

Supplemental Data

A 2-oxoglutarate dependent dioxygenase converts dihydrofuran to furan in *Salvia* diterpenoids

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Supplemental Figure S1. Aerial parts and roots of *S. miltiorrhiza* and *S. substolonifera*.

Supplemental Figure S2. UPLC profiles of enzyme activity of SmT II AS *in vitro*.

Supplemental Figure S3. Effect of temperature and pH on enzyme activities of SmT II AS using CT as substrate.

Supplemental Figure S4. Characterization of SmT II AS activities by *in vitro* assay.

Supplemental Figure S5. The metabolites altered in accumulation in SmT II AS-RANi lines.

Supplemental Figure S6. The relative content of tanshinone II A in *S. miltiorrhiza*, *S. meiliensis*, *S. bowleyana* and *S. trijuga*, respectively.

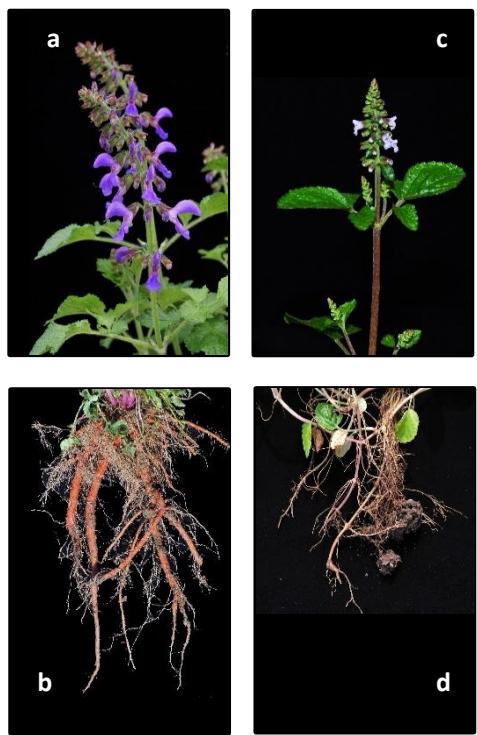
Supplemental Figure S7. Extended phylogenetic analysis of T II ASs and Sm2-ODDs with other experimentally characterized 2-ODDs.

Supplemental Table S1 The annotation ratio statistics of *S. miltiorrhiza* and *S. substolonifera* transcriptome database.

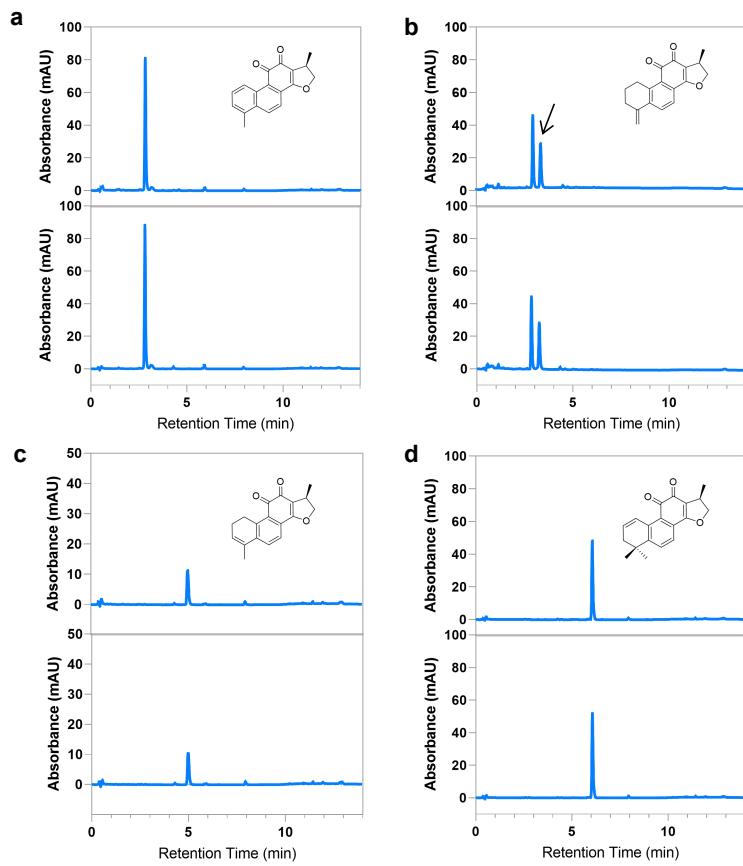
Supplemental Table S2 The sequences of 18 *Sm2-ODD* candidate genes and the homologs from Xu and Song (2017).

Supplemental Table S3 Protein sequences information for the phylogenetic tree.

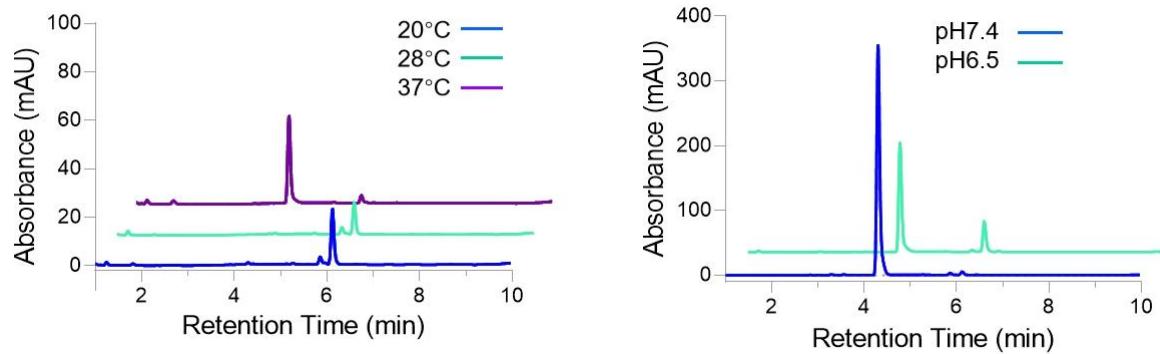
Supplemental Table S4 List of oligonucleotide primer sequences.



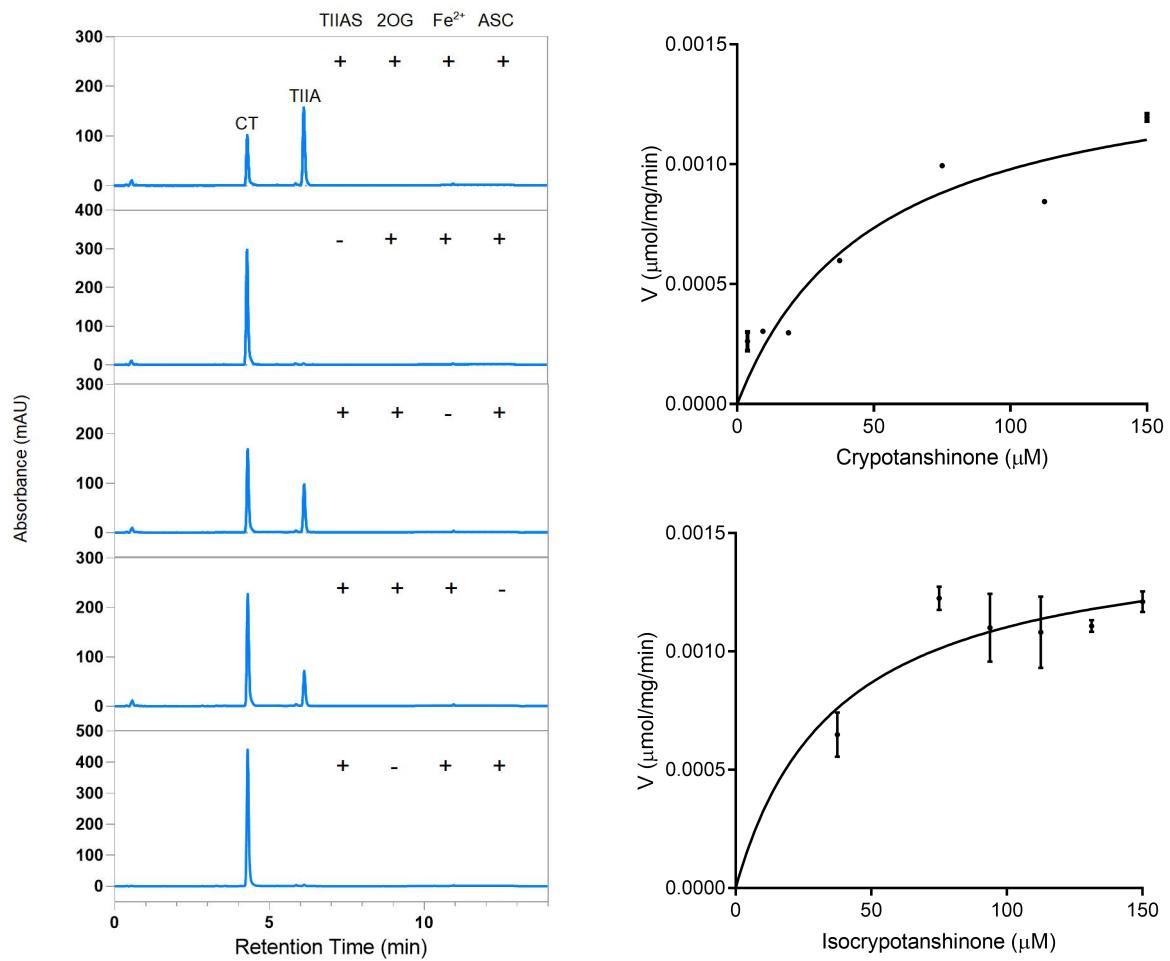
Supplemental Figure S1. Aerial parts and roots of *S. miltiorrhiza* and *S. substolonifera*. (a) *S. miltiorrhiza* aerial part. (b) *S. miltiorrhiza* root. (c) *S. substolonifera* aerial part. (d) *S. substolonifera* root.



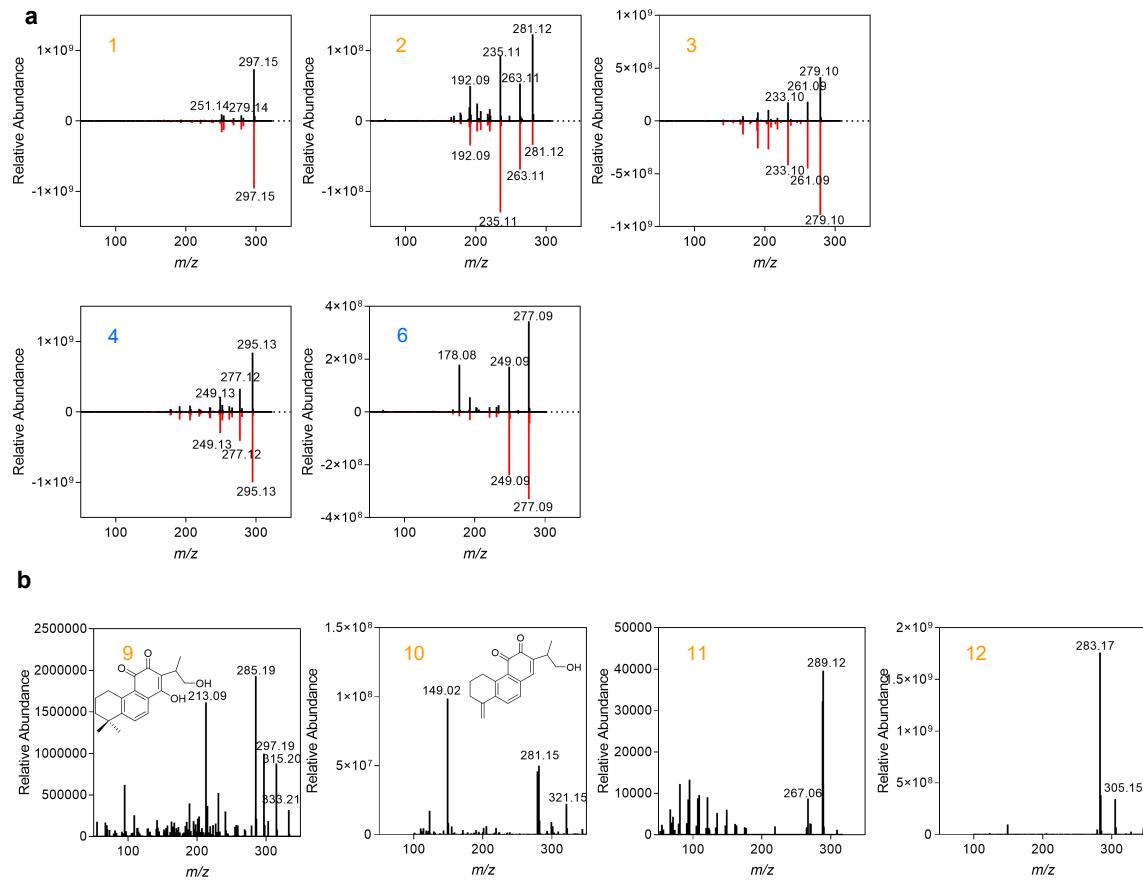
Supplemental Figure S2 UPLC profiles of enzyme activity of SmTIIAS *in vitro*. The assays were performed using 15,16-dihydrotanshinone I (a), methylenedihydrotanshinquinone (b), tetrahydrotanshinone I (c) and 1,2-dihydrocryptotanshinon (d) as substrates, respectively.



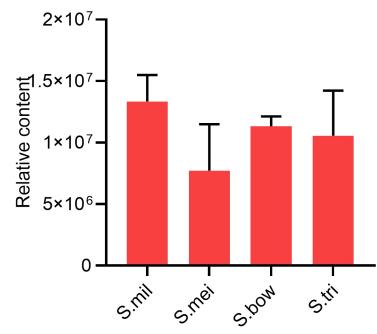
Supplemental Figure S3 Effect of temperature and pH on enzyme activities of SmTIIAS using CT as substrate. (a) The assays were carried out on different temperature of 20°C, 28°C and 37°C, respectively. (b) The reactions under the pH of 6.5 and 7.4 were compared with the substrate cryptotanshinone.



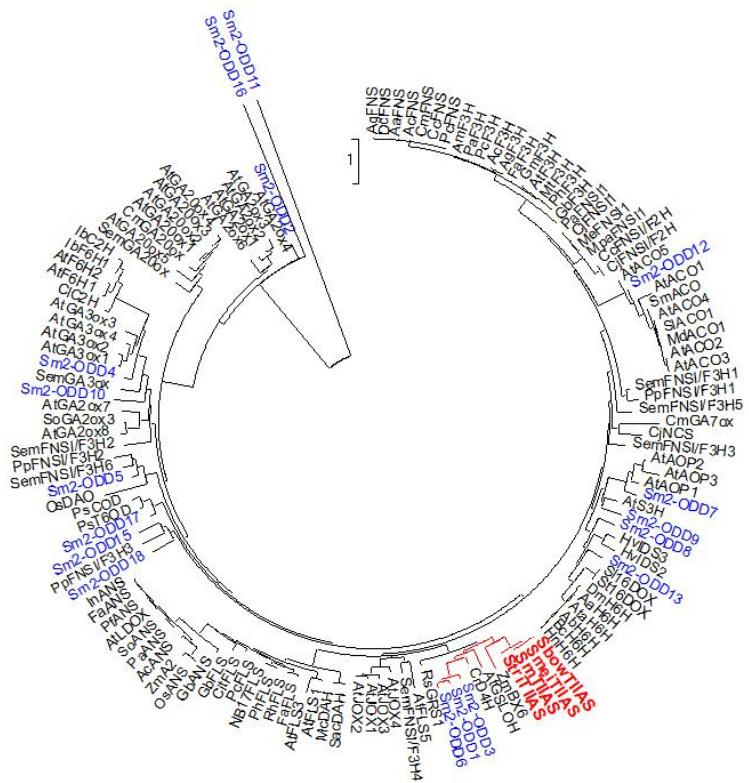
Supplemental Figure S4 Characterization of SmTIIAS activities by *in vitro* assay. (a) UPLC profiles of the catalytic products by SmTIIAS *in vitro*. The effect of 2OG, Fe²⁺ and ASC on the reaction were depicted. (b) Michaelis-Menten kinetic characterization of SmTIIAS with CT as substrate. (c) Michaelis-Menten kinetic characterization of SmTIIAS with iCT as substrate. The values represent means \pm SE of three replicates.



Supplemental Figure S5 The metabolites altered in accumulation in SmTIIAS-RANi lines. (a) The mass spectra for the compounds 1, 2, 3, 4, 6 (colored in black) and the authentic standards (colored in red) of cryptotanshinone, methylenedihydrotanshinquinone, 15,16-dihydrotanshinone I, tanshinone IIA, tanshinone I, respectively. (b) The mass spectra of unknown compounds. 9 and 10 were speculated to be neocryptotanshinone and 16-hydroxy-4-methylenemiltirone.



Supplemental Figure S6 The relative content of tanshinone IIA in *S. miltiorrhiza*, *S. meiliensis*, *S. bowleyana* and *S. trijuga*, respectively. Data represent the mean \pm SD of three replicates.



Supplemental Figure S7 Extended phylogenetic analysis of TIIASs and Sm2-ODDs with other experimentally characterized 2-ODDs. The phylogenetic tree is inferred by Maximum-likelihood method. Bootstrap statistics (1,000 replicates) are indicated at the tree nodes. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site.

Supplemental Table S1 The annotation ratio statistics of *S. miltiorrhiza* and *S. substolonifera* transcriptome database.

	Database	NR	SWISSPROT	KOG	KEGG	GO
<i>S. miltiorrhiza</i>	Annotation_numbers	66,511	50,118	42,020	16,931	45,753
	Annotation_ratio	58.94%	44.41%	37.24%	15.00%	40.54%
<i>S. substolonifera</i>	Annotation_numbers	47,174	38,307	32,565	13,268	34,274
	Annotation_ratio	52.37%	42.53%	36.15%	14.73%	38.05%

Supplemental Table S2 The sequences of 18 *Sm2-ODD* candidate genes and the homolog from Xu and Song (2017).

2-ODD	Homolog and percent identity	Sequence
Sm2- ODD1	SMil_000 18082 99.45%	ATGGCGAATTGTAGCAATGGCGATTGCGATTGGGGTAGAG CAGTTGAAGAGAGTCAGCGAGATGAAAACCGCGTGAAGG GTCTGATGGACGCCGGAGTGACGGAGCTACCGCGTCTGT TCGTTCATCCACCGGAGGATCTGCAGAGCCATCCGCC ACGGCGGACAGCTGGAGCTGCCGACAATAGACCTAGACG GGGCGGAGCGCGGAGGCGCACGGCGGAGGGAGGTGGT GGAGCAGATGGGCAAGGCAGGGCGCAGAGTGGGGTTCT TCCGCATCGTGAACCACGGGATCCCAGGGAGAAATGG ACGGCATGTTGGAGGCGGTGAAGCGATTCCACGAGCTCC CGGCAGGAGAAGCTGGCTTCTACGCAGCCGGCGCAC GACCCCCGGCCGGTGAAGCTGAACAGCAACCTCCCCGTG AGGGAGAAGGATCCGGCCAGTTGGAGGGACGTGGTGAC GTGCGTGTTCAGAGACGATCAACTGGAGGGCGCAACTCAT ACCTTCAGCCCTGAGGAAGGAAATGCTGGATTATGTCAAG TACATGATGGGACTGAGGGGGCTGATGAACGAGTTACTCT CCGAAGCCCTAGGGCTCCCCACCGACTACCTATCCAATCT GGAGTGCATGAGAACGCCAGTCGCTGGCATGTTGGTATTAT CCGGTTTGTCCAGAGCCAACAAAGACCTTAGCCTCACCCA CCCATTCCGACTTGACATTCTGACGCTGCTCATGCAAGA CACCAACCGGGCCCTCCAGATCCTCCGCGACGATCAGTG GTTCGATGTGACGCCGGTTCGCGGGGCCCTCATGCCAA CATTGCGGATCTGATGCAGATTATAAGCAATGGCAAGTTC ATAAGCGTGCAGCACAGAGTGCAGGGCGCAGGGGGTGGG GCCCAAGGATATCGGTGGCGTGCCTCTCGGTCCGAGCGT CAGAGCTACGAGCAAGGCAGTTGGTCCCAGATAACAGAGAGCT CCTTCCGACGAGAGTGGGCCAGATAACAGAGAGGTTAC CCTTCTCAATACCTCAGCAGGTACAAGAGCAAAGGCGAG CGAGTTGCCTCCGCCTGCATTATTACCAAGATAAA ATGGTTGTGATATCTAAAATCCAACCATGAAATGGAGAA AATACCGAGACGACATTGAGCTTCAATCATAAACCTCTGC AACCGATCAGAACGCCATGAAAGAGATGGTGAAGACCTGT GAAGAATATGGTTCTCAAAGTGATCAACCATGGCGTGC CGCAGGGGATAATTCTCAGGTGGAAGAAGAACCCGCG GCTTCTCGCCAAGCCGGGCCAAAAGATGCCGGCTG SMil_000 28460 99.68%
Sm2- ODD2	SMil_000 28460 99.68%	GGCCCCCTATGGCTGCAAGAACATAGGCTTGAGGGCG ACGTCGGAGAAGTCGAATATCTCATTCTCAAACCAACTC CCCCTTCATAATTCTACCGATGAATCCAATAATTCAAGGT CAGCAATAATGCGTATGTGGAAGCAGTGAGGAAGCTGG CATGTGATATATTGGATCTATTGGTGGAGGAGTGTGCGG GTCGGAGGGTGGATCGCGTGTGAGTAGGCTAATGAGAGA CAGTGAGAATGACTCAATCCTAAGGTGAATCACTACCCG GCAGGGCGACGTGAGCAAGATCGGGTTGGCGAGCACACT GACCCTCAGATCATCACCCTCCGATCCAACGGTGTGG

			AGGGCCTCCAGATCTCCGTCCAGGACGGCCTGTGGGTCC CGGTCAACCCCTACCCGGACTCTGCCTCTGCGTCAATGT GGGCGACATCTTACAGGTAAATGACGAATGGAAGGTTGTG AGCGTGAAGCACAGAGTGGCGGTGCAAGCATACGAATCA AGAATGTCGATTGCGTACTTCGTTGCTCCGGCGCTGCATG CGACGGTGAGCTGCCTCCGGGCTGGGCTGCCGCTC TACAGAAGCTTCACTTGGGGTGAATACAAGCAAGCTGTT ATACAGGCAGGCTAGCCGATAACCGCCTCAATCTCTTGC ATTGCCCTCTCATCACAAATAATTAA
Sm2- ODD3	SMil_000 18100 99.66%		ATGGTGAGTTCCAGCAATGGCGACTACGATTGGCCAAG GAAGTTAAAGAAAATCGACGAAACAAAAGCCGGTGTAAAG GTCTTGTGATAACCGGCATAACAAACATCCCCAAAATCTT CGTGCATCCGAAGACCAGTTCGAGACCCATCCTCCACCA CCCACACCCGCCAACGGCTCCGATCTGCCGACGATCGAC TTCCAAGGGCTGCGGAGCGGGCGGTGAAGGGCGGCGGT GGTGGTGGAGGAAATCCGCAAGGC GGCTCAAGAGTGGG GGTTCTTCCGCATAGTGAACCACCTCGATCCCAGTGGAGAC GATGGACGCCATGCTGGCGGGTGAAGCGCTTCCACGA GCTGCCCAACACGAGAAGGC GGCTTACACGACCGA CCAGAGGC GGAGCGTGAAGTTAACAGCAATCTGCCGGA GCGAGAAAACGAGTTGGGTGCTGGAGGGATATCCTGAG CTTCCTCTTCTACGATGACCAGCTGGAGGCCGAGGGAGATA CCCTCTGCCTGCAGGGAGCAAGTGCAGGAATACGTGAAA CACGTGATCCAGCTGAGGGAGGGTGTGGCGGAGCTGTTG TCGGAGGC GTTGGGCTCCGCAGCGACTACCTTCCAGC ATGGAGTGCATGAAAAGCGAGGGCGCTGGCGTGTGTTGATT ATCCTAAATGTCGGAGCCGCACAAGACCTCGGAAACAA GTCGCATTCCGACACCACTTCTTGTACCTTACTCATGCAA GACACCATCGCGGCCCTCCAGATTCTCACGACGCTCAAT GGGTCCACGTCCCTCCGGTCTGGGCCCTCATAGCCA ATGTTGGTGTACCTTGTACGATCATTAGCAATGACAAGTTC ATAAGCGTGGAGCACAGAGTGGCG ATGTGGCATGAGGGCTTCACTATCATGGCGGGCTCCGCC GTGGAGCATGCCAAGCAACTTGGCCTCATCACTATCACC ACTTTTGACGCAATGGACAAGTACCAAAAGAAGATGAA ATCGGTAGCCTACAAATCTTGTCTTAATCCTCAAGGCTT TGGGTGTGGATGAAGAGGAATTAAATTTCATCGTCATCATC ATTTCATGTACCAATCCGACGGTGCATTGCAGCTGAAT TCTTACCCGAGCTGCCGAACCCTAGCCGGCCATCGGG CTGGCCCCGCACACCGATTGTTACTACTGACGATCTTGC ATCAGAACGACACCGACGGCCTCCAAATCTTGCAGGAAG GGGTGGGTGGTCTGGGTCCACCCATACCAGGGGCC CTGGTCGTCAACATAGGCGACCTGATGCACATTGTCCA ACGGGAAATTCCCCACTATGTGCCATCGTGTGCTCCCTAA CGAGACGAGCCATAGATTACCATGGCTTACTTCTATGGG CCCCCGGCCGATTCCGTGGTGGCCCCGCTGCCTAATTG CCTGGGCCACCACGGTTCGGGCAGCCACGGTCAAGGAG TACATCGGCCTCAAGAACAGCATCTTGAGAAGGCTTT CTTAATCCAAATTGA
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Sm2- ODD5	SMil_000 13745		

	96.26%	GAACACCAGCCTAAACCAGGAAGTGTGAAGCCCGAGGC ATCCCATTCATCGACCTCTCCCCGCTGAACCTCCAAGAAC CCGATGCAGCTGCCCTCGATCATCTAGTGTGGAGATAG GTGATGCCTGCAAGATATGGGGCTTCTTCAGATCATCAA CCACGGGTGCCTCACGAGTCCGTAGAGAATTGAGAA AGCATAAAAGAGTTCTTGCTCTCCCCAAGGAGGAGAAG AGGAAGGCCAGCAGAGATGAGGCCAACCTCTTGGCTAT TCGGACTTTGAGATCACCAAGAGCGTCGAGACTGGAAG GAGATCTCGACTGCACCATAGAGAATCCAACGGTCATAT ACGCCTCAGATGAGCCGATGACAAGGAGCTAAAGAGC TCACTAGTCAGTGGCCTCAGTATCCTCCAAATTAAAGGGA GATATGCCAAGAATATGCTGCAGAAATGCAGAAGCTAAGT CACAAGCTACTAGAGCTGATAGCATTGAGCTTAGGCCTAA AAAAAGATCGATTTCATGGCTTAAAGAGCAGACGAG CTTCATGAGGCTGAACTAACCTACCCATCCTGCCCTGCTCCA CAACTAGCACTAGGGCTCGGGAGGCACAAGGATGCCGGT GCCATGACAGTTCTGGCTCAAGACGACGTTGGAGGGCTT GAGGTGAAGAGGAAGCCGGATGGGGAGTGGATCTTGTC AAACCTATGCCGATGTTATATTGTCACGTGGAGACC TAATCCAGGTTGGAGCAATGATAAGTAT
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Sm2- ODD13	SMil_000 27988 97.97%	ATGGAGATTCTTATGTCAAATTGGCTCGAGCCTGTGAAAG AAATACCTGAAAAATACATATTCCAGTTGATAAAAGGCCT GGTCCAATGTTTCCCAGTGATGAAACAACATTCCCTCAT CGATCTCAAAATCCCAATGCCAACAAATGATTCAAGAG ATCTTGATGCAAGCCAAGAATTGGAATCTTCAGGTGAT CAACCATGGGTTCCGGCGAGCCTAATGGAGGAGACGAT GAAGACGATGAAGGGATTCTCGCGACGACGCCGGAGCA CAAGGCCAGTTCTACTCCTCAGATATCACAAAGAAATGC AGAATTACTCTAGCACCTGAATTACCACTCTGAAGATGT CCATTATTGGAGAGATAATTCACTCACCATGCCATCCTC TTCAACATCACATTACCTCTGGCCCTAAACCCCTCCAAT TATAGAGAAGTTGTGGAGCATATTCAATTGAGACAAGGA AGCTGATGCTGAGGATTCTAGAAGCGATATCGGAAGGGC TAGGGCTAAACCTAAATATTGATGGTGAGATTAGCAA ACACAGCTGCTATCAGTGAATCATCACATCCCAGGCC ATCCGAGCCTACGCTGGGATGCCGCCACACCGATC CAAACCTTATCACCATTGCAGCAGTGCTCCGTCCGG CCTCCAAGTCCCTCGCGACCACCACTGGATAGACGTTGA GCCCAACCCCCCGCGCTTCTCGTCATCCCTGGCCTCCA ACTCAAGGTGATAAGCAATGGGAGATTTCGAGGCCGGTT CATAGAGTGGTGACGCATTGCGAGAGGGCGAGGACGACG ATCGGGACGTTCTGATTCCGTCGCCGGAGATCCTTATTG

			AGCCGGCGGAGGACTACGCCGGCACCGCCCCGTCTAC AAAGGCTTCACTTATGAAGAGTTTCAGCTGCTTCACTGG GAGCAACTGTGAAGCTGATGCTGCACTCGCCTTCTCAA AATGAGACCAATTCAAAATCAACAATGTTGCTCTAA
Sm2- ODD14	SMil_000 18668 96.92%		ATGGCCACATCCAGCTGAAAAATTGCTCGCAAGAAAACG AGGCCGATCGCGTGCACGAGCTGAACGCTTCGAGGCCA CAAAAGCCGGCGTGAAGGGGCTCACCGACTCCGGCGTCC AGAAGGTTCCGAGAATGTTCATCAGGCCAGCGACGAGC TCGTCGAGGAGCGCAACCGGAGCCGCTCCCCGCTGCAA GCTCCGGTGATAGACCTCGGCCGGATGGGGAGGGCGA GGGGCGGGAGAAGGCCGTGAGCGAGGTGAGATGGCGT CGAAGGAGCTCGGGATCTTCAGATCGTAACCCACGGGG TGGCCGTGGAGGTATGGACCGCGATGATCGACGGCGTGA GGAAGTTCACGAGCAAGATGCGGAGGCCAAGAACAGT TCCACACGCCGACGCCATCGCAAGGTGATGTACCGA GCAACGTCGATCTGTACAAGTCGCGCCCGAATTGGA GGGACACGTTCTCGGTCGCGCTATGGGTTGGACCGCG TTGAGCCGGAAGAGTTGCCGGAGATTGAGACTCAA CAATCAAGTATCTTGATGAAAGTCACGAATCTGCACACAC TCTATTGAGCTGCTCTCGGAAGCTCTGGGCTCGAACAA GGTTGCCCTAGGAGCCTGAAATGTGGCCGAGGACGCACG TTCGTCGGCCAGTACTACCCCGCGTCCCCGAGCCGGAG CTCACGATGGGCATGACCAACCACACCGATCCTGTTCC TAACTATTCTCTCCAAGATCAAATCGGAGGCCCTCCAAGC TCTGCACAATAGTCAGTACATAATGTGGAGGATATGTTGAGATTG GCTAGCTCGTCGTCAACATTGGAGGATATGTTGAGATTG TGACAAATGATGAGTTCATAGGCCAATTGATAGAGTCCA CGCAAATCGGGCCGGGCCAAGAATCTGGTTGCAGGGCTT TTTCACCGGTGATGCTATTCAAGGGACAATATATGGCCC ATCAAAGAGTTGGTATCAGAGAACAAATCGGGCTCGATACA AAGAGTTCACAGTGGGAGAGTACATGTCCTAGGAGATTG GCGGCCAATTGATAAAATCTGGTCTTGATGAAATGGAGATTG CAAGATGAAAGACAATGTCTAA
Sm2- ODD15	SMil_000 19092 96.44%		ATGGCCGGAGAATCTTATCACTGGGTTCAAAGTGAAA TTGACAAGGATGAGCAGCGAACGATACCACTCACTGTTCC TTTGGCTACAGAAATTCCGGTCATCGACCTCCGCCGATTG CTTGCCTCCACCGATGCCAACCTCTCCGACCTCCGC TCCGCTCTCAGCTCTGGGTTGCTTCAGGTAGTAAATC ATGGCATTGAAAGCTCTACTAGATGAGGTGCGCAGCAT CAGCAGAGAATTCTTCAGCTGCCATGAGCGAGAACAGCA GATATACGCCGGCGAGGAAGAAGGCTACAAATCGACCA ACTGGTCACCGACGACCAATTCCGACTGGTCCCACAAC TTACGCCTCCGTATCTTCCAGAAGATGCCGAAACCCA AATATTGGCCTCAAAATCCGATTCTTCAGAAAGGTGGT GGTGGAAATACGGTGATAAGTTGAGAGGGGTGGCAGAAGA AATACTGAAATTAAACGGGCAAGTCATTGAAGCTAGGTGAT GAGGAGAGTTGTGAAGAAAACGAGCGGAATGTACGCA CAATTCAACTACTACCCCTCCATGTCCGAATCCCGACCGAG TTCTGGGATTGAGACAACATTCTGATTTTCGATGATAACC ATTCTGCTGCAAGACGATCAAGTCCAAGGCCCTCAGCTGC TCAAAGACGACAACGGTTGAGCTCCTACAATGCCTCA

		CGCGCTCCTCGTTCGCTGGGGATCAACTCCAGGTACG CACCATCCAGATTAATTAA
Sm2- ODD16	No hits	ATGAAAGCAATCAGGCTCTGTTACTCAAGCAAGTGTC GTCTTGACAAATTACAATCAACGGCCGTCTTAAATAAT CCAAAAAAATTATTGAAGAATTAAAGAAGCGAGGACCTT TCATTATTACAATTGGTTATTGAAGTTAGGAGATTAAA ACAAGCTGTCAAGTCAGCCTTACATTTGGTGGCCAAT CCGGGGGATGATGATACTTGAGAAATGCTATTGATGAA TGGCAATATCGTCATGAGAAATTATGAAAGGCCGTAAA TGCTTATGCCATCAAGAACATGGCTATATTGTGTTAATT TTCAAGAACATTACAACAATTGGGAAGCTTGGAAAGAC TGCAGATCAGAACATGTGAATTAAACAACAAAGAGGAAT TAATGGCGGGGATGAACAAAATGAGTGGAGTGTGTTTATT ACAACAACTTATCTTCTGTTACAATGCAAACAAAGTTG TGTCTCTCAACAAAGTTCTCAATGGCGTTTACAAAAC ATTGTTGCTTCACATTATGAACATTACACCTTGCCAAT TTAATTAAAACGTGGAAGAGAACGTTGTCATCTGTGAA AATGCTTATTACTTCAACCTAAAAATATTGTGATGAGAAG AAATAAATTATTAAATTAAATTATTAAATGGAAATGTTGA GAATGATGTTCTTCAACCTAGCAAGGAAATTAAAA ATTGAGACGTGAGAAAATGGAACGTCAATTAA TTTTAGAAAAGGAAATGAATGAAGAACATTACTTCTTCT TCACCTATTGGAAAAATACAATTCCCACCTAATTCTGATGA CAACTTAATTGATCAATTAAATTATTCAAAATTACAAA TCAGCTTATTACAATCATCAGAACATTGTTATTACGTT TGCAGCAGATTCTCCCTCATCATTCCCTCTTTCAAC AACTTTAATTGAATTGTTGCTAGAACATTCTCAACTT ATGAAATTGAAGAAAACCAATTGGAGGGAAATTATTGT GTTCTAAAGGTTATTGGAAAAAGTAATTGTGAACGTCC AACATTCTGTGTCATAAAATTAAATTGTTATTGTGGTCAAAT GGGAGGGGAAGAACATTACTGGATGTGTAATTGTATTGT GAAGTGTAA
Sm2- ODD17	SMil_000 12259 100%	ATGGAATCAAAGGCGCAGATATTAGGCAGATCACTGAAGG TGCCATTGTGCAAGAACATTGCAAAGGAGAAACTGAGCAG TGTGCCGTCGAGATACATCCGACCCGATCACCAACATCTC ACCGCCGCCGATGTTCCCTCCACCTCAAATCCCCGTCA TCGATATGCAGAACGCTGCTCCTCAGATTCCATGGATT CGAGCTTCACAGGCTGCACGAAGCTTGCCTAGATTGGGG TTCTTCAGTTGATCAACCACGGCGTGGATGCGGCGCC CATAGCCAAATGAGGTCGGAAATGACGGCGTTCTTCAAC CTCCCGCCGGAAGAGAACGGACGTATTCCGGCAGAAGGAA GATGACGTGGAAGGCTACGGCCAAGCCTCGTCACATCC CAAGAGCAGAACGCTCGATTGGCGGATTATTCTGTC TCACTTCGCCTCCTATTGAGAAAGCCTCACCTAATTCCC AAGCTTCCCTGCTCGTTCAGAGATGCCATAGATGAGTATG GAGCAGATCTAAGGAAGCTAGCGATGAAGATCTTAGGT CATGGCGAAAGCACTAGGCATGAAGGGCGAAGAGATGAG GTCGGCGTTGATGAAGGGACCGCAGGCGATGAGGATGAA TTACTATCCGCCATGCCGCAGCCGGAGCTGGTGACAGG CCTCTGCCCTATTCCGACGCAGTCGCCCTACCATTCTG

CTCCAGGTTAACGACATCTCAGGCCTCAGGTAGCAAAG
ATGGCAAATGGATTCCCGTTACTCCACTCCCTCATGCATT
GTCATCAATGTTGGCGACATATTGGAGATTATGAGCAATG
GCGCTTACCGCAGCGTTGAGCATGGCGACTGTGCACA
GCGAGAAAGAGCGGTTGCCATGCCACCTCCTCAGCA
CCAGGCTGGATGGCGATATAGGTCCGGCGCAAGCCTCG
TTGGCCCCGAAACTCCGGCGAAATTCAAGACGATCAGCG
CGGTTGAATATGTCACGGGTTGTTGCAAAGGAGCTCAA
GGGTAAGTCGTATGTGGACCTCATGAGGATTAGAATTAG
ATGGCCACCGCTGTTGAGTGCCGTCCAAGAACTATCC
GGCGCCGTGAGCAGCCCGCCGGAGAAATACCTACTGAAA
GACGGGATCGCGGCCCCGAGTTCCCGTTCTCGCAGTT
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AGGGGAAAGGGAGCTGGAGAAAGCTCAAGCTTGTTCAG
CTCTTGTGGTTATTCAGGTGGTAATCATGGCATGGAT
GACGCCTTCTTGGACGAGGTGCATGGCGTGACCAAGGAG
TTCTTCTCACTCCCAATGGAAGAGAAGATGAAAAGCGCGA
GGCCCAAAGATGATATTGATGGCTATGGAATGACACTGT
TTACTCAGACACCCAAACTCTGATTGAAATGAGTTGT
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GGTGTGGCCCAAAGCGCACGGCGACGCCCTCGCCA
TGACGTACCTCTTGCAAGGATAACAAAGTGGAAAGGGCTTCA
AACTCTGAAAGACGGCGTCTGGTACAGAGTTCCCATCATT
CATATGCTATCGTCGTCAACGTTGGTATCAACTCGAGA
TAATGAGTAATGGAATATTGAGAGGCCGATACACAGAGT
TGTGACAAATCCAGAGAAAAGAGAGGATTACCATAGCAATT
TTCTTCAGCCCTGACCCAACCAGCGAAGTTGGCCTCATG
AAGGACTAATTAATGAAAAGAGGCCCAAATTATTCAAGAGT
GTTGTTGATTACACTGGTAACTATTCAGTCTTCAGAC
AGGGAAAAGGCCATTGACCTCTCAGACTTAA

Supplemental Table S3 Protein sequences information for the phylogenetic tree.

Enzyme abbreviation	Accession ID	Species
AcF3H	ABG78792.1	<i>Aethusa cynapium</i>
AcFNS	ABG78791.1	<i>Aethusa cynapium</i>
AcANS	ABM66367.1	<i>Allium cepa</i>
AmF3H	AAX21539.1	<i>Ammi majus</i>
AgF3H	AAX21540.1	<i>Anethum graveolens</i>
AaFNS	ABG78793.1	<i>Angelica archangelica</i>
AaH6H	ABM74185.1	<i>Anisodus acutangulus</i>
AtaH6H	AAQ75700.1	<i>Anisodus tanguticus</i>
AgFNS	AAX21537.1	<i>Apium graveolens</i>
AtACO1	NP_179549.1	<i>Arabidopsis thaliana</i>
AtACO2	NP_176428.1	<i>Arabidopsis thaliana</i>
AtACO3	NP_172665.1	<i>Arabidopsis thaliana</i>
AtACO4	NP_171994.1	<i>Arabidopsis thaliana</i>
AtACO5	NP_565154.1	<i>Arabidopsis thaliana</i>
AtAOP1	NP_192216.1_1	<i>Arabidopsis thaliana</i>
AtAOP2	AAL14646.1_1	<i>Arabidopsis thaliana</i>
AtAOP3	AAL14647.1_1	<i>Arabidopsis thaliana</i>
AtF3H	AEE78766.1	<i>Arabidopsis thaliana</i>
AtF6H1	NP_187970.1	<i>Arabidopsis thaliana</i>
AtF6H2	NP_175925.1	<i>Arabidopsis thaliana</i>
AtFLS1	NP_196481.1	<i>Arabidopsis thaliana</i>
AtFLS3	NP_201164.1	<i>Arabidopsis thaliana</i>
AtFLS5	NP_001032131.1	<i>Arabidopsis thaliana</i>
AtGA20ox1	NP_194272.1	<i>Arabidopsis thaliana</i>
AtGA20ox2	NP_199994.1	<i>Arabidopsis thaliana</i>
AtGA20ox3	NP_196337.1	<i>Arabidopsis thaliana</i>
AtGA20ox4	NP_176294.1	<i>Arabidopsis thaliana</i>
AtGA20ox5	NP_175075.1	<i>Arabidopsis thaliana</i>
AtGA2ox1	NP_177965.1	<i>Arabidopsis thaliana</i>
AtGA2ox2	NP_174296.1	<i>Arabidopsis thaliana</i>
AtGA2ox3	NP_181002.1	<i>Arabidopsis thaliana</i>
AtGA2ox4	NP_175233.1	<i>Arabidopsis thaliana</i>
AtGA2ox6	NP_171742.1	<i>Arabidopsis thaliana</i>
AtGA2ox7	AEE32606.1	<i>Arabidopsis thaliana</i>
AtGA2ox8	NP_193852.2	<i>Arabidopsis thaliana</i>
AtGA3ox1	NP_173008.1	<i>Arabidopsis thaliana</i>
AtGA3ox2	NP_178150.1	<i>Arabidopsis thaliana</i>
AtGA3ox3	NP_193900.1	<i>Arabidopsis thaliana</i>
AtGA3ox4	NP_178149.1	<i>Arabidopsis thaliana</i>
AtGSLOH	NP_180115.1	<i>Arabidopsis thaliana</i>
AtLDOX	NP_194019.1	<i>Arabidopsis thaliana</i>
AtS3H	NP_192788.1	<i>Arabidopsis thaliana</i>
AtJOX1	NP_187728.1	<i>Arabidopsis thaliana</i>
AtJOX2	Q9FFF6.1	<i>Arabidopsis thaliana</i>
AtJOX3	Q9LY48.1	<i>Arabidopsis thaliana</i>
AtJOX4	AEC09512.1	<i>Arabidopsis thaliana</i>
AbH6H	ABR15749.1	<i>Atropa baetica</i>
BcH6H	ACB40931.1	<i>Brugmansia candida</i>
CrD4H	AAB97311.1	<i>Catharanthus roseus</i>
CIC2H	AER36089.1	<i>Citrus limetta</i>
CitFLS	BAA36554.1	<i>Citrus unshiu</i>

CmFNS	AAX21538.1	<i>Conium maculatum</i>
CcFNSI/F2H	QEP99660.1	<i>Conocephalum conicum</i>
CjFNSI/F2H	QEP99661.1	<i>Conocephalum japonicum</i>
CjFNSI1	QEP99662.1	<i>Conocephalum japonicum</i>
CjNCS	BAF45337.1	<i>Coptis japonica</i>
CmGA20ox	AAB64345	<i>Cucurbita maxima</i>
CmGA7ox	AAB64346	<i>Cucurbita maxima</i>
CcFNS	ABG78790.1	<i>Cuminum cyminum</i>
DmH6H	AAQ04302.1	<i>Datura metel</i>
DcFNS	AAX21536.1	<i>Daucus carota</i>
FaANS	AAU12368.1	<i>Fragaria x ananassa</i>
FaF3H	AAU04791.1	<i>Fragaria x ananassa</i>
FaFLS	AAZ78661.1	<i>Fragaria x ananassa</i>
GbANS	ACC66092.1	<i>Ginkgo biloba</i>
GbF3H	AAU93347.1	<i>Ginkgo biloba</i>
GbFLS	ACY00393.1	<i>Ginkgo biloba</i>
GmF3H	AAT94365.1	<i>Glycine max flavanone</i>
HvIDS2	BAA03647.1	<i>Hordeum vulgare</i>
HvIDS3	BAA75493.1	<i>Hordeum vulgare</i>
HnH6H	AAA33387.1	<i>Hyoscyamus niger</i>
IbC2H	BAL22346.1	<i>Ipomoea batatas</i>
IbF6H1	BAL22344.1	<i>Ipomoea batatas</i>
InANS	BAB71811.1	<i>Ipomoea nil</i>
MdACO1	Q00985.1	<i>Malus domestica Borkh.cv. Golden deliciou</i>
MeFNSI1	QEP99659.1	<i>Marchantia emarginata</i>
MpaFNSI1	QEP99658.1	<i>Marchantia paleacea</i>
MtF3H	ACR15123.1	<i>Medicago truncatula</i>
McDAH	QJD15033.1	<i>Menispermum canadense</i>
NB17FLS	BAC10995.1	<i>Nierembergia sp.</i>
OsANS	CAA69252.1	<i>Oryza sativa</i>
OsDAO	NP_001053075.1	<i>Oryza sativa</i>
PsCOD	ADD85331.1	<i>Papaver somniferum</i>
PsT6OD	ADD85329.1	<i>Papaver somniferum</i>
PfANS	BAA20143.1	<i>Perilla frutescens</i>
PcF3H	AAP57394.1	<i>Petroselinum crispum</i>
PcFLS	AAP57395.1	<i>Petroselinum crispum</i>
PcFNS	AAP57393.1	<i>Petroselinum crispum</i>
PhF3H	AAC49929.1	<i>Petunia hybrida</i>
PhFLS	CAA80264.1	<i>Petunia hybrida</i>
PpFNSI1/F3H	XP_001780809.1	<i>Physcomitrella patens</i>
PpFNSI2/F3H	XP_001781297.1	<i>Physcomitrella patens</i>
PpFNSI3/F3H	XP_001785619.1	<i>Physcomitrella patens</i>
PaANS	BAE54521.1	<i>Phytolacca americana</i>
PaF3H	AAX21535.1	<i>Pimpinella anisum</i>
PafNSI1	MK557763	<i>Plagiochasma appendiculatum</i>
RsGRS1	BAW81934.1	<i>Raphanus sativus</i>
RhFLS	BAC66468.1	<i>Rosa hybrida</i>
SmACO	AFJ75398.1	<i>Salvia miltiorrhiza</i>
SemFNSI1/F3H	XP_002985262.1	<i>Selaginella moellendorffii</i>
SemFNSI2/F3H	XP_002967867.1	<i>Selaginella moellendorffii</i>
SemFNSI3/F3H	XP_002963905.1	<i>Selaginella moellendorffii</i>
SemFNSI4/F3H	XP_002963353.1	<i>Selaginella moellendorffii</i>
SemFNSI5/F3H	XP_002965940.1	<i>Selaginella moellendorffii</i>

SemFNSI6/F3H	XP_002965430.1	<i>Selaginella moellendorffii</i>
SemGA20ox	ABX10768.1	<i>Selaginella moellendorffii</i>
SemGA3ox	ABX10776.1	<i>Selaginella moellendorffii</i>
SacDAH	QJD15032.1	<i>Sinomenium acutum</i>
Si16DOX	BBD17782.1	<i>Solanum lycopersicum</i>
SIACO1	NP_001234024.2	<i>Solanum lycopersicum</i>
St16DOX	BBD17781.1	<i>Solanum lycopersicum</i>
SoANS	BAE54520.1	<i>Spinacia oleracea</i>
SoGA2ox3	AAX14674.1	<i>Spinacia oleracea</i>
ZmA2	CAA39022.1	<i>Zea mays</i>
ZmBX6	NP_001105100.1	<i>Zea mays</i>

Supplemental Table S4 List of oligonucleotide primer sequences.

2ODD7-BamHI-F	CGGGATCCATGGTTGTGGAGAGCCTTGG
2ODD7-NotI-R	ATAAGAATGCGGCCGCCTAGTGTCCACAATATTCTT
2ODD8-BamHI-F	CGGGATCCATGGAAAATCATGCGTCAAA
2ODD8-NotI-R	ATAAGAATGCGGCCGCTTAATTAAATTGAAGGGATATA
2ODD14-BamHI-F	CGGGATCCATGGCCACATCCAGCTTGAA
2ODD14-NotI-R	ATAAGAATGCGGCCGCTTAGACATTGTCTTCATCTT
2ODD16-BamHI-F	CGGGATCCATGAAAGCAATCAGGCTCTC
2ODD16-NotI-R	ATAAGAATGCGGCCGCTTACACTCACAAAATACAA
2ODD17-BamHI-F	CGGGATCCATGGAATCAAAGGCGCAGAT
2ODD17-NotI-R	ATAAGAATGCGGCCGCTAATTCTGAATCCTCATGA
2ODD18-BamHI-F	CGGGATCCATGGCCACCGCTTGTGAG
2ODD18-NotI-R	ATAAGAATGCGGCCGCTTAAAGTCTGAGAAGGTCAA
SmT II AS-RNAi-F	GGGGACAAGTTGTACAAAAAAAGCAGGCTCAAGATGCGGAGGC GAAGAACGAGTT
SmT II AS-RNAi-R	GGGGACCACTTGTACAAGAAAGCTGGTTTGAGAAGAATAGT TAGGAAACAA
qRTSmActin-F	CTGACAGGATGAGCAAGGAG
qRTSmActin-R	GCGAACGAAAGAGTTGATT
qRTSmT II AS-F	ACCGATCCTGTTTCCTAAC
qRTSmT II AS-R	CCGATTGCGTGGACTCTAT
SmeiT II AS-BamHI-F	CGGGATCCATGGCCACATCCAGCTTGGAAAAATT
SmeiTIIAS-NotI-R	ATAAGAATGCGGCCGCTTAGACATTGTCTTCATCTTGCAAT
SbowTIIAS-BamHI-F	CGGGATCCATGCAGGAAGACGATCGCGTGAAGG
SbowTIIAS-NotI-R	ATAAGAATGCGGCCGCTTAGACATTGTCTTCATCTTGCAAT
StrITIIAS-BamHI-F	CGGGATCCATGGCGGCAGACGATCGCGTGGAGG
StrITIIAS-NotI-R	ATAAGAATGCGGCCGCTTAGACATTGTCTTCATCTTGCAAC
Sm-N144D-2F	GTACGCGAGCGACGTCGATCTGT
Sm-N144D-1R	ACAGATCGACGTCGCTCGCGTAC
Sm-L147A-2F	CAACGTCGATGCGTACAAGTCGC
Sm-L147A-1R	GCGACTTGTACGCATCGACGTTG
Sm-R156W-2F	CGCGAATTGGTGGGACACGTTCT
Sm-R156W-1R	AGAACGTGTCCCACCAATTGCG
Sm-P245M-2F	CCACACCGATATGTGTTCTAA
Sm-P245M-1R	TTAGGAAACACATATCGGTGTGG
Sm-F314W-2F	GGTTGCGGGCTGGTCACCGGTG
Sm-F314W-1R	CACCGGTGAACCAGCCCCGCAACC
Sm-F352A-2F	CATGTCCAAGGCTTAGAGCGGC
Sm-F352A-1R	GCCGCTCTAAAGCCTGGACATG