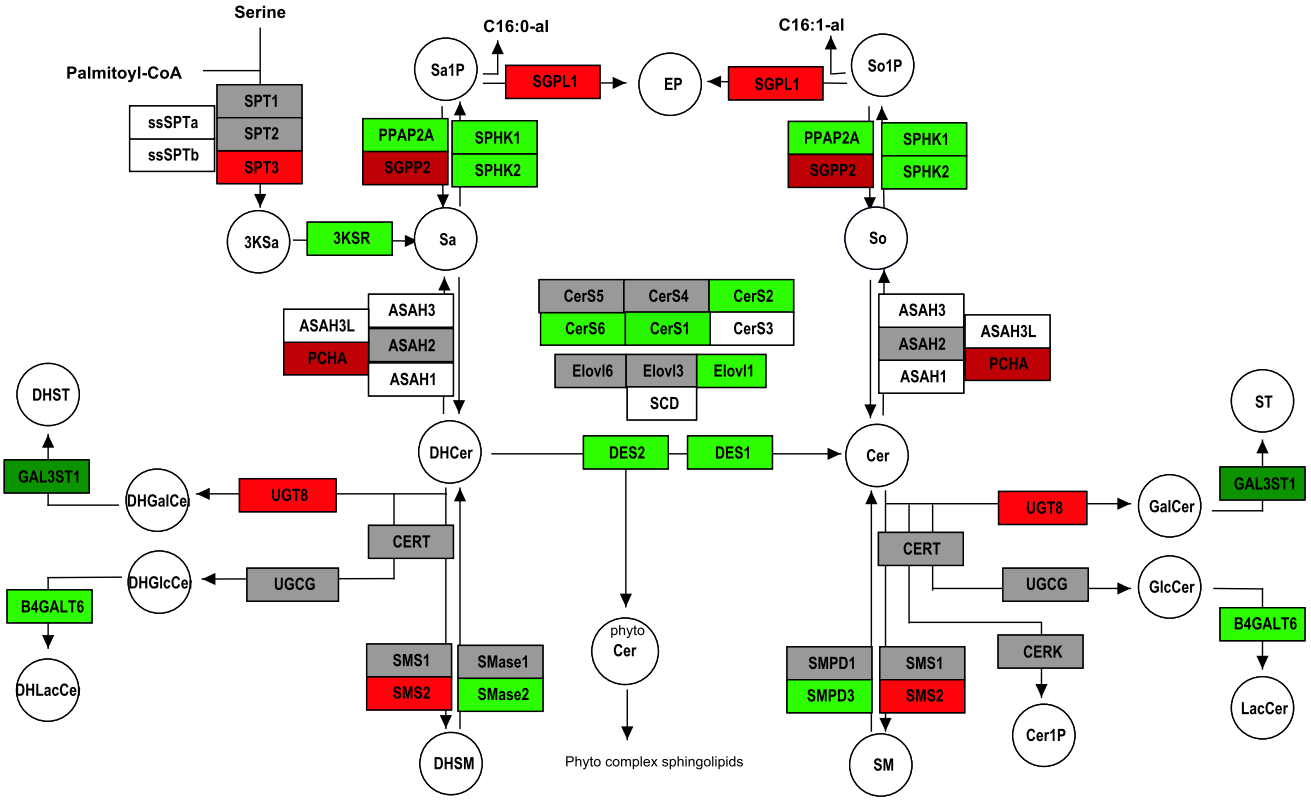

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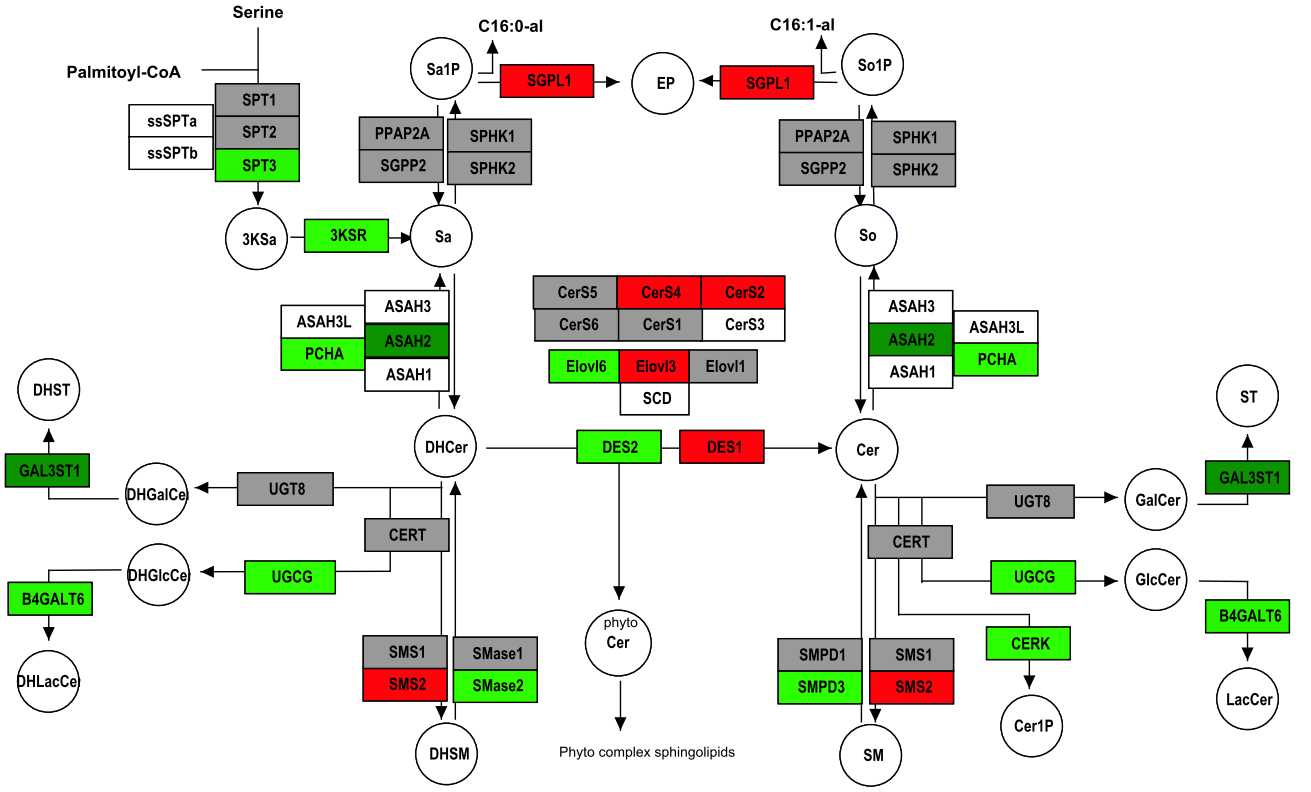

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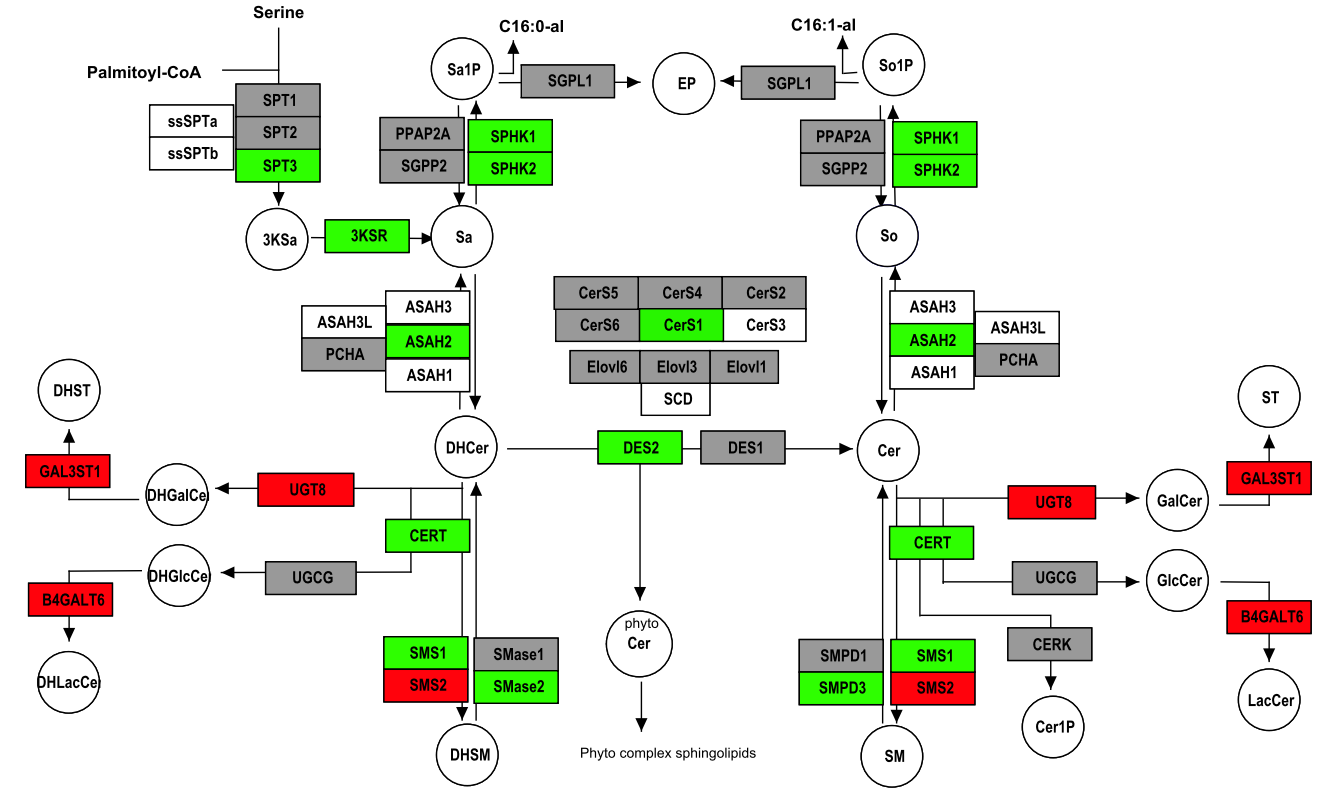

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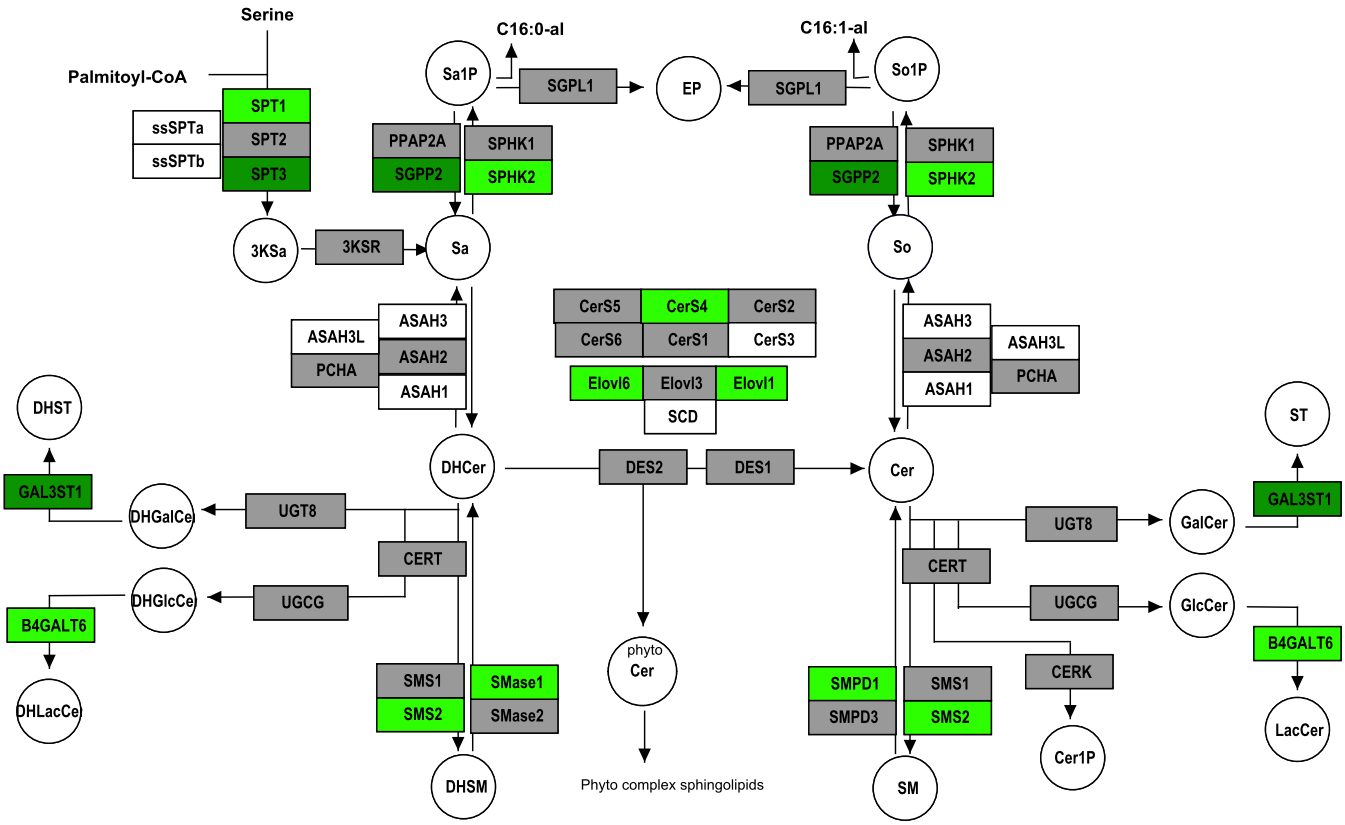

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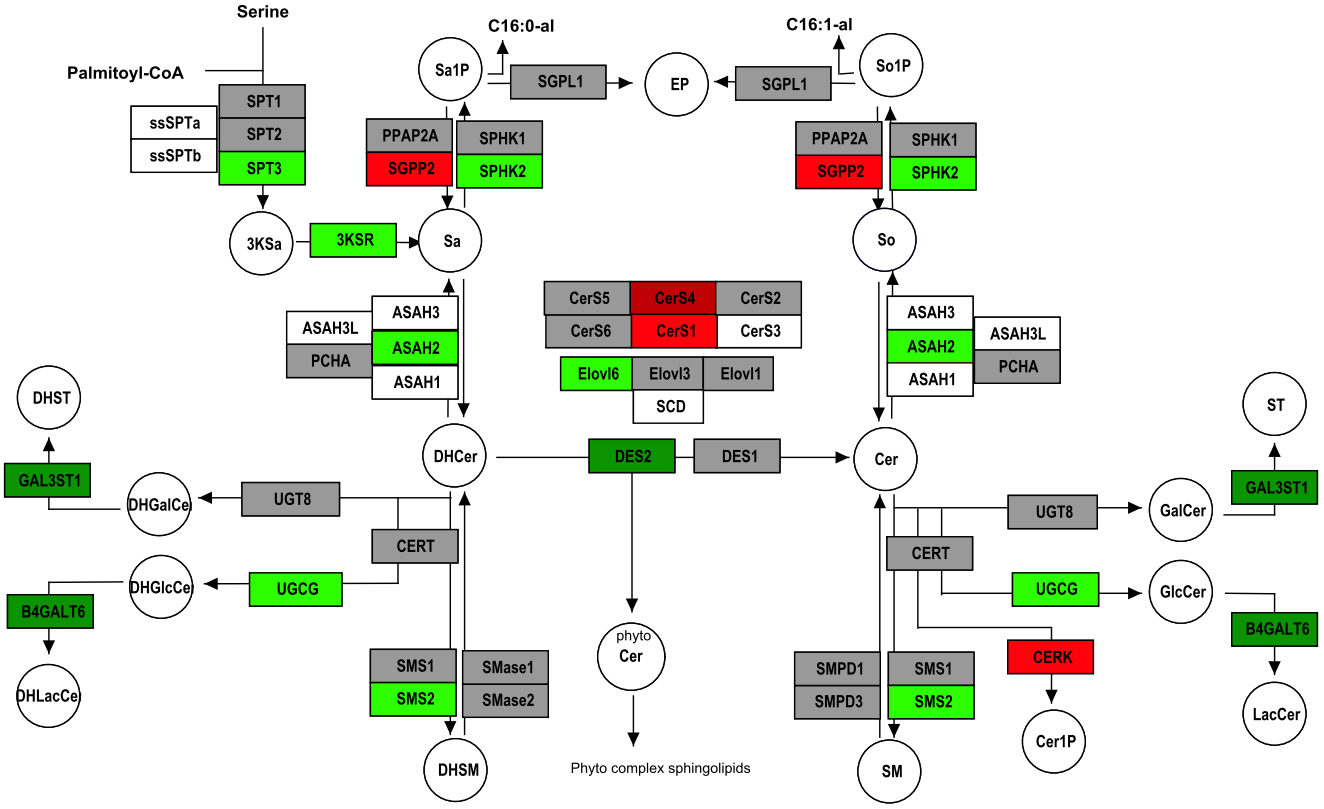
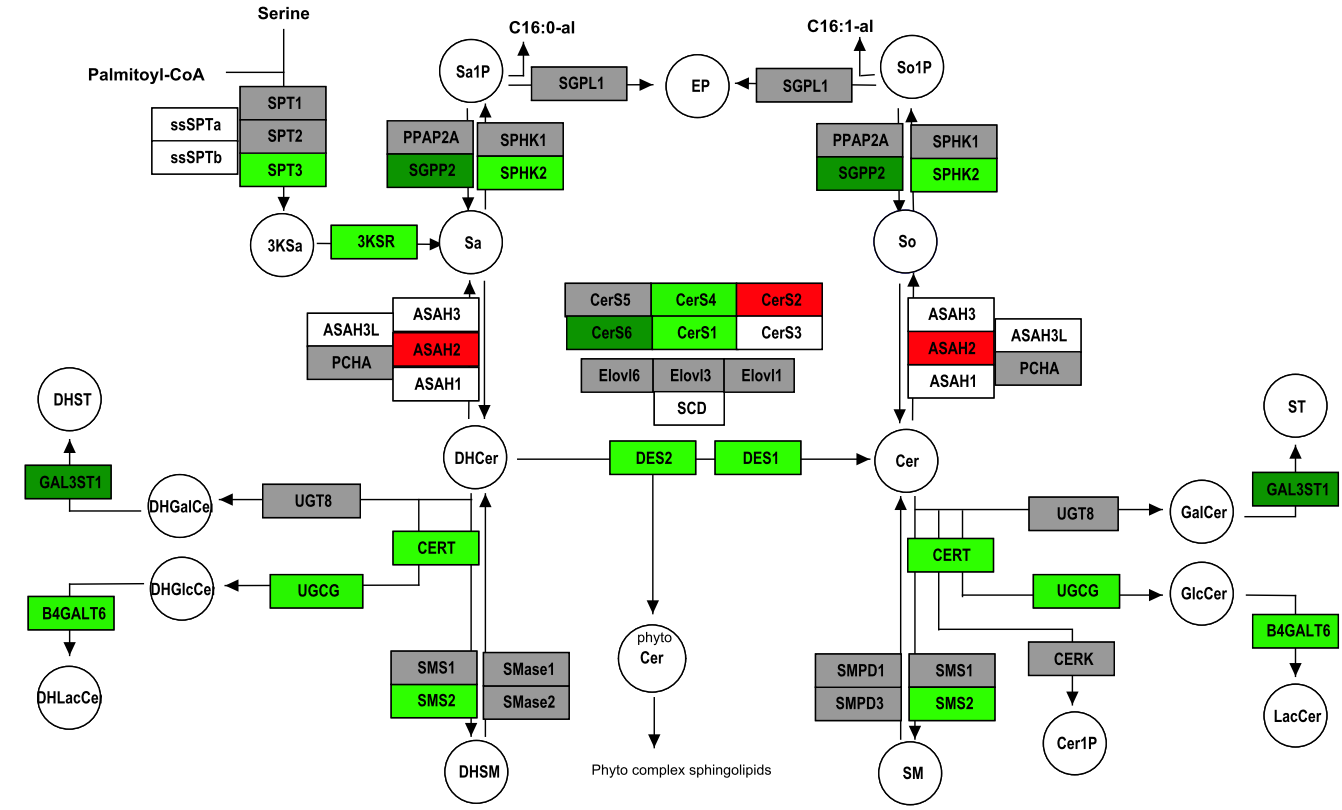
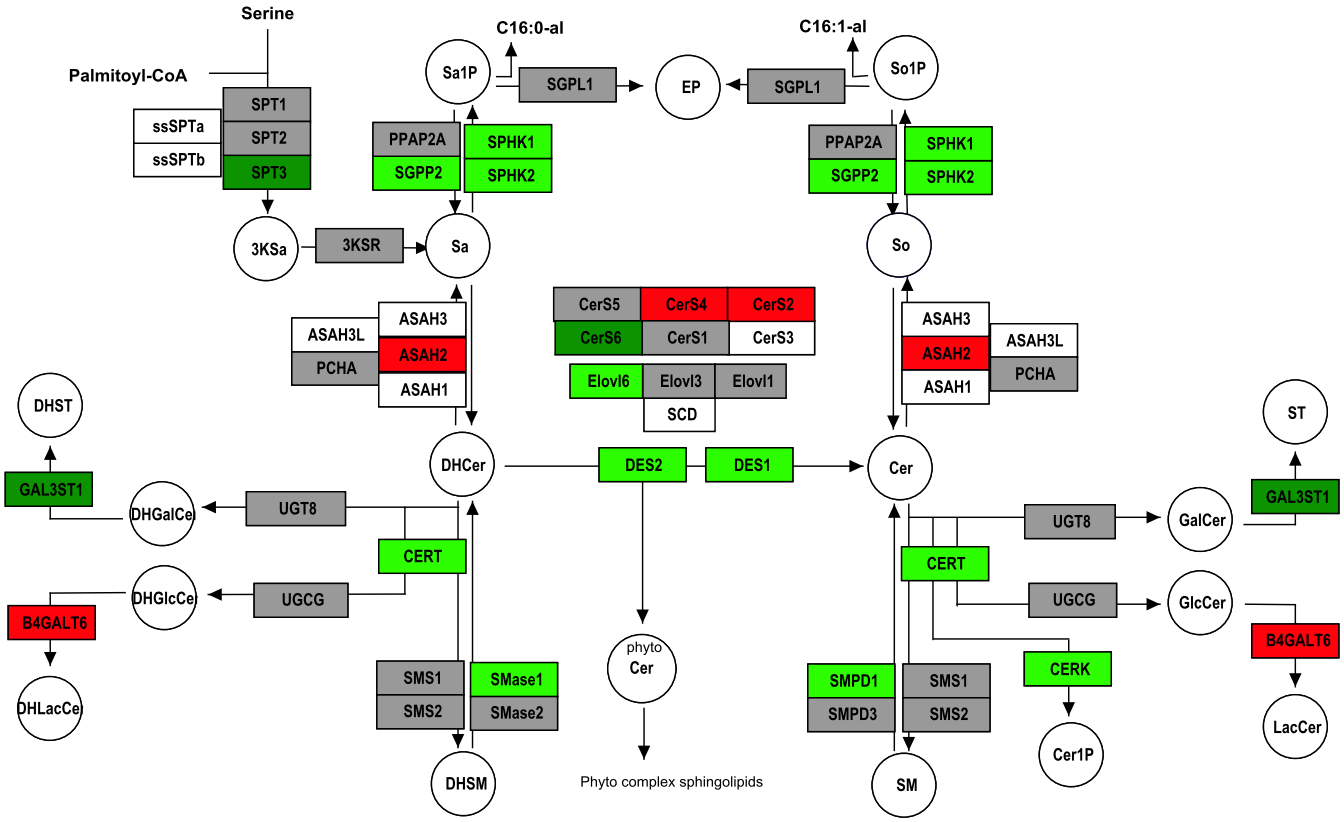


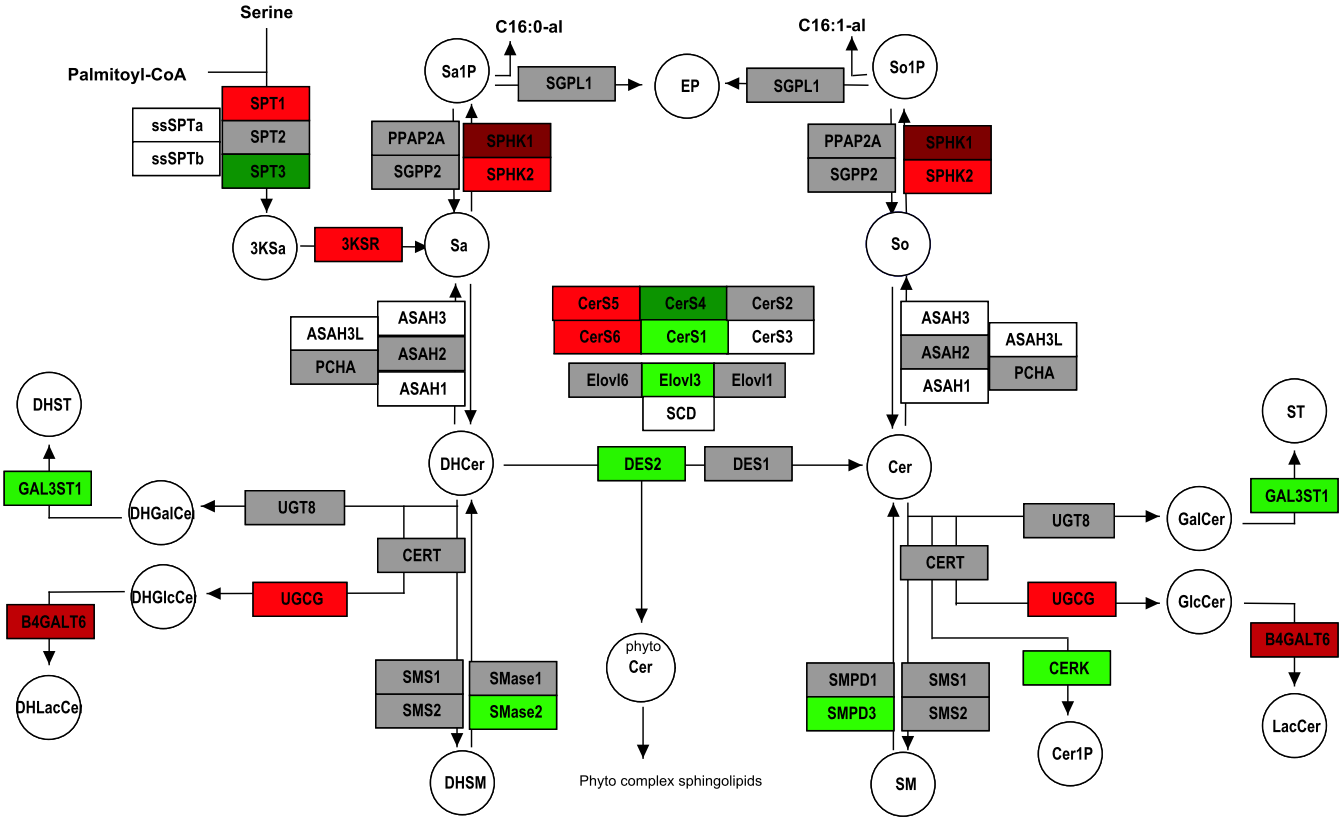
Sf 47: SK_OV_3



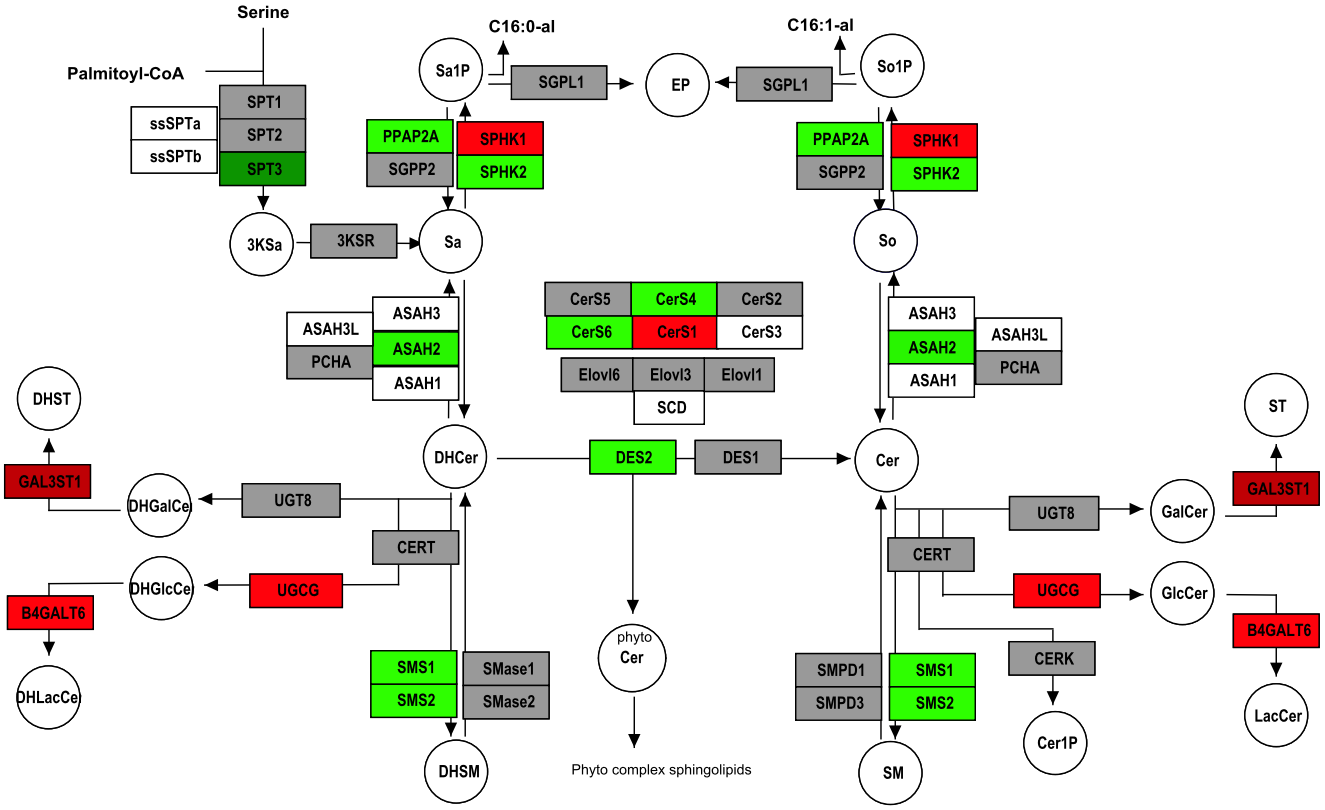
Sf 48: PC_3



Sf 49: DU_145



Sf 50: LOXIMVI



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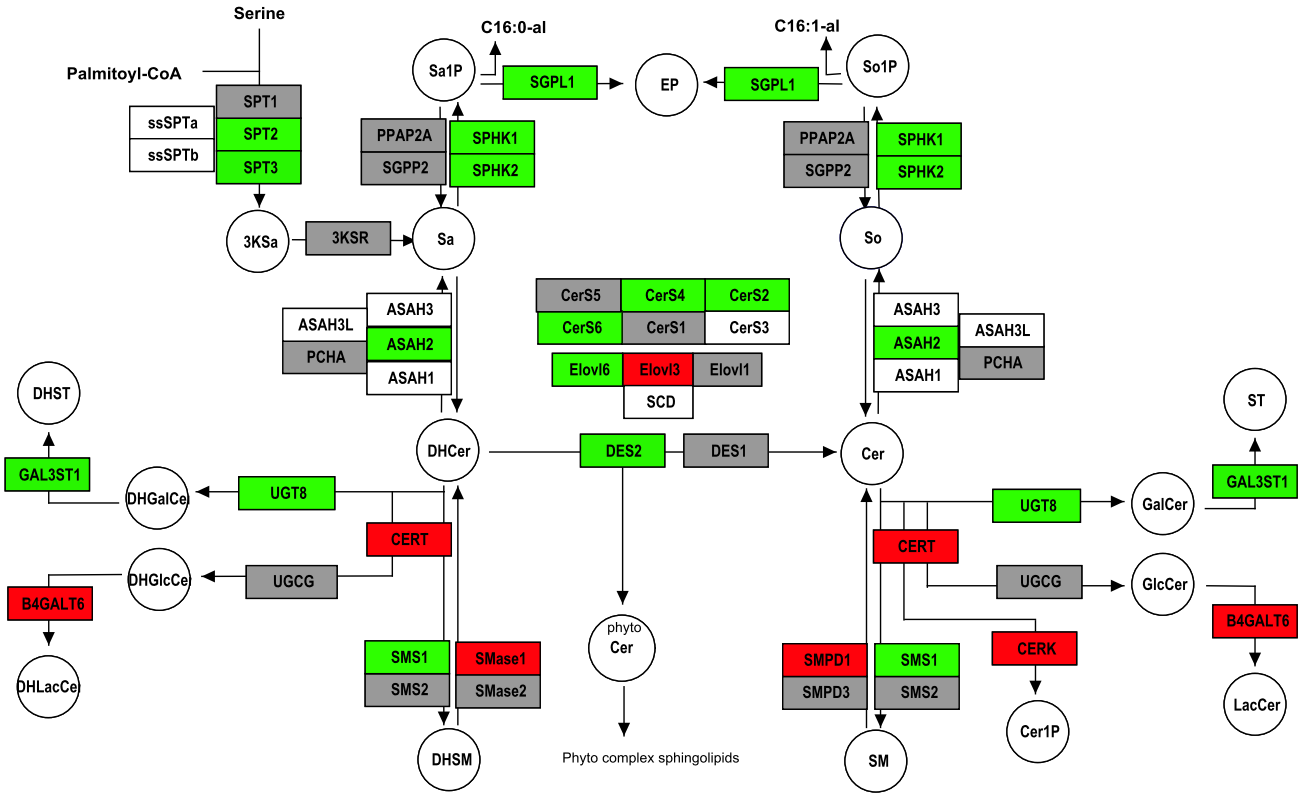
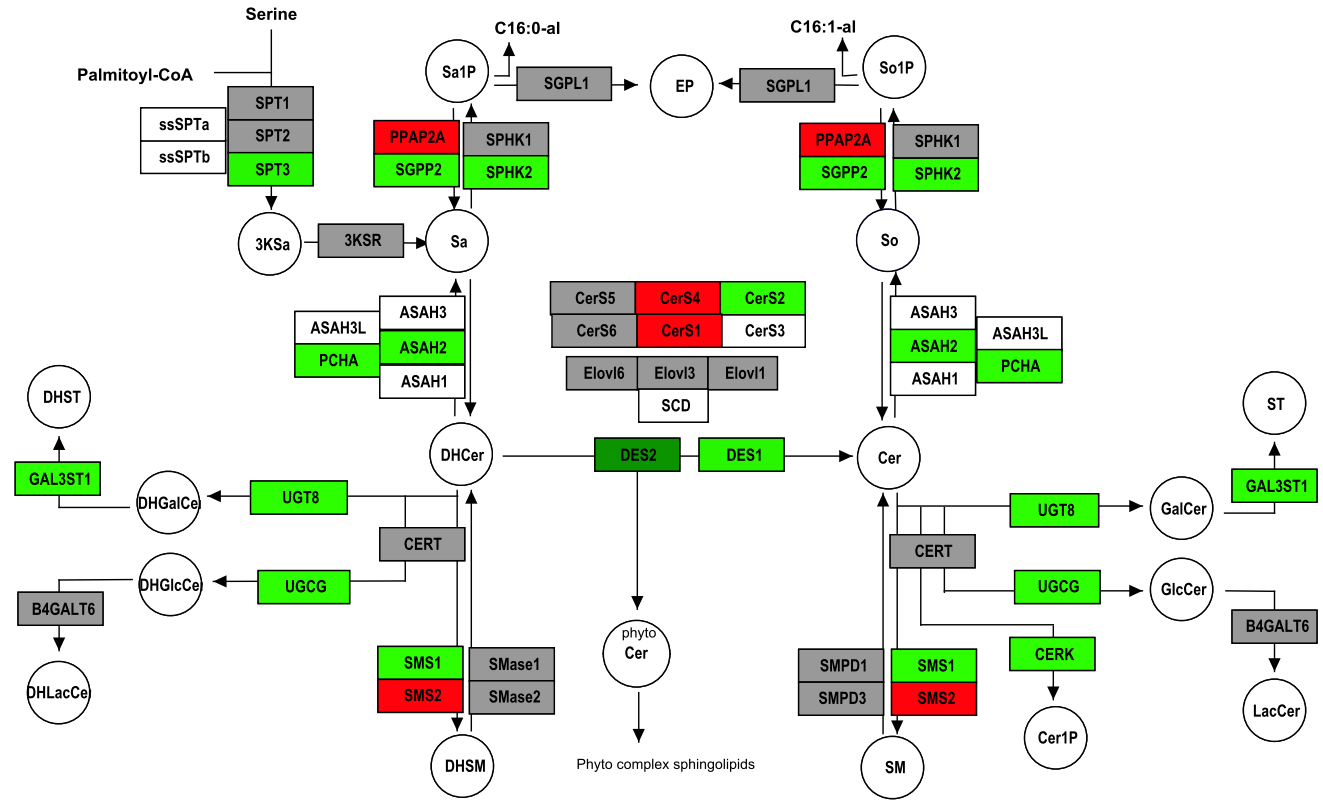


Sf 52: M14



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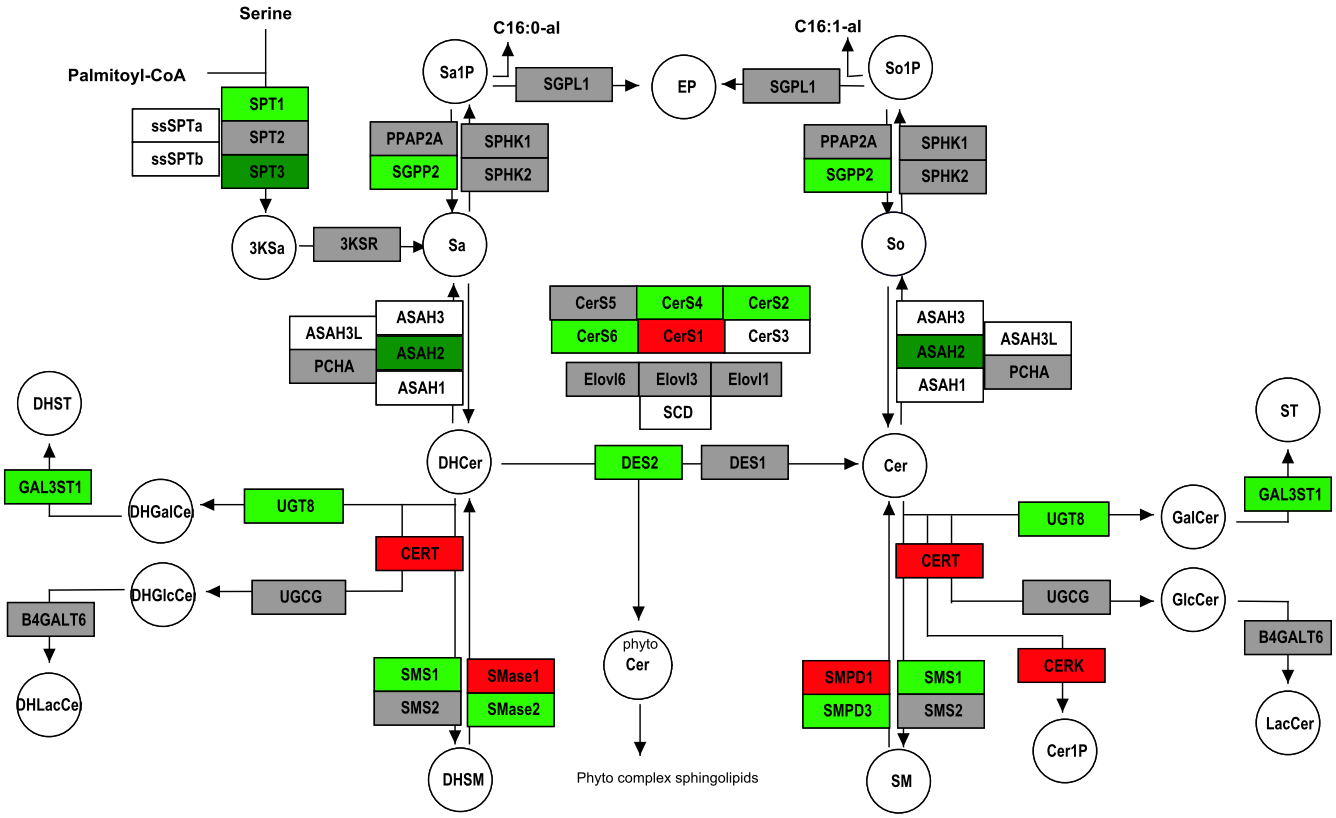
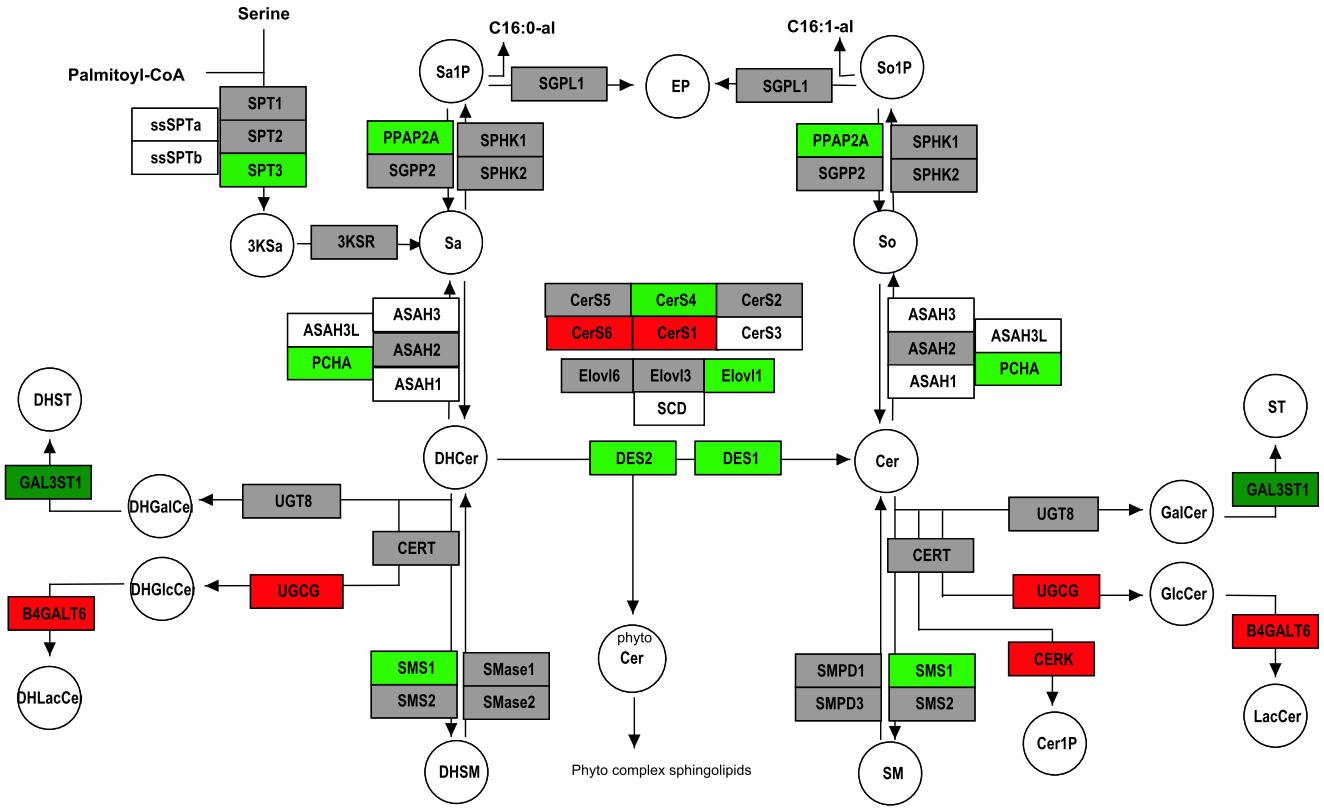
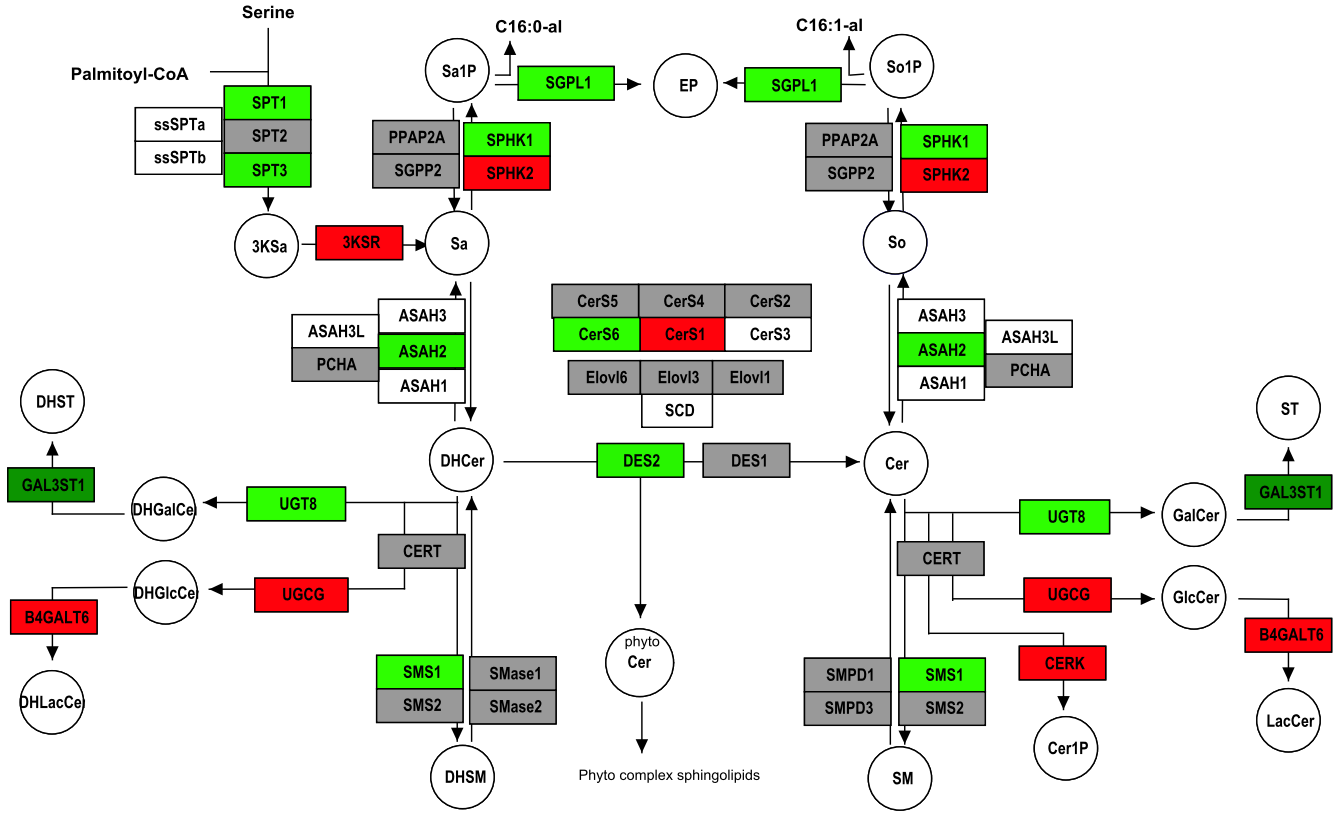


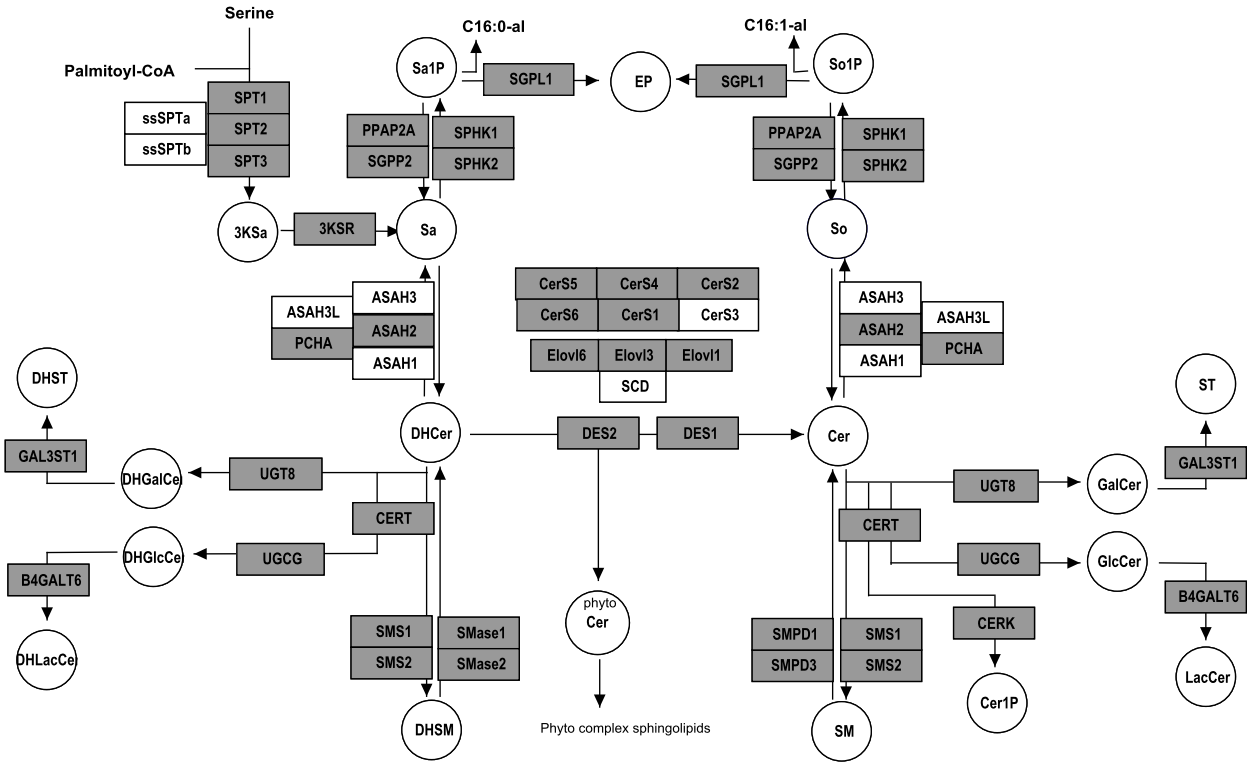
Sf 54: SK_MEL_28



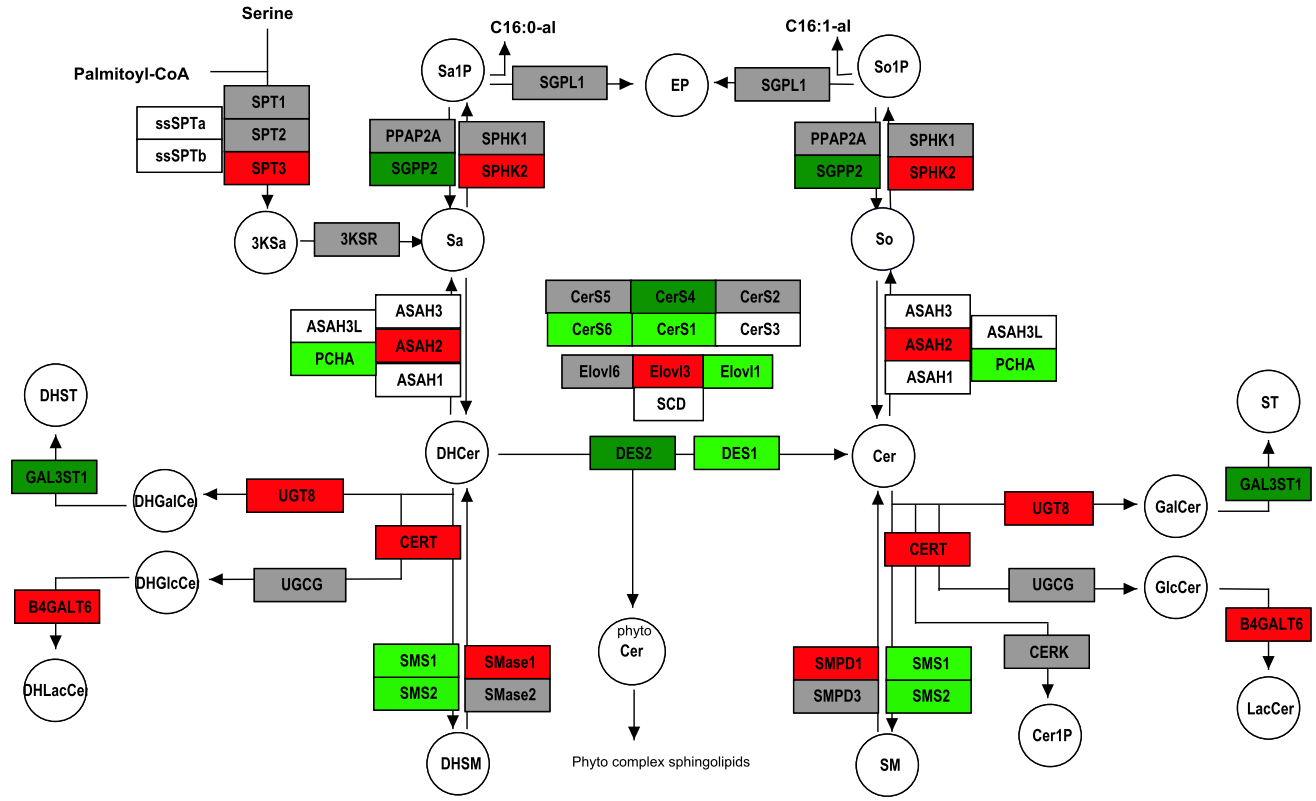
Sf 55: SK_MEL_5



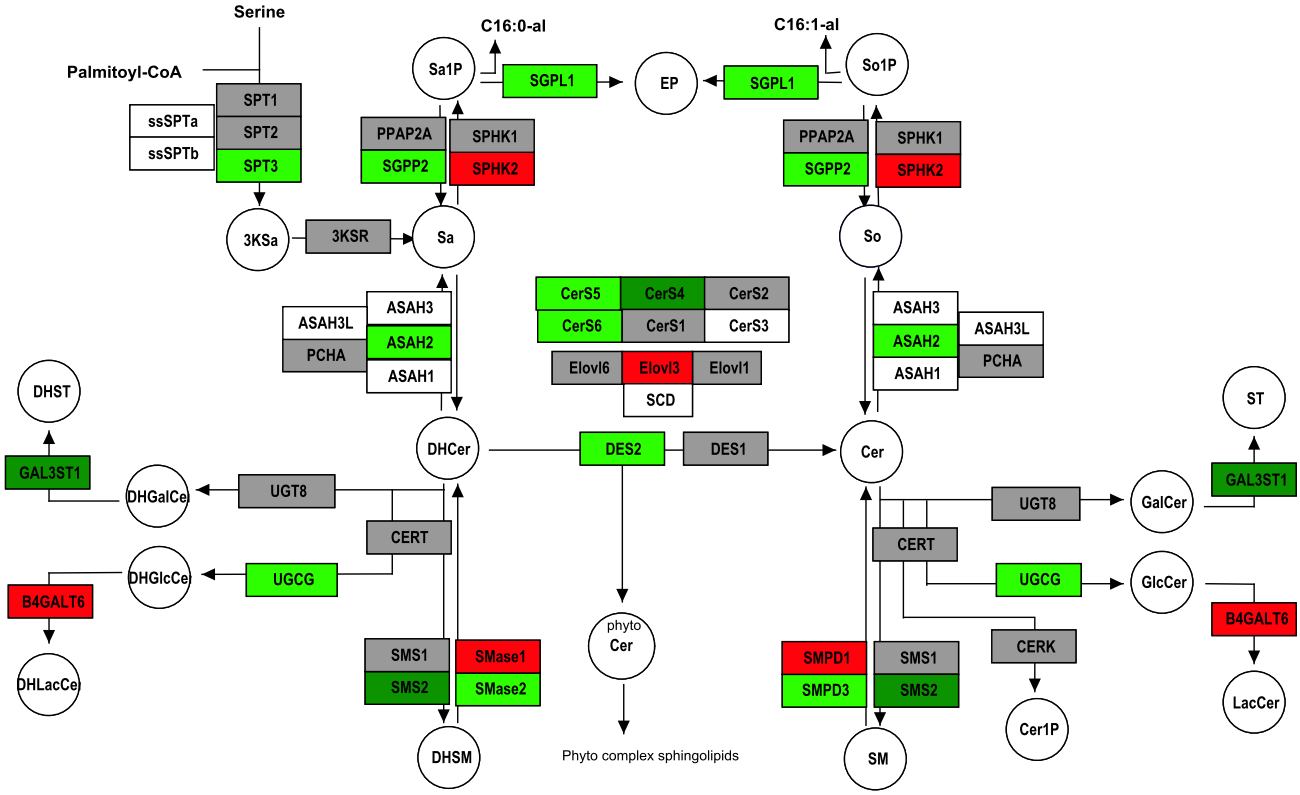
Sf 56: UACC_257



Sf 57: UACC_62



Sf 58: MDA_MB_435


Serine
Palmitoyl-CoA
SPT1
ssSPTa
SPT2
ssSPTb
SPT3
3KSa
3KSR
Sa
C16:0-al
Sa1P
SGPL1
EP
SGPL1
C16:1-al
So1P
PPAP2A
SGPP2
SPHK1
SPHK2
PPAP2A
SGPP2
SPHK1
SPHK2
So
ASAH3
ASAH3L
ASAH2
PCHA
ASAH1
CerS5
CerS4
CerS2
CerS6
CerS1
CerS3
Elovl6
Elovl3
Elovl1
SCD
ASAH3
ASAH2
ASAH3L
PCHA
ASAH1
DHST
GAL3ST1
DHGalCe
UGT8
CERT
DHGlcCe
UGCG
B4GALT6
DHLacCe
DHCer
DES2
DES1
Cer
ST
GAL3ST1
GalCer
UGT8
CERT
UGCG
GlcCer
B4GALT6
LacCer
CERK
Cer1P
phyto
Cer
SMS1
SMase1
SMS2
SMase2
SMPD1
SMPD3
SMS1
SMS2
DHSM
Phyto complex sphingolipids
SM

Sf 59: MDA_N