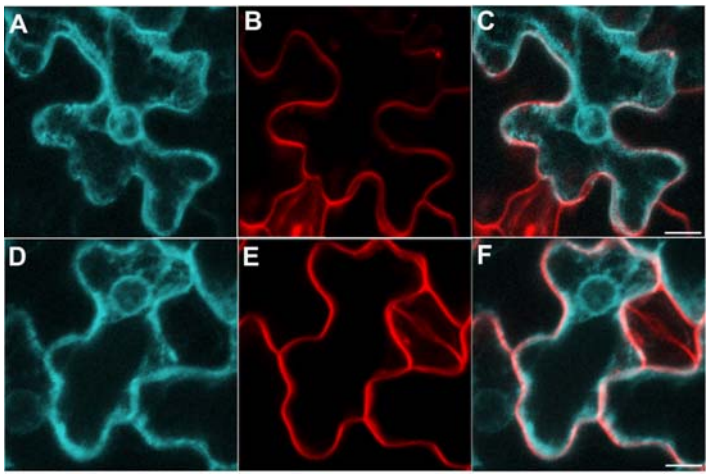
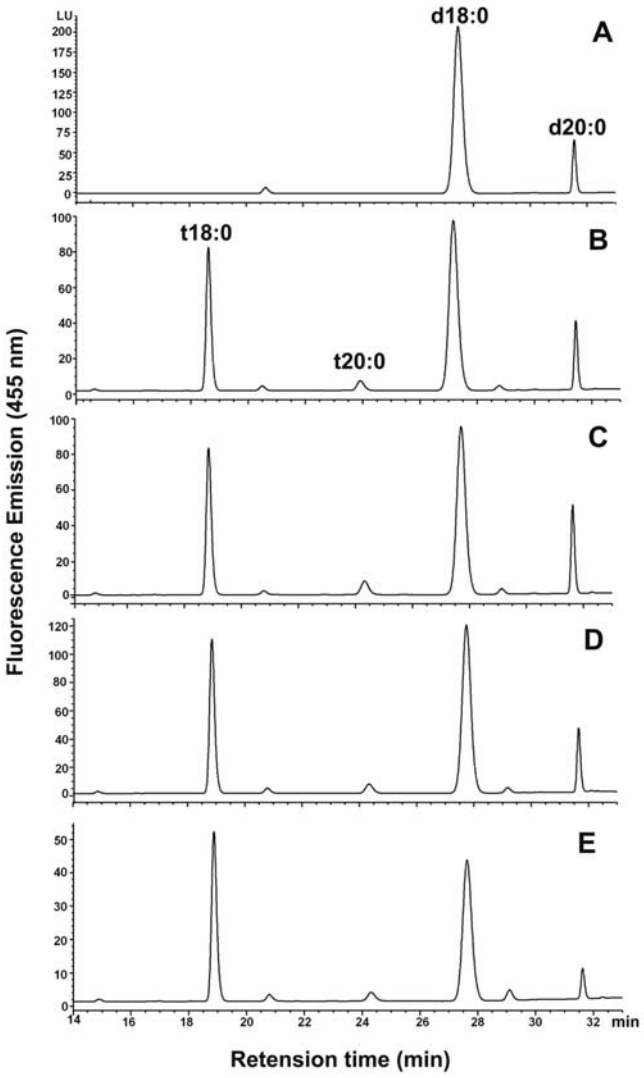
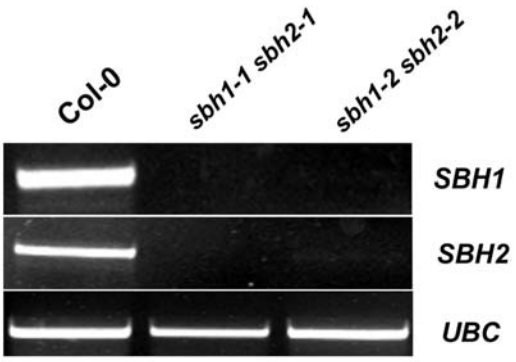


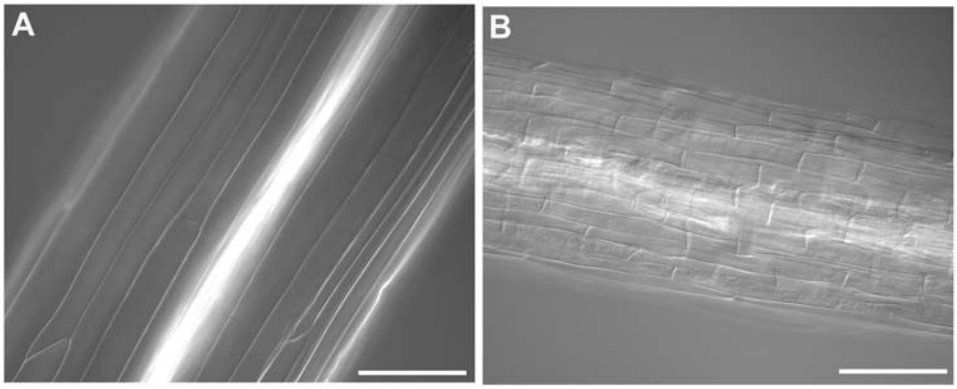
Supplemental Figure 1. Comparison of the ER localization of SBH1-CFP and SBH2-CFP versus a plasma membrane marker dye. Leaves of tobacco transiently expressing SBH1-CFP or SBH2-CFP were used for co-localization studies with the plasma membrane marker dye FM4-64. The micrographs presented here show a different localization pattern for SBH1-CFP and SBH2-CFP relative to FM4-64. All images were projection of ten×0.67 μm optical sections of either CFP or FM4-64 fluorescence through transformed leaf epidermal cells. Bar = 10 μm. (A) SBH1-CFP. (B) FM4-64 staining of the plasma membrane of the cell in (A). (C) Merge of A and B. (D) SBH2-CFP. (E) FM4-64 staining of the cell in (D). (F) Merge of D and E.



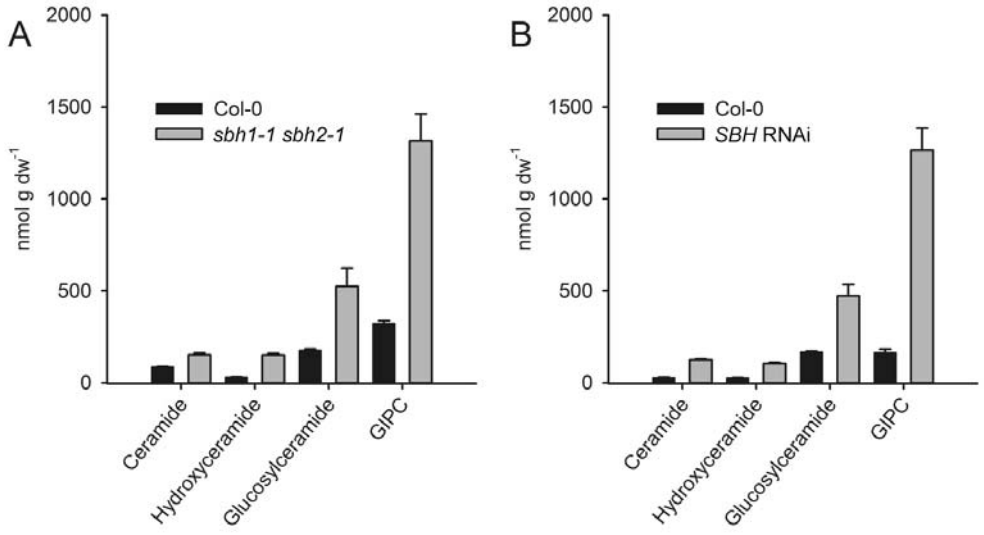
Supplemental Figure 2. LCB C-4 hydroxylase activity of fluorescent protein fusions of SBH1 and SBH2. The activity of N- or C-terminal fusions of SBH1 or SBH2 with YFP or CFP in the *S. cerevisiae sur2*Δ mutant was examined. As shown, both N- and C-terminal fusion proteins were able to restore C-4 LCB hydroxylation ability to the *sur2*Δ mutant as indicated by the production of C18- and C20-tri-hydroxy LCBs in *sur*2Δ cells upon galactose induction of fusion protein expression. (A) Yeast *sur2*Δ transformed with the empty vector pYES2. (B) Yeast *sur2*Δ expressing SBH1-CFP (C′-fusion). (C) Yeast *sur2*Δ expressing SBH2-CFP (C′-fusion). (D) Yeast *sur2*Δ expressing YFP-SBH1 (N′-fusion). (E) Yeast *sur2*Δ expressing YFP-SBH2 (N′- fusion).



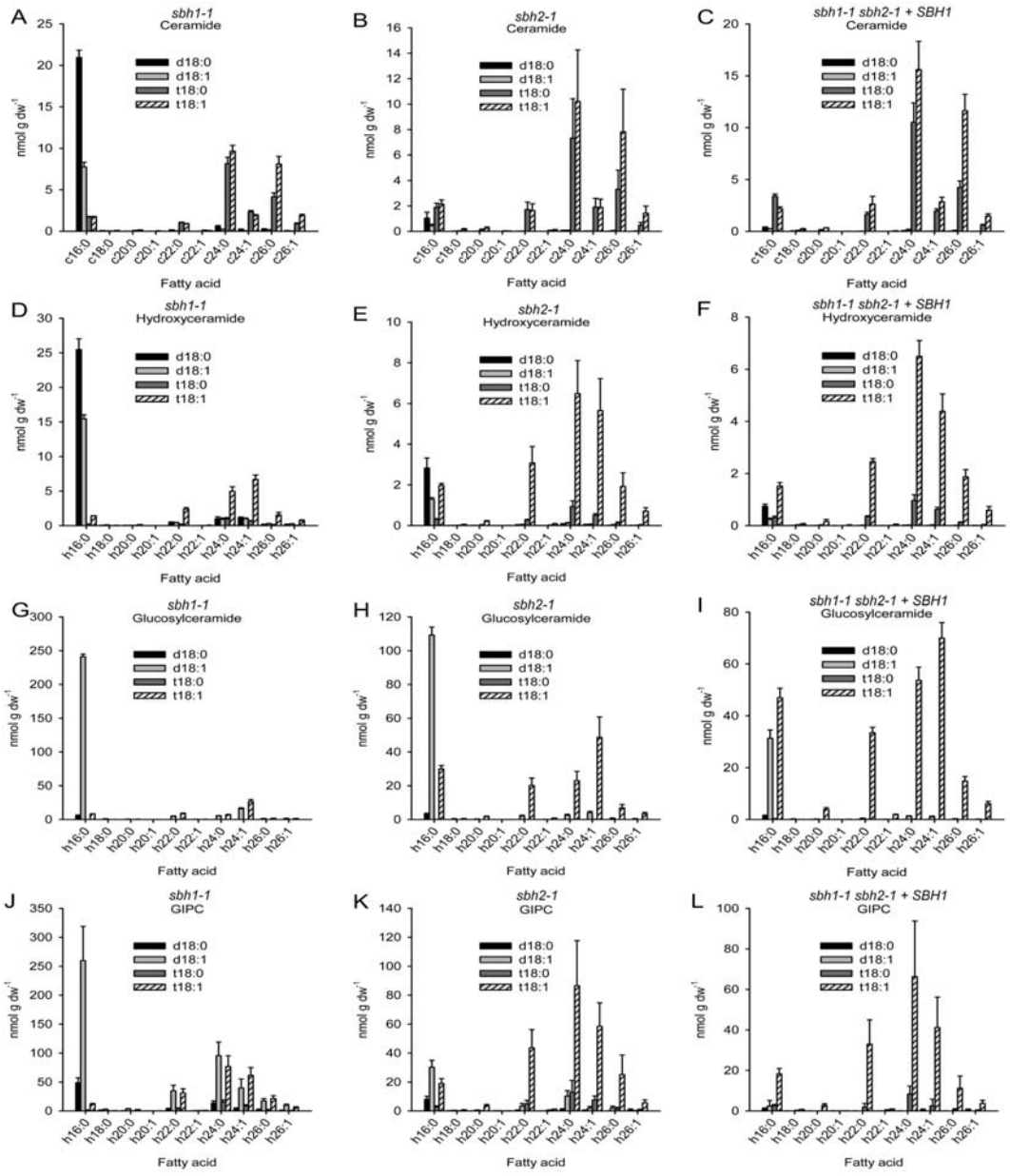
Supplemental Figure 3. Expression analysis of *SBH1* and *SBH2* in *sbh1 sbh2* double mutants. RT-PCR analyses using gene specific primers show that the T-DNA double mutants *sbh1-1 sbh2-1* and *sbh1-2 sbh2-2* are null mutants for both *SBH1* and *SBH2*. An ubiquitin-conjugating enzyme gene (UBC, At5g25760) was used as a positive control. Shown are representative results from three independent analyses.



Supplemental Figure 4. Epidermal cell length in *Arabidopsis* Col-0 hypocotyls versus that in the *sbh1-1 sbh2-1* double mutant. Epidermal cells from the middle part of hypocotyls of (A) wild-type and (B) *sbh1-1 sbh2-1* plants grown on vertical MS plates in the dark for one week. Images were taken with DIC microscopy. Bar=100 μm.



Supplemental Figure 5. Content of sphingolipid classes in *sbh1-1 sbh2-1* double mutant and RNAi suppression lines. Total amounts of each sphingolipid class detected by LC-MS/MS profiling of wild-type Col-0 control plants (*Col-0*), *sbh1-1 sbh2-1* double mutant (*sbh1-1 sbh2-1*), and a C-4 LCB hydroxylase RNAi line with approximately 20% tri-hydroxy LCBs in total sphingolipid extract (*SBH RNAi*). Panel (A) shows the sum of the data for each class shown in Figure 9, and Panel B shows the sum of the data for each class in Figure 10. Bars represent the mean (n=3) and error bars show one standard deviation.



Supplemental Figure 6. Sphingolipid composition of leaves from *sbh1-1, sbh2-1* and the *SBH1*-complemented *sbh1-1 sbh2-1* double mutant. Panels show ESI-MS/MS measurements of the sphingolipid composition and content of *sbh1-1* (A, D, G and J), *sbh2-1*, (B, E, H and K) and the complemented double mutant *sbh1-1/sbh2-1+SBH1* (C, F, I and L). Each panel shows measurements of individual molecular species for different sphingolipid classes as follows: (A-C) ceramides, (D-F) hydroxyceramides, (G-I) glucosylceramides, and (J-L) GIPCs (n=3 ± SD).

Supplemental Table 1. Oligonucleotide primer sequences used for confirmation of T-DNA insertion sites of *SBH1* and *SBH2* mutants.

Primer	**Oligonucleotide Sequence**
LBa1*	5'- TGGTTCACGTAGTGGGCCATCG-3'
LB1*	5'-GCCTTTTCAGAAATGGATAAATAGCCTTGCTTCC-3'
SALK 090881 LP†	5'-TTCTGTGATTTGGCAATAGGG-3'
SALK 090881 RP§	5'-ATCCATTTCTCCAACCTCAGG -3'
SAIL 1292E_09 LP	5'-ATTTGTCAACTCAACAACGCC-3'
SAIL 1292E_09 RP	5'-TCCAGTTCAACAAAAGAACCG-3'
SALK 032139 LP	5'- TATAACGGTCTCCCATGCAAG-3'
SALK 032139 RP	5'-TTTCTAATCATATTTTCACCATTCG-3'
SALK 047916 LP	5'-GCTGCGAGAAGTTGTATTTGG-3'
SALK 047916 RP	5'-CCTTTTGTTGTCCTGAAGCTG-3'
SALK 024105 LP	5'-ACAAGAAGCCCCAAAAGAAAG-3'
SALK 024105 RP	5'-TGAATCTCCTGCTTCTTGCTG-3'

*The LBa1 and LB1 oligonucleotides were used for screening of left T-DNA borders of SALK and SAIL mutants, respectively.

†LP, left gene-specific primer for genotyping of corresponding SALK or SAIL mutant.

§RP, right gene-specific primer for genotyping of corresponding SALK or SAIL mutant.

Supplemental Table 2. Oligonucleotide primer sequences used for RT-PCR, complementation, subcellular localizations, promoter-GUS assays, and Northern blot analyses.

Primer Name	Oligonucleotide Sequence	Restriction Site Added
P1	5'-ATGCGGCGCGCCTGGTTTAGATTTAAACCCTTGGGTTCCC-3'*	*Asc*I
P2	5'-ATGCTTAATTAAGGCTTCGCAGGTACTGTTTTATTTAGTC-3'*	*Pac*I
P3	5'-CACCATGATGATGGGTTTTGCTGTATCGGA-3'	None
P4	5'-ATCGTCTTTGAATTCTTTAGTCGGGCGAGCT-3'	None
P5	5'- TTAATCGTCTTTGAATTCTTTAGTC-3'	None
P6	5'-CACCATGATGAGTTTCGTGATTTCAGATGAATT-3'	None
P7	5'-CTCATCTTTGGATACTTTGATTGGCCGTGTTT-3'	None
P8	5'- TTACTCATCTTTGGATACTTTGATT-3'	None
P9	5'- AGATTTTGGAGTTTGGATATATGTTATGTATCTGC-3'	None
P10	5'- AGAATGGCCTCGTAGATGGATAGATTATAAAA -3'	None
P11	5'- TTTCTCTTTCTTTCTTTTGGGGCTTCTT-3'	None
P12	5'- GGTCTCAAAACCTGGAATTATAAAGCAGATAA-3'	None
P13	5'-GCGCGACTGTTTAAAGAATACAAAGAG-3'	*None*
P14	5'-TCACCAGATCTTAGAAGATTCCCTGAGT-3'	*None*
P15	5'-GCCTCGAGAAACATATCCACTCTCAACACCACC-3'*	*Xho*I
P16	5'-ATGGTACCTTCTCAAGCGAGTAAGGCATGTAA-3'	*Kpn*I
P17	5'-GCTCTAGAAAACATATCCACTCTCAACACCACC-3'	*Xba*I
P18	5'-GCAAGCTTTTCTCAAGCGAGTAAGGCATGTAA-3'	*Hind*III
P19	5'- GCAAGCTTTCTTCCGTTCTCTCTCCTTTCTCTC-3'	*Hind*III
P20	5'- ACTCTAGACCTAAATCTCAGCTTCTTCAGATCCTC-3'	*Xba*I
P21	5'-GCAAGCTTTTTGTTGTTCTCCGACTCTAGTCCC-3'	*Hind*III
P22	5'-ACTCTAGACTCTAATTCTAATCAATCTCTCCCCC-3'	*Xba*I
P23	5'- ATGCAAGCTTACCATGGCAATGATGATGGGTTTTGCTGTATCGGA-3'	*Hind*III
P24	5'- ATGCTCTAGATTACTTGTACAGCTCGTCCATGCCGAGA-3'	*Xba*I
P25	5'- ATGCAAGCTTACCATGGCAATGATGAGTTTCGTGATTTCAGATGA-3'	*Hind*III
P26	5'- ATGCAAGCTTACCATGGTGAGCAAGGGCGAGGAGCTGT-3'	*Hind*III
P27	5'- ATGCTCTAGATTAATCGTCTTTGAATTCTTTAGTC-3'	*Xba*I
P28	5'- ATGCTCTAGATTACTCATCTTTGGATACTTTGATT-3'	*Xba*I

*The underlined sequences correspond to introduced restriction enzyme sites.

Supplemental Table 3. Oligonucleotides and numbers of PCR cycles used for expression analysis of cell death and HR related genes.

Oligonucleotide	Oligonucleotide Sequence	# of Cycles
PR-2(At3g57260)5'	5'-AGCCTCACCACCAATGTTGATGAT-3'	35 cycles
PR-2(At3g57260)3'	5'-GTTCTCGATGTTCTGCATTGCTTGT-3'	
PRXc(At3g49120)5'	5'-CAACATCGTCCACTTGGACAATCTT-3'	30 cycles
PRXc(At3g49120)3'	5'-CCTGCCAAAGTGACAGATTGTTGAG-3'	
SAG13(At2g29350)5'	5'-GAAACTCAGCTTCAAGAACGCTTACGTG-3'	30 cycles
SAG13(At2g29350)3'	5'-TCGCCCATTCGCAAGCTAAGTTT-3'	
FMO(At1g19250)5'	5'-CGTATTCGAAGCCTCGGATTCAGTC-3'	35 cycles
FMO(At1g19250)3'	5'-GGTATTCTTGGAACGTCGCCGTATT-3'	
SAG12(At5g45890)5'	5'-TTGACTGGAGGAAGAAAGGAGCTGT-3'	35 cycles
SAG12(At5g45890)3'	5'-CTTCAATTCCAACGCTAACCGGT-3'	
PR-3(At3g12500)5'	5'-AACGGTCTATGCTGCAGCGAGTT-3'	30 cycles
PR-3(At3g12500)3'	5'-GCGCTCGGTTCACAGTAGTCTGA-3'	
ERD11(At1g02930)5'	5'-ATGGCAGGAATCAAAGTTTTCGG-3'	25 cycles
ERD11(At1g02930)3'	5'-CCTCTTCTTCTTCAACAACGGTTTTG-3'	
UBC(At5g25760)5'	5'-ATGCAGGCATCAAGAGCGCGACTGT-3'	30 cycles
UBC(At5g25760)3'	5'-CACCGCCTTCGTAAGGAGTCTCCGA-3'	